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The Effect of Spirulina as Feed Additive to Myocardial Necrosis and Leukocyte of Chicken with Avian Influenza (H5N1) Virus Infection

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Abstract

The aim of this research was to examine the effect of *Spirulina sp.* as feed additive to myocardial necrosis and leukocytes which were infected by Avian Influenza H5N1 virus. This research comprised three level treatment of Spirulina 0%, 10%, 20% of the fresh water algae as a liquid supplement, each of which consisted of seven replicates given to 7 day to 32 day old broiler chicken. Artificial infection of Avian Influenza virus H5N1 by entering the respiratory tract (nose drops) using a dose of 0.1 ml inoculum. Blood samples were collected from brachialis vein 0.5–1 ml to calculate leukocyte cell. Heart tissue of chicken were taken to histopathologic and immunohistochemistry examination. The results showed that there was no significant difference ($p>0.05$) in myocardial necrosis and significant difference ($p<0.05$) in leukocyte in the treatment of *Spirulina sp.* The result indicates that *Spirulina sp.*can be used as feed additive to increase immunity in broiler chicken.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: feed additive; *Spirulina sp;* Avian Influenza H5N1 virus; chicken; myocardial necrosis; leukocyte

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Nomenclature

EID	embryo infectious dose
EDTA	ethylenediaminetetraacetic acid
HPAI	high pathogenecity avian influenza
TL1A	TNF-*like ligand* 1A

Introduction

The primary focus of determining nutrient requirements in poultry production is to improve performance characteristics such as growth and yield. However, nutritional factors are important in improving disease resistance and health. *Spirulina platensis* is a well-known commercial cyanobacterium used as dietary and feed supplementation. It has high contents of proteins, other substances such as polyunsaturated fatty acids (PUFA), vitamins, phycocyanin, β-carotene and chlorophyll pigments that have been used as food and drink, cosmetic and pharmaceutical colorants [1, 2, 3], and minerals. It has been shown to activate the mononuclear phagocytic system of chickens. The mechanism for macrophage function modulation in response to most of these nutrients is not well defined. However, it is clear that dietary components can have a significant influence on the ability of the host to mount an effective immune response[4].

In microalgae, the micronutrient or trace element such as Cu, Mn, Zn, Co, is important in enzymatic reactions and the biosynthesis of many compounds[5]. It is also incorporated into the cells in a range of 0.001 to 0.25 µg mg^{-1} dry weight[6]. In bacteria B, it is an essential part of signal molecules required for quorum sensing[7,8].

The produced biomass of *Spirulina* sp can be used as animal feed, energy production, fertilizers or to produce fine chemistry products such as pigments, polysaccharides, carotenes, sterols, vitamins, polly-unsaturated fatty acids and lipids[9,10]. It is thus necessary to examine the use of different levels of *Spirulina* sp on chicken which Avian Influenza H5N1 virus infected to necrosis myocard and leukocyte.

2. Methods

Twenty one broiler chickens were divided into three treatments of *Spirulina* sp, i.e: P_0:0% *Spirulina* sp, P_1:10% *Spirulina* sp, P_2:20% *Spirulina* sp of the fresh water algae, each of which consisted of seven replicates, in drinking water. The treatment of Spirulina was given to broiler from the age of 7 days old to 32 days old. Artificial infection of Avian Influenza virus H5N1 (A/Ck/Indonesia/BL/03 from isolate collection of Adi Prijo Rahardjo M.Si., DVM) was given to 26 day old chicken by enfecting the respiratory tract (nose drops) using a dose of 0.1 inoculum containing 10^7 EID50. Inoculated chicken were observed for 6 days after inoculation, during which the clinical signs were recorded. Blood samples were collected from brachialis vein for as much as 0.5 – 1 ml in tube containing EDTA (1 mg/tube) to calculate leukocyte cell. Dead chickens in this research were necropsied for the determination and heart tissues of chicken were collected for histopathologic and immunohistochemistry examination. Heart tissues were fixed by submersion in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections were made at 7 µm and were stained with hematoxylin and eosin (HE) and a duplicate 7 µm section was conducted immunohistochemically with primer antibody (anti H5N1).

3. Results and discussion

Based on the results of the variance analysis, the addition of *Spirulina* sp showed a significant difference ($p<0.05$) in the total number of leukocytes between treatments. The Duncan's multiple range test showed that treatment which produced the highest amount of leukocyte was obtained at P_2 treatment (*Spirulina* sp 20%), which was not different from the treatment P_1 (*Spirulina* sp 10%). The treatment which produced the lowest number of leukocytes was obtained at the P_0 treatment (*Spirulina* sp 0%), which was not different from the P_1 treatment (*Spirulina* sp 10%) (Table 1 and Fig 1).

Table 1. Average and standart deviation of leukocyte (10^3/mm^3)

Treatment	Average and standart deviation
Spirulina sp 0% (P_0)	20.51 ^{a} ± 1.73
Spirulina sp 10% (P_1)	21.75 ^{ab} ± 0.85
Spirulina sp 20% (P_2)	22.50 ^{b} ± 1.29

Note: numbers with ^{a,b} are significantly different between treatments ($p<0.05$).

Spirulina platensis containing calcium-Spirulan, an intracellular polysaccharide, inhibits the replication of several viruses *in vitro* by inhibiting the penetration of the virus into the different host cells used[11,12], activates the production of nitric oxide, and immunostimulates the production of cytokines in macrophages[13]. On the other hand, inhibition of leukocyte migration seems to be related to the anti-inflammatory activity of the polysaccharides[14]. As leukocyte movement to the site of injury contributes to additional cytokine release and production of nitric oxide, therapeutics has to be effective against this over-inflammation.

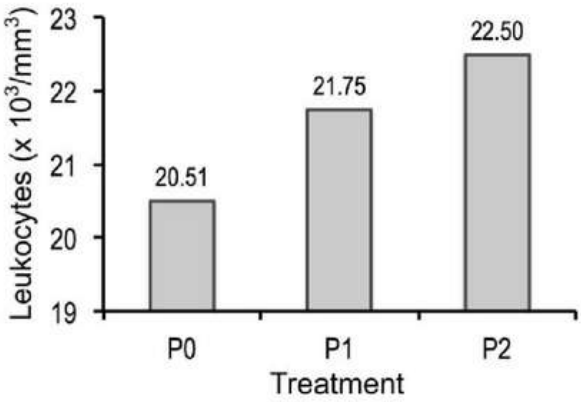

Fig. 1. Broiler leukocyte with *Spirulina* sp treatment. See main text for further information.

The normal range leukocyte of cock is 19.8 x 10^3/mm^3. In this research, the treatments of *Spirulina* sp 10% (P_1) and 20% (P_2) could increase leukocyte compared to control treatment without *Spirulina* sp (P_0). The result of this research showed that *Spirulina* sp could increase leukocyte associated with an immunological function. Lymphocytes are involved in a variety of immunological functions, such as immunoglobulin production and modulation of immune defence[15]. The remaining 20% of the leukocytes represents a combination of eosinophils, which play a role in the inflammation process. Low lymphocyte counts (which the authors of these studies attribute to elevated glucocorticoids) reliably predict the risk of mortality associated with a range of ailments, such as coronary artery disease, heart failure or myocardial infarction. High H : L ratios have been associated in birds with susceptibility to infection, slow growth rates[16].

Based on the results of the variance analysis on myocardial necrosis, the addition of *Spirulina* sp did not show significant difference ($p>0.05$) among the treatments of P_0, P_1 and P_2 (Table 2).

Table 2. Average and standard deviation of myocardial necrosis

Treatment	Average of myocard necrosis (%)
Spirulina sp 0% (P_0)	65.35^{a} ± 3.55
Spirulina sp 10% (P_1)	67.73^{a} ± 3.48
Spirulina sp 20% (P_2)	68.22^{a} ± 4.40

Note: numbers with ^{a,b} are significantly different between treatments ($p<0.05$).

In the examination of cardiac muscle histophatologic, many lesions were found. Myocardium underwent multifocal necrosis characterized by the number of empty space within the remains of myosit cells. Indeed, it is one thing that often occurs in the cardiac by High Pathogenecity Avian influenza (HPAI) H5N1 infection[17,18,19]. The necrosis of cardiac cells considerably affect the ability of the heart to pump blood throughout the body. Cardiac failure will reduce blood pressure so that in the level of the arterioles, blood which carries oxygen and nutrients for the tissues of the body organs would be reduced. The cell will be without oxygen and nutrients will not be able to produce the ATP for the organs. Many essential organs will undergo malfunctions. The blood supply to the heart through the coronaria artery will decline and this cause necrosis in the cardiac muscle and causes cardiac failure[20]. The brain, which is the main organ to coordinate all the organs of the body, would lost a lot of its cells because ischemia that will eventually worsen cardiac failure under the coordination.

Myocardial necrosis of broiler found in all levels of *Spirulina* sp treatment does not show significant difference. In treatment of 20 % *Spirulina* sp, necrosis does not lead to mortality. It might be caused by the fact that the rates of TNF-*like ligand* 1A (TL1A) in chicken on 20 % *Spirulina* sp treatment were lower than those in the other traetments. As a result, the hypovolemic shock was not as severe as that in the other treatment which experienced heavier cytokine storm. Mortality found in treatments of 0% *Spirulina* sp (P_0) and 10 % *Spirulina* sp (P_1) can be due to impaired blood supply, because shock and a disorder in myocardial system can reduce blood pressure throughout the body. The blood supply can decline or even stop and would eventually destruct the other essential organs. Anoxia experienced by the brain will cause mortality in a short time. In this research, anoxia can be observed on wattle and comb of chicken that experienced cyanosis. If it is related to mortality level, a dose of *Spirulina* sp 20% showed 0% mortality (Fig. 2) so that the dose can decrease mortality caused by Avian Influenza H5N1 virus.

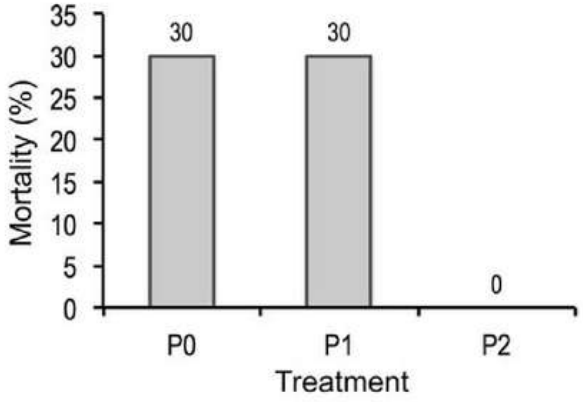

Fig. 2. Chicken mortality by *Spirulina* sp treatment. See main text for further information.

4. Conclusion

The results showed that there was no effect in myocardial necrosis but leukocytes could be increased with the treatment of *Spirulina sp*. This result indicates that *Spirulina sp.*could be used as feed additive to increase immunity in broiler chicken.

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