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The Effect Of Turmeric As Immunostimulator Against Avian Influenza (AI)Vaccine

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***Abstract*—This study was conducted to know the effect of turmeric as immunomodulator in layer chicken against Avian influenza vaccine. This research used DOC that were given turmeric ethanol extract 50% concentration at day 8 until day 21, and Avian influenza vaccine was given at day 15. The bloods sample were collected at day 7, 14, and 21 at brachial vein. It was an experimental research and data were analyzed using t test paired sample. The result of total heterophils before AI vaccine was given showed an increased significant effect ($p<0.05$) (12.6563 ± 4.60) %, and after vaccine was (19.0000 ± 9.22) %. The results of total basophils before AI was given showed there was no significant effect ($p>0.05$) (2.9688 ± 1.96) %, and after vaccined was (3.2813 ± 1.76) %. It can be concluded that turmeric has an immunomodulatory effect.**

Keywords—curcumin, Avian influenza, heterophil, basophil, immunostimulator

I. INTRODUCTION

The Avian Influenza H5N1 virus has spread widely in more than 60 countries. To control the disease, poultry vaccination has been implemented in some countries where the H5N1 virus is already enzootic in poultry and wild birds [1]. Although vaccination programs have been performed, failure factors may occur, either because of internal factors and also because of external factors. Therefore, the utilization of herbal plants that have immunostimulatory effects is expected to provide synergistic effects with the vaccination program.

Turmeric (*Curcuma longa* Linn.) is a plant in which the main active ingredient is curcumin. Several studies have shown that curcmin has an immunostimulatory effect;this can be proved by Umar et al.'s [2] study which gave curcumin in turkey feed. All groups were challenged with H9N2 Avian Influenza virus, and the treatment group showed mild clinical symptoms, the lowest viral shedding and higher titer than the control group. Research conducted by Hartati et al. [3] showed that giving curcumin in layer chicken feed stimulates lymphocyte activity. In the histopathological picture of the bursa fabricius, there was an increased number of lymphocytes in the lymphoid follicles and the thymus were also seen in widening of the cortex compared to the medulla. Curcumin has been shown to increase adaptive immunity, either humoral or cellular immunity. Therefore, research will conduct observations of how effective curcumin is in increasing innate immunity, in this case in the number of heterophil and basophil cells.

II. EASE OF USE

A. To know the effect of turmeric on heterophil and basophil count in layer chicken.
B. To know the effect of turmeric as an immunostimulator against Avian Influenza vaccine in layer chicken.

III. METHODS

Curcumin extract is made by maceration method. Selected turmeric, which is an old orange color, is then washed, sliced, dried and ground until it becomes powder. The powder is mixed with 96% ethanol, 1: 3 ratio in the container, then shaken every two hours for 48 hours. The filtered shake results are then placed in a nitrogenous waterbath until a viscous extract is obtained. 32 DOC CP 909 is divided into two groups of treatment, P0 (control) and P1, group given curcumin extract concentration 50% with dose 0.1 ml, once daily orally. After adaptation for seven days, at 8 days treatment was administered. The Avian Influenza (AI) vaccine was administered after the chicken reached two weeks of age with

a dose of 0.2ml per cutaneous. Blood sampling through the brachial vein was done three times when the chicken is aged 7 days (before curcumin extract administration), 14 days (before vaccination) and at age 21 days (after vaccination). Blood samples were then used to prepare blood smear preparations by dropping blood into a glass object, then using another glass object as a thruster with an angle of 30-40°, resulting in a 3-4cm long smear on the glass object. Blood was dried, then fixed with 96% methanol for three minutes, then immersed in 5% Giemsa dye for 20-30 minutes, then removed and doused with running water; after drying, the number of heterophil and basophil cells were counted under a microscope with magnification 1000x.

IV. RESULTS

Neutrophil is one of the leucocyte types in mammals and called heterophil in avian. The data of this study were analyzed using t-test. Results in Table I show that the increasing number of heterophil was significant ($p<0.05$) between treatment group (9.0000 ± 2.54)% and control (16.0625 ± 3.77)% and so was basophil; its number was also increased (2.8125±1.80)% compared with control (2.0000±1.13)%, although the increase was not significant ($p>0.05$).

TABLE I. HETEROPHIL AND BASOPHIL CELL COUNT BEFORE AND AFTER CURCUMIN EXTRACT ADMINISTRATION

Treatment	X± S.D	
	Heterophil	***Basophil***
Before	9.0000 ± 2.54%	2.0000 ± 1.13%
After	16.0625 ± 3.77%	2.8125 ± 1.80%

TABLE II. HETEROPHIL AND BASOPHIL CELL COUNT BEFORE AND AFTER AVIAN INFLUENZA VACCINE ADMINISTRATION

Treatment	X±S.D	
	Heterophil	***Basophil***
Before	12.6563 ± 4.60%	2.9688 ± 1.96%
After	19.0000 ± 9,22%	3.2813 ± 1.74%

This is similar to that of Kumari et al.'s [4] study in broiler chickens given by curcumin in their feed and which showed an increased number of heterophil; this finding is in line with Essam et al.'s [6] study which showed that curcumin in mice not only increases the number of neutrophils, but also increases the number of eosinophils and lymphocytes. This is in contrast to Napirah et al.'s (2011) findings whose research on quail given turmeric starch feeds resulted in a decrease in the percentage of neutrophils at age 35 and 42 days compared with 28 days, whereas the percentage of lymphocytes increased and the percentage of monocytes increased, but not in excessive amount. In mice given oral curcumin for 45 days, it showed a decrease in the number of neutrophils, a significant increase in the number of lymphocytes and increase in the number of monocytes, but not in excessive amounts [7]. Effects of curcumin on heterophil and basophil counts in chicken before and after vaccination in Table II showed that heterophil increased in number from (12.6563 ± 4.60)% before vaccination to (19.0000 ± 9.22)% after vaccination, and showed significant differences ($p <0.05$), whereas, in basophil counts, there was no significant difference ($p> 0.05$) between the result before vaccination (2.9688 ± 1.96)% and after vaccination (3.2813 ± 1.74)%. Previous research has shown that treatment with curcumin before and after virus entry will cause damage to the viral infectivity, so the possible curcumin mechanism of action is through the binding of viral particles to the sialic acid receptors on the cell surface. To ensure this, Hemagglutination Inhibition assays were performed to ensure that curcumin was able to inhibit hemagglutination by the Avian Influenza virus. The results obtained show that curcumin prevents the PR8 virus linkage to red blood cells of the chicken, which is indicated by a point-resembling appearance in non-hemagglutinated cells. Interestingly, curcumin also prevents the association of other subtype Avian Influenza viruses (H6N1 strains) against red blood cells [8]. Netrophil or heterophil play an important role in the antiviral immune response, and high amounts of neutrophil accumulate early in the infection because of their role as the ultimate cellular defense in innate immune responses. In addition to neutrophils, the presence of other immune cells, such as mast cells and basophils, in terms of their interactions with influenza viruses is not as greatly explored [8].

Conclusion

The study concluded that turmeric as a feeding supplement to layer chicken has increased the number of heterophil and basophil as well as having immunostimulatory effect against Avian Influenza vaccine.

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