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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.

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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 pathogenicity 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 response⁴.

In microalgae, the micronutrient or trace element such as Cu, Mn, Zn, Co, is important in enzymatic reactions and the biosynthesis of many compounds⁵. It is also incorporated into the cells in a range of 0.001 to 0.25 $\mu\text{g mg}^{-1}$ dry weight⁶. 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, poly-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: P₀:0% *Spirulina* sp, P₁:10% *Spirulina* sp, P₂:20% *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 entering the respiratory tract (nose drops) using a dose of 0.1 inoculum containing 10⁷ 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 P₂ treatment (*Spirulina* sp 20%), which was not different from the treatment P₁ (*Spirulina* sp 10%). The treatment which produced the lowest number of leukocytes was obtained at the P₀ treatment (*Spirulina* sp 0%), which was not different from the P₁ treatment (*Spirulina* sp 10%) (Table 1 and Fig 1).

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