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# TABLE OF CONTENTS

Table of Content	v
Preface	ix
Advisory Board	x
Organizing Committee	xi

## CONTRIBUTED PAPERS 1 : BIOTECHNOLOGY FOR SUSTAINABLE DEVELOPMENT AND HUMAN WELFARE

Variation on The Growth Performance of Tiger Shrimp ( <i>Penaeus monodon</i> ) Cultured on Monoculture and Polyculture System of Silvofishery Pond Rini Budihastuti	1
The Proximate Analysis of Berenuk Leaves ( <i>Crescentia cujete</i> ) Potential as Good Nutrition and Medicine for Humans and Animals Umarudin, Munifatul Izzati and Endah Dwi Hastuti	6
Monocyte Proliferation Activity of Broiler Chickens After Vaccinated Avian Influenza and Feeding Virgin Coconut Oil Enny Yusuf Wachidah Yuniwanti	11
Potential Of Endospore-Forming Rhizobacteria From Rice Plant As Biocontrol Of Phytopathogen <i>Xanthomonas oryzae</i> Maerani Sumarno, Anto Budiharjo and Sri Pujiyanto	15
Assessment of Water Quality Using Macrobenthos as Bioindicator and Its Application on Abundance-Biomass Comparison (ABC) Curves Suci Wulan Pawhestri, Jafron.W.Hidayat and Sapto P. Putro	24
Development of Japanese Quail ( <i>Coturnix coturnix japonica</i> ) Embryo Tyas Rini Saraswati and Silvana Tana	29
Influences of Dietary Supplementation of Brown Algae ( <i>Sargassum cristaefolium</i> ) Extract on Growth Performance and Feed Utilisation of Juvenile White Shrimp ( <i>Litopenaeus vannamei</i> ) Agung Sudaryono, A. Harjuno C. Haditomo and Alim Isnansetyo	34
Affect of Plasma Corona Irradiation at Atmospheric Conditions on The Growth of Seaweed <i>Gracilaria verrucosa</i> (Hudson) Papenfuss Filemon Jalu N Putra, Riche Hariyati, Zaenul Muhlisin, Agung Sudaryono and Sapto Purnomo Putro	40

# Monocyte Proliferation Activity of Broiler Chickens After Vaccinated Avian Influenza and Feeding Virgin Coconut Oil

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## ABSTRACT

This research aimed at feed modulation to increase body immune system of broiler chicken as alternative to preventing avian influenza (AI) disease in broiler chicken by AI vaccination and feeding virgin coconut oil. Monocyte was the first cell that enter peripheral blood would directly react to phagocyte antigen during 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 IL-2, furthermore IL-2 would stimulated proliferation of monocyte so that increasing body immune system of broiler chicken. 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 monocyte in chickens given 10 mL per kg feed and vaccinated with AI was higher than that in chickens given VCO without AI vaccine.

**Keywords:** Avian influenza, broiler chicken, monocyte , VCO

## INTRODUCTION

The health of chicken becomes important for humans who consume meat chicken, as a healthy food if free from infection so that safe for consumption, because it does not contain the disease and cause illness. During this time, vaccination AI becomes an important tool in the control of highly pathogenic avian influenza in poultry, vaccination may increase resistance to infection, prevent disease and death, and to reduce transmission to other birds [1]. Avian influenza (AI) is still an endemic in Indonesia. Highly mutative characteristic of AI causes AI vaccination is not always successful to prevent chicken mortality, therefore feed modulation alternatives are sought to raise body weight and body immune as well. Modulation of feed to increase immune system of broiler chickens is expected to reduce the death rate from the disease of broiler chickens [2], one way is to add the feed modulation vco on feed.

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 caprylic 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 [3]. Saturated fat in VCO especially palmitic and myristic acids are phospholipids component of T cell [4], so that giving VCO especially 10 mL/kg of feed, able to increase the number of lymphocyte [5], thus might also be able to increase monocyte replication so that the number of monocytes increased.

Monocytes are mononuclear phagocytic cells found in circulation. During hematopoiesis, progenitor cells differentiate into premonosit that leave the bone marrow into the circulation and subsequently differentiate into mature monocytes. Monocytes are widely distributed in lymphoid organs and migrate to various tissues to differentiate as macrophages. Monocytes also act as antigen presenting cells (APC) which recognize and attack the microbes in response to infection [6]. This study aims to find ways to increase endurance of chicken by providing VCO and AI vaccination resulting in increased proliferative activity of monocyte phagocytic cells which are infectious agents

## MATERIAL AND METHOD

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 monocyte. The amount of monocyte was determined from blood smear. Chicken's blood was collected at the end of the treatment, taken from the wing vein and put in 2 ml 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 monocyte obtained [7]. Furthermore percentage of monocyte multiplied by the number of leukocytes to obtain actual number of monocyte.

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 [8].

## RESULTS AND DISCUSSION

The amount of monocyte in AI-vaccinated broilers was higher than the unvaccinated ones, accordingly, VCO intake as much as 5mL/kg feed and 10mL/kg feed in AI-vaccinated chickens showed higher amount than the AI-unvaccinated chickens (Figure 1)

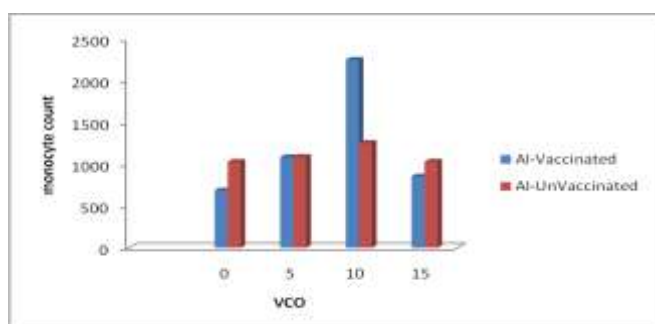


Figure 1. The Amount of Monocyte on AI Vaccinated and Unvaccinated Chicken after Feeding VCO.

Statistical analysis with factorial design showed significant difference ( $P < 0.05$ ) between the treatments with VCO and without VCO, and monocyte increased in 10 mL VCO/kg feed (Table 1). Result on VCO giving and AI vaccine towards broilers' monocyte did not show any difference among the group of AI-vaccinated and AI-unvaccinated, with or without VCO, although there was no significant, monocyte value tended to be increased in broiler chicken by vaccinated AI. In this research AI vaccine could increase the number of monocyte since vaccine using inactive AI virus could activate the antigen [9] and therefore would stimulate the innate immune response through the expression of various receptors involved in pathogenesis [10]

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

VCO	AI-vaccinated	AI-unvaccinated	Total VCO
0	687	1031	1718 <sup>a</sup>
5	1086	1090	2176 <sup>b</sup>
10	2252	1257	3509 <sup>c</sup>
15	856	1033	1889 <sup>a</sup>
Total vaccine	4881	4411	

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 ( $P < 0.05$ )

Virgin coconut oil is medium chain fatty acid. Fatty acids have been found to modulate phagocytosis, reactive oxygen species production, cytokine production and leukocyte migration, also interfering with antigen presentation by macrophages. Several groups have investigated the changes induced by fatty acids in cell membrane functioning, transduction pathways, protein acylation, and calcium release in leukocytes.. The study of cell metabolic regulation is therefore a fundamental approach in immunology and in the study of inflammation. Fatty acids are associated with a series of metabolic pathways, being synthesized from amino acids and glucose, esterified to glycerol to form phospholipids and triacylglycerol or broken down to acetyl CoA or CO<sub>2</sub>, generating energy. Probably because of this key position in cell metabolic pathways, fatty acids can regulate their own synthesis and breakdown, as well as interfere with neighboring metabolic routes. As mentioned before, lipids are important membrane components, sources of energy and of cell mediators, being essential for cell survival and function. There are many feedback loops that guarantee controlled fatty acid metabolism [11]. Increasing the number of monocytes could be due to the presence of multiple responses from AI vaccination and administration of VCO in the feed. AI vaccination and VCO can increase the number of Th-Lymphocyte [5] which in turn stimulates B-lymphocyte resulting in increased activity of macrophage phagocytosis [12]. B lymphocytes can not produce antibodies without the help of Th, while vaccination may increase the frequency of Th [13] activity of macrophage phagocytosis is supported by a number of monocytes in the circulation [14] so that the increase in phagocytosis macrophage activity can stimulate the replication of monocyte.

The increase in Th-lymphocyte caused by saturated fatty acids in virgin coconut oil (VCO) especially palmitate and myristate acid was the phospholipid component of the T cell. Subsequent studies showed that administration of VCO in chicken feed at 10 mL per kg of feed is able to increase the production of interleukin-2 (IL-2) [15]. T lymphocyte mainly Th lymphocyte to produce IL-2, it functions as an autocrine. The constitutive expression of IL-2 receptors on regulatory T cells is consistent with their requirement for IL-2 in order to survive. Interleukin-2 also promotes survival of cells by inducing the anti apoptotic protein, IL-2 increases production of other effector cytokines [16]. Increased production of IL-2 further stimulates lymphocytic proliferation [17]. Glyceromonolaurat from lauric acid in VCO can be converted into monolauric acid or glyceromonolauric to increase lymphocyte sensitivity towards IL-2 receptor so that resulting in lymphoproliferation [18]. The biological activity of IL-2 is done by binding to membrane receptors are only expressed on the active cell while the cell inactive is not found IL-2 receptor. Some other cells such as monocytes also have receptors for IL-2 [19], thus allegedly IL-2 also stimulates the proliferation of monocytes.

Recently, demonstrated that nutritional fatty acids can activate the Toll-like receptor-4 (TLR-4) signalling in monocytes. Fatty acid-induced induction of Toll-like receptor-4/nuclear factor- $\kappa$ B pathway in adipocytes links nutritional signalling with innate immunity [20], so that the fatty acids in VCO can stimulate the proliferation of monocytes through activation of TLR-4. Summary of this study, that can increase the monocytes replication by feeding vco and AI vaccination in several ways, the first of fatty acids in VCO able to activate TLR-4 which in turn can stimulate the proliferation activity of monocyte. Another way through stimulation of vaccines and VCO, which will increase the Th lymphocytes and IL-2 thus increasing the proliferation of monocytes.

## **CONCLUSION**

Based on the research result, it could be concluded that VCO was able to increase the amount of monocyte on broiler chicken either vaccinated or unvaccinated with AI. Therefore, VCO was potential to increase body immune system.

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