THE EFFECT OF VIRGIN COCONUT OIL ON LYMPHOCYTE AND CD4 IN CHICKEN VACCINATED AGAINST *Avian Influenza* VIRUS

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ABSTRAK

Penelitian ini bertujuan untuk mencari alternatif pencegahan penyakit avian influenza (AI) pada ayam pedaging melalui peningkatan daya tahan tubuh. Limfosit T akan bereaksi secara langsung melawan antigen yang telah dipresentasikan ke permukaan sel oleh *antigen presenting cell* (APC). Interaksi Th-CD4 berperan membantu mampertahankan ikatan Th-APC agar tetap menyatu pada saat aktivasi antigen spesifik berlangsung. Asam lemak pada virgin coconut oil (VCO) berpotensi sebagai immunostimulan sehingga mampu meningkatkan daya tahan tubuh ayam melalui peningkatan limfosit T dan Th-CD4. Penelitian ini menggunakan 40 ekor ayam broiler umur satu hari. Desain penelitian yang digunakan adalah Rancangan Acak Lengkap pola faktorial dengan faktor pertama adalah 2 level vaksin yaitu kelompok ayam yang divaksin AI dan kelompok ayam yang tidak divaksin AI. Faktor kedua menggunakan 4 level VCO yaitu 0, 5, 10 dan 15 mL/kg pakan. Ayam broiler dikelompokkan dalam 8 kelompok perlakuan dan dilakukan pengulangan dalam 5 unit percobaan. Pakan dan minum diberikan *ad libitum* selama 4 minggu. Hasil penelitian menunjukkan bahwa jumlah limfosit dan Th-CD4 pada ayam yang diberi VCO sebesar 10 mL/kg pakan dan divaksin AI lebih tinggi dibanding pemberian VCO tanpa divaksin AI.

Kata kunci: avian influenza, ayam pedaging, CD4, limfosit, VCO

ABSTRACT

This research aimed to find preventing alternative of avian influenza (AI) disease in broiler chicken by increasing body immune. Lymphocyte T would directly react to antigen presented to the cell surface by antigen presenting cell (APC). Th-CD4 interaction functioned to maintain Th-APC bond intact during specific antigen activation. Fatty acid in virgin coconut oil (VCO) was potential as immunostimulant, which therefore could increase chicken immunity through the increase of lymphocyte T and Th-CD4. This research used 40 one-day-old broiler chickens. The method applied was Completely Randomized Factorial Design in which the first factor was two levels of vaccine, namely groups of AI vaccinated and unvaccinated. The second factor was four levels of VCO namely 0, 5, 10, 15 mL/kg feed. Day Old Chick (DOC) were divided into eight treatment groups and repeated five times. Feed and water were given *ad libitum* for four weeks. The result showed that the number of lymphocyte and Th-CD4 in chickens given 10 mL per kg feed and vaccinated with AI was higher than that in chickens given VCO without AI vaccine.

Key words: Avian influenza, broiler chicken, CD4, lymphocyte, VCO

INTRODUCTION

Chicken as other vertebrate contain two kinds of main lymphocyte namely B lymphocyte or B cell and T lymphocyte or T cell. B cell and T cell were specified for different antigen and both cells performed different defense activity but completing each other. Some researches reported that lymphocyte played significant role in chicken body immune against infection. B lymphocyte derived from fabricated supply that would produce antibody, while T lymphocyte was from thymus and grew into T cell (Davidson, 2008). T

lymphocyte plays an important role in the stimulation of the immune system against certain diaseas and stressor (Hussain *et al*., 2004). Lymphocyte Th (T-helper) would recognize antigen through MHC class II (*major histocompatibility complex)* on the surface of macrofag cell (Gordon, 2003). The physiological function of MHC molecul was the presentation of peptides to T cells, while macrofag was the Antigen Presenting Cell (APC). Interaction between Th and APC would increase due to surface protein on T cell named CD4 (*cluster of differentiate*) on most T helper cell (Veillette and Ratcliffe, 1991).

Saturated fatty acid in virgin coconut oil (VCO) especially palmitate and myristate acid was the phospholipid component of T cell, therefore the decrease of T lymphocyte would activate macrofag as celluler immunity response towards infection with intracelluler patogen (Gordon, 2003). Some research showed that VCO was potential as agent of antivirus and antibacteria (Bergsson *et al*., 1998; Bartolotta *et al*., 2001). Body immune improvement was preventing alternative against AI in broiler chicken because H5N1 virus could easily undergo mutation (Peiris *et al*., 2007) and tended to cause disease in stricted area (Suarez and Cherry, 2000). This characteristic of AI made AI vaccination to chicken was not always perfectly protect chicken from AI (Perkin and Swayne, 2003). This research aimed to investigate whether VCO could increase lymphocyte and CD4 in broiler chickens vaccinated or unvaccinated AI, which therefore potential as immunomodulator.

MATERIALS AND METHODS

Chicken Maintenance and Feed Treatments

Forty broiler DOC were used in the research. The cage used was collective cage for 10 chickens kept until they reached three weeks old, then they were moved to individual cage up to five weeks. The cage was equipped with feed and water containers. Chickens were placed randomly in the cages. The control feed used were manufactured BR1 pellet, while treatments of feed were mixed of control feed and different level of VCO, namely 5, 10 and 15 mL of VCO/kg feed. VCO used was from factory so the quality consistency was guaranteed. Feed and water were given *ad libitum* for four weeks. AI vaccination with sub-type H5N1 was given intra-musculary at 0.5 mL .

Lymphocyte Parameter and CD4

The observed variables were the number of lymphocyte and CD4. Lymphocyte was determined from blood smear preparat. The blood was collected from the wing vena at the end of treatment and placed in 2 mL tube. Blood smear preparat was done firstly by smearing blood on glass object, then fixated with methanol, coloured with may grunwald and giemsa, cleaned with water and left to dry at room temperature. The dried matter was observed using microscope to count the lymphocyte percentage (Bain and Path, 2005). CD4 was determined using flowcytometry. This method was done by examining total blood reaction with antibody monoclonal conjugated fluorochrome that would be bound specifically to the cell surface antigen. The colored sample was then added with FACS ((flowcytometry activated cell sorter) solution to make lysis of erythrocyte in hypotonic condition but safe for leucocyte, then sample was analyzed using flowcytometry. Sample preparation was started by taking sample aseptically from wing vena, then placed in vacuntainer tube with K3EDT anticoagulant. Blood sample was ready to examine, then the specimen was reversed to falcon tube containing 50 µL beads, added with 4 µL CD4 PE anti chicken, mixed homogenously in vortex mixer, then incubated for 15 minutes at 20-25^0 C in dark room. FACS solution was diluted by mixing 50 µL lysis 10 times FACS with 450 µL reagen FACS (1x), mixed homogenously and incubated for 15 minutes at 20 - 25^0 C in dark room. After incubation, it was analyzed using FACS flowcytometer (Alexander, 1998).

Statistical Analysis

This research used factorial design with two factors. Factor one was two levels of vaccine, namely vaccine+, chicken vaccinated with AI, and vaccine–, which was unvaccinated chicken. Factor two was four levels of VCO namely 0, 5, 10, 15 mL VCO/kg feed. Chickens were grouped into eight and treatment was repeated five times during four weeks. At the end of research, data was collected and then analyzed using ANOVA and continued with LSD test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The Amount of Lymphocyte

The research result showed that the amount

of lymphocyte in AI vaccinated chickens was higher than non-vaccine chickens. Accordingly, the addition 10 mL and 15 mL VCO/kg feed to vaccinated chicken showed higher amount than chicken without AI vaccine (Figure 1).

Statistical analysis with factorial design showed significant difference (P<0.05) between the treatments with VCO and without VCO, and lymphocyte increased in 10 mL VCO/kg feed (Table 1). The amount of lymphocyte was significantly affected by the interaction between VCO addition and AI vaccination (P<0.05).

In this research, the addition of 10 mL VCO/kg feed increased the amount of lymphocyte, but that of 15 mL VCO/kg feed showed decrease. This was assumed that VCO increased lymphocyte proliferation through phospholypid formation and stimulation in receptor IL-2. Lymphocyte increase was also likely due to vaccine and VCO because the increase of T lymphocyte stimulated by VCO would therefore increase Th which later led to stimulation of antibody secretion from lymphocyte B cell. The decrease of lymphocyte in 15 mL VCO/kg feed addition was due to the change of lipid structure that would change the membrane fluidity. Consequently, sensitivity of receptor IL-2 decreased and led to the obstruction of lymphocyte proliferation.

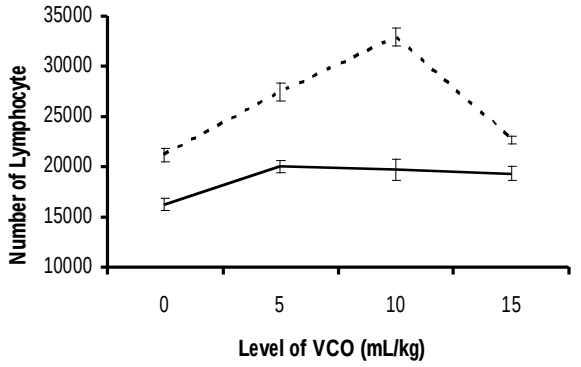

Figure 1. The Amount of Lymphocyte on AI Vaccinated and Unvaccinated Chicken after Feeding VCO as much as 0, 5, 10, 15 mL/kg feed. Error bars represent standard deviation of the mean (— : unvaccinated; ---: vaccinated)

Swayne and Kapczynski (2008) stated that vaccine would stimulate humoral antibody response secreted by B lymphocyte supported this result. The intensity of antibody response varied in every aves. Immune response toward neuraminidase protein could contribute to protection but immunity against virus internal protein was generally not protective. Humoral antibody response from B lymphocyte was the main source of protection because it had several protective effects that could slow down the virus spread. Antibody against AI virus could be stimulated through vaccination (Gioia *et al*., 2008) and AI virus was proven to increase chicken's lymphocyte activation (Holt, 2002).

Virgin coconut oil contained a number of lauric acid which would be turned into monogliceryd of lauric acid or monolaurin (Enig, 1997). Monolaurin was very potential against toxic of glutamic acid (Dave *et al*., 1997). Monoglyceride from caproat acid, caprylat acid, caprat acid, laurat acid, and miristat acid could inactivate virus (Isaacs *et al*., 1995). Monolaurin worked at all viruses and decreased ineffectiveness by breaking the virus envelope. Lipid structure determined the work of anti-ineffective lipid dealt with its structure

Table 1. The Average Number of Lymphocyte on AI Vaccinated and Unvaccinated Chickens after Feeding VCO

Level of VCO (mL/kg)	V-	V+	Total VCO
0	16225	21155	37380[a]
5	19994	27402	47396[c]
10	19670	32855	52525[d]
15	19314	22653	41967[b]
Total Vaccine	75203[a]	104065[b]	

V-: treatment group without AI vaccine, V+: treatment group with AI vaccine. Different superscript on the same row and column indicate significantly difference (P<0.05).

(Hierholzer and Kabara, 2007). Glicerolmonolaurat of low concentration could modulate lymphocyte proliferation which later caused lymphoproliferation and toxin inhibition. Meanwhile, high concentration would delay lymphocyte proliferation and block the proliferative effect of T lymphocyte. The delaying effect was due to general toxication of all process of cell physiology, moreover high concentration could also change lymphocyte sensitivity towards receptor IL-2 which led to delaying effect. Interleukin-2 (IL-2) was cytocine secreted by T lymphocyte and Tc and would stimulate T lymphocyte proliferation (Witcher *et al*., 1996). Fatty acid concentration on feed could also modify response of body immune system through the change of membrane fluidity to induce change of surface protein expression (Pablo and Gienfuegos, 2000).

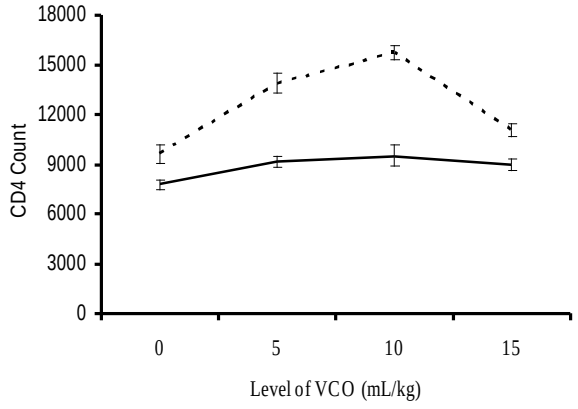

Figure 2. CD4 count on AI Vaccinated and Unvaccinated Chicken after Feeding VCO as much as 0, 5, 10, 15 mL/kg feed. Error bars represent standard deviation of the mean (─ : unvaccinated; ---: vaccinated)

The CD4 Count

The amount of CD4 in this research showed that broiler chicken vaccine with AI had higher CD4 than unvaccinated chicken, accordingly, VCO addition as much as 10 mL/kg feed to chicken vaccinated with AI showed greater amount than that to unvaccinated chicken (Figure 2).

Statistical analysis with factorial design showed significant difference ($P<0.05$) on CD4 count between AI vaccinated and AI unvaccinatedd. In treatment with VCO, there was also significant difference ($P<0.05$) between treatment with VCO and without VCO (Table 2).

CD4 was molecule marking on surface of T helper lymphocyte cell (Th), functioned as co-receptor from T receptor related to peptide antigen presented by MHC molecule (Li *et al*.,1999). In this research, AI vaccinated chickens had more CD4 than unvaccinated ones, accordingly addition of 10 ml VCO/kg feed increased CD4 amount, while 15 ml VCO/kg feed decreased CD4 amount. This could be explained due to relevant relation between CD4 and T lymphocyte as in research by Luo *et al*. (2011) showing that CD4 coded glicoprotein on the surface of Th cell through the interaction with MHC grade II. CD4 activated Th cell that the level of transcription of CD4 directly related to the development of T lymphocyte cell. Witcher *et al*. (1996) found that glicerolmonolaurat given in low concentration could modulate lymphocyte proliferation, while high concentration would block lymphocyte proliferation and block the proliferative effect of T lymphocyte, but not affected B cell. B cell could not produce antibody without Th-CD4,

Table 2. Average of CD4 Count on AI Vaccinated and Unvaccinated Chickens after Feeding VCO

Level of VCO (mL/kg)	V-	V+	Total VCO
0	7746	9609	17355[a]
5	9145	13835	22980[c]
10	9504	15720	25224[d]
15	8948	11005	19953[b]
Total Vaccine	35343[a]	50169[b]	

V-: treatment group without AI vaccine, V+: treatment group with AI vaccine. Different superscript on the same row and column indicate significantly difference ($P<0.05$)

while vaccination could increase the frequency of CD4 (Giogia *et al*., 2008).

CONCLUSION

Based on the research result, it could be concluded that VCO was able to increase the amount of lymphocyte and CD4 on broiler chicken either vaccinated or unvaccinated with AI. Therefore, VCO was potential as immunomodulator.

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