Virgin Coconut Oil Increases the Productivity of Broiler Chicken Post Avian Influenza Vaccination

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Abstract. Chicken productivity is not only determined by body weight increase and feed efficiency but also disease resistance. Avian influenza (AI) is still an endemic in Indonesia. Highly mutative characteristic of AI causes unsuccessful vaccination to preventing chicken mortality; therefore, feed modulation alternatives are sought to raise body weight and body immune as well. Virgin coconut oil (VCO) contains fatty acid potential as antimicrobe and antivirus; VCO intake is therefore expected to increase chicken body immune. This research aimed at feed modulation to increase broiler chicken productivity. Forty broiler chicken of one day old (DOC) were used and the research applied Completely Randomized Factorial Design in which factor one was two vaccine levels namely AI-vaccinated chickens and AI-unvaccinated chickens. Factor two used four levels of VCO: 0, 5, 10, 15 mL/kg feed. DOC chickens were divided into eight treatment groups and repeated in five experiment units. Feed and water were given *ad libitum*. The result demonstrated that in spite of heterophile increase in AI-vaccinated VCO-given chickens, heterophile/lymphocyte ratio and feed intake were not significantly different among all treatment groups. With the highest body weight found in AI-vaccinated chickens given 10ml/kg feed VCO, it could therefore be concluded that VCO intake of 10mL/kg feed could raise body weight.

Key words: heterophile, heterophile/lymphocyte ratio, feed intake, body weight

Abstrak. Produktivitas ayam tidak hanya ditentukan oleh kenaikan bobot badan dan efisiensi pakan, tetapi juga ketahanan terhadap penyakit. Avian influenza (AI) masih merupakan wabah endemis di Indonesia. Sifat AI yang mudah bermutasi menyebabkan vaksinasi tidak selalu berhasil untuk mencegah kematian ayam, sehingga dicari alternatif modulasi pakan untuk meningkatkan bobot badan dan kekebalan tubuh. Virgin coconut oil (VCO) mengandung potensi asam lemak sebagai antimikroba dan antivirus, sehingga asupan VCO diharapkan dapat meningkatkan kekebalan tubuh ayam. Penelitian ini bertujuan untuk modulasi pakan untuk meningkatkan produktivitas ayam broiler. Empat puluh ayam broiler umur satu hari (DOC) digunakan dalam penelitian yang menerapkan Rancangan Acak Lengkap Faktorial dengan faktor pertama dua level vaksinasi yaitu ayam divaksin AI dan tidak divaksin AI. Faktor kedua adalah empat level VCO: 0, 5, 10, 15 mL kg pakan. DOC ayam dibagi menjadi delapan kelompok perlakuan dan diulang dalam lima unit percobaan. Pakan dan air minum diberikan ad libitum. Hasilnya menunjukkan bahwa meskipun adanya kenaikan heterofil pada ayam yang divaksin AI dan diberi VCO, rasio heterofil/limfosit dan konsumsi pakan tidak berbeda secara signifikan pada semua kelompok perlakuan. Dengan bobot badan tertinggi ditemukan pada ayam yang divaksin AI dan diberi VCO 10 ml/kg pakan, maka dapat disimpulkan bahwa asupan VCO 10 mL/kg pakan dapat meningkatkan bobot badan.

Kata kunci: heterophil, rasio heterophil/limfosit, konsumsi pakan, bobot badan

Introduction

For two decades, AI vaccine has become a significant tool in controlling Avian Influenza in poultry because vaccine can increase virus resistance, prevent disease and mortality, and minimize contagion (Swayne and Kapczynski, 2008). To date, AI in chickens especially broiler is still endemic in several area in Indonesia, resulting in decrease of chicken population and production of eggs and meat (Kementan, 2012). Science development makes broiler productivity be regarded not only from body weight increase and feed efficiency but also health. Feed modulation to increase broiler's body immune is also expected to increase productivity by means of body weight rise and decrease in chicken mortality due to disease (Kidd, 2004).

Virgin coconut oil (VCO) is food supplement producible in Indonesia and safety proven for human consumption, hence it is assumed to be safe for chicken. Nutritional value test showed VCO contains 51.23% lauric acid, 17.13% myristic acid, 7.30% palmitic acid, 9.18 caprilic acid, 7.07% capric acid, 5.42% oleic acid, 2.17 stearic acid and 0.51% caproic acid. 90% fatty acid in VCO is saturated fat and only 10% is unsaturated fat (Setiaji, 2009). Saturated fat in VCO especially palmitic and myristic acids are phospholipids component of T cell, the decrease of T lymphocyte is therefore able to fix by giving palmitic and myristic acids (Enig, 2004). Lymphocyte Th will activate macrophage as cellular immunity response against infection with intracellular pathogen (Gordon, 2003). Some research showed that VCO was potential as antiviral and anti bacterial agents (Bergsson et al., 1998). Fatty acid in VCO is also potential as antivirus (Bartolotta et al., 2001). Body immune enhancement is an alternative to prevent AI in broiler because H5N1 virus is easily to mutation (Peiris et al., 2007) and it tends to develop disease in particular area (Suarez and Cherry, 2000). This characteristic of AI makes AI vaccine given to the chickens not highly protective against AI virus attack (Perkin and Swayne, 2003).

Heterophile is essential component of innate body immune, working rapidly in detecting and killing pathogen and directing signal towards other mechanisms of immune response. Heterophile holds an important role at the early stage of infection, with rapid activation through chemotaxis process that enables heterophile to kill the pathogen. Detection of bacteria molecule is through receptor that later stimulate heterophile to phagocytosis and induce cytokine expression. Heterophile contains anti microbial components, able to release through degranulation to kill bacteria through phagocytosis process (Redmond et al., 2011). Heterophile is the front line defense mechanism activated during inflammation and therefore has an important role in chickens' disease resistance (Harmon, 1998). Heterophile is able to respond to pathogen within 30 minutes in early stage of inflammation. The increase of innate immune response will reduce disease which later leads to productivity rise (Farnell et al., 2006). Heterophile, the first cell to migrate to infection spot, is an essential cellular component from innate immunity because it serves as a more effective sign to choose chickens with better immunity (Ferro et al., 2004).

This research aimed to figure out the feed modulation by giving VCO in order to increase chicken productivity through parameters of the amount of heterophile, heterophile/lymphocyte ratio, feed intake and body weight.

Materials and Methods

Forty broiler chickens were used in this research, kept in collective cages per ten heads to

three week old age, then moved to individual cage up to 5 week old. Chickens were randomly placed in the cages equipped with feed and water containers. Control feed used was manufactured BR1 pellet, while treatment feed was control feed mixed with VCO manually based on each treatment namely 0, 5, 10, and 15mL VCO/kg feed. VCO used was manufactured one with consistent quality warranty. Feed and water were given ad libitum. AI vaccine sub type of H5N1 was given to the chickens intra-muscularly as much as 0.5mL per head.

The observed variables were the amount of heterophile, heterophile/lymphocyte ratio, feed intake and body weight. The amount of heterophile was determined from blood smear. Chicken's blood was collected at the end of the treatment, taken from the wing vein and put in 2ml tube for blood smear. Blood smear was initiated by smearing blood on glass object, then fixated with methanol, colored with Giemsa, washed with water and let dry at room temperature. After dry it was then observed under microscope to count the percentage of heterophile obtained (Bain and Path, 2005). Determining heterophile/lymphocyte ratio was by having the number of heterophile divided by the number of lymphocyte. Chicken's feed intake per head was obtained from summing up the amount of feed intake per week for four weeks. Body weight was figured out from weighing the chickens' living body weight every week until the chickens reached four week old.

This research applied factorial design in which factor one was two vaccine levels namely AI-vaccinated chickens and AI-unvaccinated chickens. Factor two used four levels of VCO, 0, 5, 10, 15 ml/kg feed. Chickens were divided into eight treatment groups and repeated in five experiment units. Treatment was done within four weeks and sample was collected on the fifth week. The obtained data were then analyzed using ANOVA and continued with LSD test (Gomez and Gomez, 1995).

Results and Discussion

The amount of heterophile in AI-vaccinated broilers was higher ($P<0.05$) than the unvaccinated ones, accordingly, VCO intake as much as 10mL/kg feed and 15mL/kg feed in AI-vaccinated chickens showed higher amount than the AI-vaccinated chickens without VCO (Table 1). Result on VCO giving and AI vaccine towards broilers' heterophile/lymphocyte ratio did not show any difference among the group of AI-vaccinated and AI-unvaccinated, with or without VCO (Table 2).

This result showed that the increase of heterophile followed by the increase of lymphocyte would not result in an increase in heterophile/lymphocyte ratio, accordingly, no treatment group underwent physiological stress due to AI vaccination or VCO giving. This condition was proven by no different level of feed intake in all treatment groups (Table 3).

Chicken body weight is a sensitive indicator on body response against disease (Dehkordi et al., 2011). In this research body weight increase was observed in AI-vaccinated chicken group, and the heaviest one was on AI-vaccinated group given 10ml VCO/kg feed (Table 4).

In this research AI vaccine could increase the number of heterophile since vaccine using inactive AI virus could activate the antigen (Holt, 1990) and therefore would stimulate the innate immune response through the expression of various receptors involved in pathogenesis (Kogut et al.,2003). Heterophile is the front line of cellular defense against pathogenic microbe invasion, white blood cell main component, having granule, and responding to acute

Table 1. The amount of heterophile from AI-vaccinated chickens and AI-unvaccinated chickens after feeding Virgin Coconut Oil

Level of virgin coconut oil (ml/kg feed)	Unvaccinated AI	Vaccinated AI	Total VCO
VCO 0	8498±923.20	10364± 695.93	18862[a]
VCO 5	11739±585.03	14049±1588.51	25788[c]
VCO 10	13159±468.66	20784±619.94	33943[d]
VCO 15	10374±639.24	12932±858.00	23306[b]
Total Vaccine	43770[a]	58129[b]	

VCO 0, 5, 10, 15: virgin coconut oil with level of 0, 5, 10, 15 mL/kg feed.
Values bearing different superscript at the same column differ significantly (P<0.05)

Table 2. Ratio heterophile to lymphocyte ratio of AI-vaccinated and unvaccinated chickens after feeding virgin coconut oil

Level of virgin coconut oil (ml/kg feed)	Unvaccinated AI	Vaccinated AI	Total VCO
VCO 0	5.07±0.33	4.99±0.18	10.054
VCO 5	5.20±0.11	4.98±0.28	10.182
VCO 10	5.48±0.22	5.35±0.15	10.830
VCO 15	5.10±0.18	5.16±0.18	10.254
Total Vaccine	20.847	20.473	

VCO 0, 5, 10, 15: virgin coconut oil with level of 0, 5, 10, 15 mL/kg feed

Table 3. Feed intake of AI-vaccinated chickens and unvaccinated chicken after feeding virgin coconut oil(gram)

Level of virgin coconut oil (ml/kg feed)	Unvaccinated AI	Vaccinated AI	Total VCO
VCO 0	10835.8±41.3	10336.6±59.8	21172.4
VCO 5	10409.4±44.6	10332.3±56.6	20741.7
VCO 10	10353.4±47.3	10571.0±35.4	20924.4
VCO 15	10413.9±65.4	10248.4±39.4	20662.3
Total Vaccine	42012.5	41488.3	

VCO 0, 5, 10, 15: virgin coconut oil in level of 0, 5, 10, 15 ml/kg feed

inflammation through the activity of phagocytosis and having anti microbial activity with wide spectrum (Harmon, 1998). Heterophile would rapidly be taken to the infection spot and phagocytosis took place (Hea et al., 2005). Heterophile in the artery would interact with endothelial receptor, emigrated through the artery wall following gradient chemotactic and accumulated in inflaming cell. Chemotactic factor resulted from the mix of serum or plasma with bacteria. In heterophile granule was found beta-defensin, group of peptide cationic, lysozyme, and phosphatase acid. In the granule hydrolysis of glucoronidase, cathepsin, and glucocidase acids took place. The finding of granule content showed that heterophile could have phagocytosis

Table 4. Body weight of AI-vaccinated chickens and unvaccinated after feeding Virgin Coconut Oil (gram)

Level of virgin coconut oil (ml/kg feed)	Unvaccinated AI	Vaccinated AI	Total VCO
VCO 0	6563±43.09	6479±42.82	13042^{b}
VCO 5	6524±45.71	6586±59.18	13110^{c}
VCO 10	6582±37.51	6799±101.16	13381^{d}
VCO 15	6217±82.45	6245±59.63	12462^{a}
Total Vaccine	25886	26109	

VCO 0, 5, 10, 15: virgin coconut oil with level of 0, 5, 10, 15 mL/kg feed. Values bearing different superscript at the same column differ significantly (P<0.05)

by releasing the granule content. Heterophile would undergo respiratory burst and oxidate glucose when met with phagocytosis stimulation. Defensins had wide spectral activity towards positive gram bacteria, negative gram bacteria, protozoa, fungi and several enveloped viruses (Harmon, 1998). Peptide cationic is the strongest molecule for microbe destruction (Bennoune et al., 2009).

The intake of 10 ml VCO/kg feed increased the heterophile amount but decreased in that of 15 ml VCO/kg feed. It was assumed that VCO intake could increase the lysis in enveloped virus and bacteria, as research on human that the number of white blood cell and neutrophlile would increase after given with Medium Chain triglycerides (MCT) saturated chain due to membrane disintegration on enveloped virus resulting in virus became lysis (Versleijen et al., 2008). VCO contained lauric acid which in the body would be turned into monoglyceride of lauric acid or monolaurin (Enig, 1997). Monoglyceride from caproic acid, caprylic acid, lauric acid and myristic acid could activate virus (Isaacs et al, 1995). Monolauric acid worked on all virus and decreased ineffectivity through the destruction of virus envelope. The decrease of heterophile amount in 15ml VCO/kg feed intake was due to the change of lypid structure that in turn changed the membrane fluidity, the lypid structure accordingly determined the anti-infectious work of lypid related to its structure (Hierholzer and Kabara, 2007).

The increase of heterophile amount in AI-vaccinated chickens given with 10ml VCO/kg feed did not show any infection that might lead to physiological stress. The presence of physiological stress was detectable using heterophile/ lymphocyte ratio (Maxwell and Robertson, 1998), because the function of heterophile could be expressed by nitric oxide synthesis (Gudev et al., 2011). The increasing heterophile/lymphocyte ratio therefore showed an increasing level of stress as well (Cetin et al., 2011). Stress in chicken would obstruct the appetite and later decreased the feed intake (Rybkin et al., 1997), consequently feed intake that was not different in all treatments showed that physiological condition of the chickens was fit.

Chicken body weight increased in the group vaccinated with AI compared to the unvaccinated ones despite the statistically insignificant increase (P>0.05). VCO giving however increased body weight significantly (P<0.05). The highest body weight increase was in group with 10ml VCO/kg feed intake which then decrease at the giving of 15ml VCO/kg feed. This related to the increasing lymphocyte amount at 10ml VCO/kg feed intake

and it decreased at 15 ml VCO/kg feed because glycerolmonolauric from VCO at low concentration could modulate lymphocyte proliferation leading to lymphoproliferation and toxin inhibition. While at high concentration, it would restrain the lymphocyte proliferation and block the proliferative effect of T lymphocyte (Witcher et al. 1995). Lymphocyte activation was initiated by splitting up phosphatidyl inositol into inositol triphosphate and diacylglycerol. This second messenger would induce calcium increase and therefore activate the protein kinase C. Protein kinase C increase would as well increase phosphorylation of various cellular protein and transcription of some genes (Kaplan and Cohen, 1991). The increase of gene transcription would increase protein synthesis process that led to body weight increase.

Conclusions

Virgin coconut oil and AI vaccine could increase body weight of broiler chickens without any disease that might cause physiological stress because there was no increase in heterophile/lymphocyte ratio increase nor feed intake decrease despite the increase of heterophile amount. Virgin coconut oil therefore could modulate feed to increase the productivity of broiler chicken.

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