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Virgin Coconut Oil Supplementation Increased the Survival of Avian Influenza Virus (H5N1) Infected Chicken

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ABSTRACT

The purpose of this study was to increase the viability of avian influenza infected chicken through a Virgin Coconut Oil (VCO) administration in feed. A number of live chicken and chicken duration of survival after the challenge test with virus influenza A subtype H5N1 and supporting of immunological data including antibody titers, lymphocyte count, CD4 count and number of CD8 have been analysed for the effect of VCO. This study was conducted in a biosafety level 3 (BSL 3) laboratory using twenty-four broiler chickens that were divided into two groups: vaccinated AI and unvaccinated AI. All groups were fed with the supplementation of VCO (10 mL kg⁻¹ of feed). The chicken without administration of VCO was used as a control. The results showed that supplementation of the VCO could increase chicken viability, antibody titers, lymphocyte count, CD4 count and number of CD8 after challenge test against AI virus.

Key words: Broiler chickens, avian influenza, VCO

INTRODUCTION

Herbal medicine has been known for a long time in Indonesia to prevent the disease in humans and animals. As a consequence, the demand on the use of herbal medicines has increase. Herbal medicine was also received various attention from researcher to study on the herbal medicine to treat a variety of diseases in humans including Virgin Coconut Oil (VCO). VCO has drawn the attention of researcher due to its biological activities, such as anti-inflammatory, analgesic and antipyretic (Intahphuak *et al.*, 2010) and antioxidant (Nevin and Rajamohan, 2006; Marina *et al.*, 2009). The use of VCO has also been well developed to prevent the disease in animal and human due to preventory activities such as hepatoprotective activity (Zakaria *et al.*, 2011), antihypercholesterol and antithrombotic (Nevin and Rajamohan, 2004) and antiviral properties (Van Immerseel *et al.*, 2004).

VCO has been known in Indonesia as a food supplement due to its rich in fatty acid i.e., lauric acid (51.23%), myristic acid (17.13%), palmitic acid (7.30%), caprylic acid (9.18%), capric acid (7.07%), oleic acid (5.42%), stearic acid (2.17%) and caproic acid (0.51%) (Yuniwarti *et al.*, 2012). Saturated fatty acid content in VCO has been reported about 90% while unsaturated fatty acids

content reaches 10% (Yuniwanti *et al.*, 2012). Oleic acid has also been existed in VCO (Yuniwanti *et al.*, 2012). The deficiency in T lymphocytes can be neutralized by the administration of VCO (Enig, 2004). Lymphocytes has been known as an important key to protect the body health against infection. B lymphocytes derived from the bursa fabricius which will make the antibodies are derived from thymic and develop into T lymphocytes. Number of lymphocytes could be directly indicated the degree of endurance. It has been reported that the lower in the number of lymphocytes, the easier for the virus to cause the infection to the body due to the weakening of immune system (Davison, 2008).

Outbreaks of avian influenza caused by the H5N1 subtype, in Indonesia has erupted around early 2004 and spread to almost all provinces, covering 295 districts. Government paid high attention and hold several prevention programs resulting in the remarkable decrease in cases of avian influenza in poultry. The program for prevention is now continuously applied however avian influenza in poultry, particularly in chicken broiler, remained endemic in various regions in Indonesia. The impact of the bird flu outbreak led to a huge decline in the population of chicken and meat products and egg (Nugroho, 2013).

Avian influenza viruses have an ability to perform genetic mutation and reassortment thus allowing the virus to change the antigenic properties, host pathogenicity and specificity resulting in the rapid replication of virus. Antigenic variation on the bird flu virus can be variously found and occurred in two ways, namely shift and drift, therefore the vaccinations has not always able to protect chickens from avian influenza viruses (Peiris *et al.*, 2007). Administration of VCO has been studied to increase thyroid hormone secretion, resulting in the increase in metabolic process and function of the cells. Thus, it finally helps the body to protect a state of pain and accelerates healing (Fife, 2009). Event though VCO has been reported as an agent to increase the immune system but its application remained a question in the benefit of using VCO against avian influenza in chicken. Therefore, this research was aimed to investigate the effect of VCO administration via feed to the viability of chicken after challenge test with AI virus.

MATERIALS AND METHODS

This research was conducted in the Laboratory Biosafety Level 3 (BSL3) Tropical Disease Center, Surabaya, Indonesia, using twenty-four broiler chickens that were divided into two groups, each group consisted of 12 chickens. The first group was treated with AI vaccination while second group was untreated with AI vaccination. Each group has been administered with 10 mL kg⁻¹ VCO of feed. Control with no administration of VCO for each group has been applied (VCO 0). Avian Influenza vaccination was given intramuscularly at pectorales musculus at 5th day old. Feed and drink were provided *ad libitum*. The VCO treatment was started at seventh day old of chickens until the end of the experiment (30 days).

Challenge test of AI has been performed from day fifth until day thirtieth. The virus influenza A virus subtype H5N1 that was isolated from the Tropical Disease Center Surabaya, Indonesia and was given 10⁴EID₅₀ dose of 100 mL by intratracheal using sterilized micropipette. Observation of the onset of clinical symptoms and mortality have been done microscopically every day. Blood samples for determination of the amount of lymphocytes, CD4, CD8 and antibody titers were taken from the vein pectorales of live chicken and three days after the challenge test. Chicken viability has been analysed statistically based on a long life after challenge test. For chicken viability test, the number indicated the duration of chicken that remained alive.

Determination antibody titers: ELISA (Enzyme-linked immunosorbent assay) was used to determine antibody titers against AI. The main principle of the use ELISA technique is the use of an enzyme indicator for immunological reactions. Antigen bound to a polystyrene microtiter plate first. Antiserum containing anti-peptide antibody was added to the wells. The second antibody specific for the first antibody to be detected was labeled and added to the wells. Second antibody in the form of enzymes and the enzyme catalyzes the formation of the substance color. The colors on the substance is then measured and the number of existing antibodies can be calculated (Bion, 2010).

The number of lymphocytes were calculated from blood smears. Chicken blood were collected at the end of treatment was taken from the wing vein and was collected in 2 mL tubes for the manufacture of blood smears. Preparation of blood smears begins with a blood smear on a glass object, then fixed with methanol, stained with Giemsa, washed with water and allowed to dry at room temperature. Observations were made under a microscope and were counted for the percentage of lymphocytes. The number of lymphocytes were then multiplied by the number of leukocytes (Bain and Path, 2005).

Determination of the number of CD4 and CD8 by flow cytometry method. This method begins with a complete blood reaction with fluorochrome conjugated monoclonal antibodies which bind specifically to cell surface antigens. Stained samples were then added to solution containing formaldehyde and diethylene glycol to lyse erythrocytes in a safe condition for leukocytes hypotonic, then the samples were analyzed flow cytometry. Samples of whole blood (50 mL) were taken and put into tube, then were mixed with CD4 or CD8 reagents. The solution was shaken for 15 min and added 450 mL lyse solution and shaken again for several minutes. Finally, the solution was incubated for 15 min and prepared for analysis using flow cytometer (Becton-Dickinson, 2003).

Statistical analysis: The design used in this experiment was a completely randomized factorial design to determine the viability of chickens after challenge test, the effect of VCO on antibody titers, lymphocyte, CD4 and CD8 after challenge test used the t test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Viability of broiler chicken: The duration of chickens that could survive after challenge test with influenza. A virus subtype H5N1 was called as the viability. Scores were made of the numbers one to fourteen days which indicated the duration of observation (Fig. 1). Statistical analysis showed a significant effect of VCO administration to the viability of chicken with AI vaccination (Table 1). The high number in viability could be connected with the positive effect of VCO in suppressing the number of chicken's death. The elevation of immune system of chicken's body after feeding VCO might be a dominant factor in reducing mortality.

Table 1: Viability of chickens post challenged test with influenza A subtype H5N1 after administration of VCO in through feed

Replications	Unvaccinated		Vaccinated	
	VCO 0	VCO 10	VCO 0	VCO 10
1	1	3	3	14
2	1	3	3	14
3	2	3	14	14
4	3	3	14	14
5	3	3	3	14
Sum	10 ^a	15 ^a	37 ^a	70 ^b

VCO 0: Group of chicken without VCO treatment and VCO 10: Group of chicken with the VCO treatment at 10 mL kg⁻¹ of feed

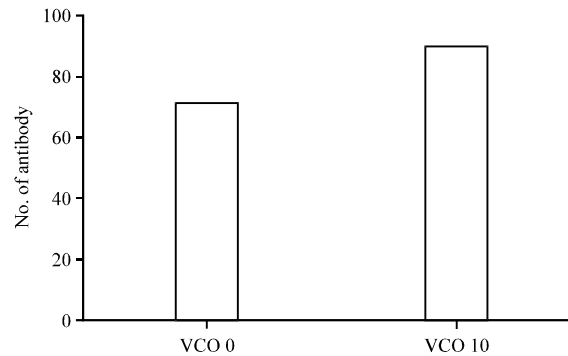


Fig. 1: Number of antibody in AI vaccinated chicken without VCO (VCO 0) and with VCO (VCO 10) administration after challenge test with influenza A virus subtype H5N1

Table 2: Number of lymphocytes, CD4 and CD8 in without VCO (VCO 0) and with VCO (VCO 10) administration after challenge test with influenza A virus subtype H5N1

Parameters	VCO 0	VCO 10
Lymphocyte	13836.7 ^a	17833.0 ^b
CD4	213.7 ^a	422.8 ^b
CD8	169.8 ^a	254.9 ^b

VCO 0: Group of chicken without VCO treatment and VCO 10: Group of chicken with the VCO treatment at 10 mL kg⁻¹ of feed

Antibody against AI virus: The result of antibody's number of vaccinated AI chicken with and without administration of VCO is showed in Fig. 1. The administration of VCO through feed significantly increased the number of antibody in vaccinated AI chicken from 73 to 91. The number of antibody's test has only been applied in the vaccination group since the viability chicken of unvaccinated group was negligible. AI vaccination intended to provide protective immunity against AI virus and it was proved that providing the VCO could increase the number of antibody resulting in the high number of chicken's viability.

It was well studied that the increase in antibody number by the influence of the VCO occurred indirectly through Th-lymphocytes. Saturated fatty acids in VCO, particularly palmitic acid and myristic acid is a phospholipid component of T cells, thus decreasing the number of T lymphocytes can be corrected by administration of palmitic acid and myristic acid (Enig, 2004). The increase in T lymphocytes stimulating production of antibodies from B lymphocytes (Gioia *et al.*, 2008).

The number of Lymphocytes, CD4 and CD8: The results of lymphocytes number, CD4 and CD8 in vaccinated chicken is showed in Table 2. The test has been applied in the vaccinated group of chicken since the unvaccinated group showing the negligible number in viability. As can be seen of Table 2, the number of lymphocytes in vaccinated AI chicken with VCO administration were higher than those of without VCO administration. The results indicates that the VCO increased the number of lymphocytes. Lymphocytes plays an important role in the body's immune system to fight various infections in chicken (Davison, 2008). T lymphocytes stimulates the immune system in fighting various diseases and stressors (Hussain *et al.*, 2004).

Statistical analysis by t-test showed that administration of VCO significantly increased the CD4 and CD8 (Table 2). Avian Influenza vaccine could increase the frequency of CD4-Th cell reactivity

thereby increasing the neutralization of the influenza A virus subtype H5N1. Challenge test with the H5N1 virus showed that the CD4-Th cell has a more response specific to NA peptide compared to HA peptide. Th-CD4 cells also stimulated the secretion of antibodies from B lymphocytes, so the presence of CD4 cells is indispensable for specific influenza antibody secretion (Gioia *et al.*, 2008). Based on these results, it appears that the VCO was able to increase the survival of chicken through the increase in the number of antibody, lymphocytes, CD4 and CD8. Vaccination AI would produce protective antibodies but without feeding VCO, the antibody was limited in number due to the less number of Th cells-CD4 which was known as stimulator of antibody secretion. As previously mentioned, the administration of VCO increased the number of lymphocytes. It was likely due to the elevation of palmitic acid and myristic acid intake after VCO administration. Furthermore, VCO could also provide greater energy to the lymphocyte synthesis purposes.

Lymphocytes Tc-CD8 of chickens vaccinated AI were increased along with the VCO's administration after challenge test with influenza A virus subtype H5N1 (Table 2). CD8 is a co-receptor of T lymphocytes and very helpful to improve the immune system in poultry (Li *et al.*, 1999). It has been documented that the role of CD8 in the clearance of influenza virus is through the production of antiviral cytokines and lyse target cells in infected epithelial cells. Lysis of infected epithelial cells is mediated by exocytosis granules containing perforin and granzymes. The release of perforin and granzymes of Tc-lymphocytes strictly regulated and occurred shortly after the contact between Tc-lymphocytes with infected target cells (Thomas *et al.*, 2006; O'Neill *et al.*, 2000; Jameson *et al.*, 1999). Number of CD8 together with CD4 is increased along with the increase in antibodies (Riberdy *et al.*, 1999). This research was inline with this statements, since the administration of VCO increased the number of CD4 and CD8 in vaccinated AI chicken resulting in the a much greater number in the viability.

Several studies indicate that to prevent an outbreak of bird flu in Indonesia, improved management of avian influenza through enhanced biosecurity, vaccination, depopulation, control traffic of poultry, surveillance and tracking, increased public awareness, monitoring and evaluation is required (MoA., 2014). Since Indonesia produce huge number of coconut, the VCO administration is suggested along with the requirement of the strategy to suppress the cases of Avian Influenza.

CONCLUSION

Administration of VCO through the feed increased viability, the number of antibodies, lymphocytes, CD4 and CD8 in chickens with AI vaccination after challenge test with Avian influenza A virus subtype H5N1.

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