

# Evaluation of Immunomodulatory Activity of the Herbs Formula Viranur, Turmeric (*Curcuma heyneana* Val.) and Phyllanthus (*Phyllanthus niruri* L.) in Layer Chicken Vaccinated with Avian Influenza

Sri Hartati<sup>1</sup>, Tri Untari<sup>2</sup>, Ida Fitriyani<sup>3</sup> and Bambang Sutrisno<sup>4</sup>

1. Department of Veterinary Clinical Science, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia

2. Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia

3. Department of Pharmacology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia

4. Department of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia

**Abstract:** The aim of this research was to know the effect of herbal as immunomodulator on chicken layer vaccinated with avian influenza. A total of 60 chickens were allotted into three treatment groups: control group (KA), group KB and group KC, with 20 chicken each group. All the chickens were vaccinated with Newcastle disease (ND) in the age of one week, and a week later they were vaccinated with avian influenza (AI). The chickens in group KB were drunken with herbal solution containing of 5 g turmeric (*Curcuma heyneana* Val.) and 25 g phyllanthus (*Phyllanthus niruri* L.) and group KC were drunken with herbal solution containing of 36 g the herbs formula Viranur and 25 g phyllanthus (*Phyllanthus niruri* L.), respectively for four weeks. Thirty days after AI vaccinated, all of chicken were weighed and necropsied. Samples from bursa of Fabricius, thymus and spleen were taken for weighing and histopathological examination. The weight indexes of bursa of Fabricius, thymus and spleen were not significantly different between control group and treatment group in the considered statistically significance ( $P > 0.05$ ), but the treatment groups (KB and KC) had higher weight index. The histopathologically changes of spleen in both control group and treatment groups were not different, although in the group KC, in bursa of Fabricius, there was lymphocyte increase in its lymphoid follicles; and in the group KB and KC, the thymus were more widening in the cortex than medulla. The conclusion of this study showed that the herbs can stimulate lymphocyte activity.

**Key words:** Herbal, immunomodulator, histopathologic, bursa of Fabricius, thymus, spleen, layer chicken, AI.

## 1. Introduction

The herbs formula Viranur remedies are made from a mixture of turmeric (*Curcuma heyneana*), cubeb (*Piper cubeba*) and fennel (*Foeniculi fructus*), and they are widely used to treat patients with dengue fever, but its efficacy as immunomodulator against avian influenza (AI) is not yet known. One of the active substance in turmeric rhizome is curcumin [1].

Curcumin has activity preventive against the virus, such as vesicular stomatitis virus (VSV), HSV 1 and 2, parainfluenza-3, reovirus-1, feline corona virus, feline herpesvirus and other viruses [2]. Based on the research results of Ade et al. [1], the methanol extract of turmeric rhizomes has inhibitory against Newcastle disease (ND) virus with  $IC_{50}$  value of 7.295  $\mu$ g/mL. Previous researchers have made use of garlic [3] and virgin coconut oil [4] as immunomodulator.

AI or better known to the public with avian influenza (bird flu) is a viral disease that can infect humans and poultry, caused by the infection of

---

**Corresponding author:** Bambang Sutrisno, Ph.D., research field: pathological study of lymphoid organ caused by the infection and mycotoxin.

Orthomyxovirus included in the family Orthomyxoviridae and genus influenza type A. This family is grouped into three types: A, B and C, based on antigenic nucleoprotein (NP) and matrix (M). Type A is classified into several subtypes based on the surface antigens, i.e. 16 subtypes of HA (1-16) and nine subtypes of NA (1-9) [5, 6]. Type A of influenza is not only responsible for disease problem in birds, humans and pigs, but also the aquatic mammals, namely seals and whales [7]. Avian influenza virus subtype H5N1 has also been reported to infect dogs and cats [8]. Type B and C infect humans, but it showed mild in clinical sign [9]. The government has issued a policy for AI prevention, including vaccination, biosecurity, stamping out and limited traffic control of livestock, especially poultry. The policy was not yet successfully, because the thousands of ducks were still death in East Java. The high rate of infection provides the opportunity of mutation, so as to cause the antigenicity of the virus changes. Changes in antigenicity of the virus will affect the immune response. Vaccines not homologous to the infected virus can lead to failure in the field of vaccination, so that vaccination can not be relied on for AI control. The immune response in chickens is not only dependent on the humoral system, but the cellular immune system also plays a role. It encourages researchers to make a breakthrough to resolve the problem, namely the use of medicinal plants which are expected to trigger a cellular and humoral immune responses. The herbals formula Viranur raw material mixture of turmeric, cubeb, fennel and phyllanthus much as immunomodulators has not been reported. Thus, this study aimed to obtain herbal medicine that can boost the immune response against AI.

## 2. Materials and Methods

Sixty chickens were randomly divided into three treatment groups: control group (KA), group KB and group KC, with 20 chickens each, respectively. After adaptation for a week, then all the chickens were

vaccinated against ND and AI a week later. One week post-vaccination, all chickens in the group of KB were orally given with herbal medicine: turmeric (*Curcuma heyneana*) + phyllanthus (*Phyllanthus niruri*) in the dose of turmeric 5 g and phyllanthus 25 g mixed with water 200 mL, and in the group of KC in the dose of herbals formula Viranur 36 g and phyllanthus 25 g mixed with water 200 mL. Thirty days post-vaccination of AI, all of chicken were weighed and necropsied. They were taken bursa of Fabricius, thymus and spleen for weighing and histopathological processing.

### 2.1 Preparation of Herbal Medicine

The herbals formula Viranur and the production were done at the Laboratory of Pharmaceutical Chemistry, Faculty of Science, Department of Chemistry, State University of Yogyakarta with standard methods. All the ingredients (turmeric, fennel and cubeb) were washed, respectively, then dried in a dryer cabinet at a temperature of 40 °C. Once drying, ingredients are blended and filtered. The preparation procedure of phyllanthus as the same as in herbals Viranur, so that it becomes a product that is ready to use.

### 2.2 Lymphoid Organs Weight Index

Bursa of Fabricius, thymus and spleen weight index were done by measuring the weight of each organ divided by the body weight of each chicken, as in the following Eq. (1):

$$\text{Weight index} = \frac{\text{Organ weight}}{\text{Body weight}} \quad (1)$$

where, organ weight = weight of each chicken lymphoid organs; body weight = weight of each chicken.

### 2.3 Data Analysis

The results of lymphoid organs weight index were analyzed using a statistical software package SPSS version 12. Data were expressed as mean  $\pm$  standard

error of the mean (mean  $\pm$  SEM). The statistically significant level was  $P < 0.05$ . The microscopically lymphoid organs were analyzed using descriptive-qualitative approach.

### 3. Results

#### 3.1 Measurement of Lymphoid Organs Weight Index

Measurement of lymphoid organs weight index is intended to better represent the actual organ weights, because chickens used for the treatment amount is not uniform, so that only the weight of the organ would have caused variations unfair. Lymphoid organ weights indicate the content of the lymphocyte cells in it; the more weight, the more the lymphoid organs content lymphocyte cell numbers in it. The chicken immune response can also be seen in the content of existing cells in lymphoid organs through the examination organ weights. Examination of weight index of lymphoid organs, such as the bursa of Fabricius, thymus and spleen, showed no significant difference ( $P > 0.05$ ) between the control group and the treatment group, but there is a tendency that thymus mass index was higher in treatment group compared with the control group shown in Fig. 1.

In examination of bursa of Fabricius weight index in KC group (herbals formula Viranur + phyllanthus),

there is a higher tendency when compared with the control group KA and the group KB (turmeric + phyllanthus), while KB group tended to be lower than the control group KA as shown in Fig. 2.

Examination of spleen weight index showed only a slight difference, though not statistically significantly different. KB group tendency was higher than the control group KA and the group KC as shown in Fig. 3.

#### 3.2 Histopathological Examination of Lymphoid Organs

Results of microscopic examination of the primary lymphoid organs, such as bursa of Fabricius and thymus, and secondary lymphoid organs, such as spleen, show that both treatment and control chickens have differences. Microscopic examination of bursa of Fabricius organs is not much different from the examination of weight index bursa of Fabricius in the control group KA; while the microscopic changes of KB group were the same as control group (KA) that the lymphocyte cells in medulla lymphoid follicles do not fill the full and still looks loose; it indicates that the lymphocyte cells are relatively less in lymphoid follicles as shown in Figs. 4 and 5. While in the KC group (herbals formula Viranur + phyllanthus), part of the medulla of lymphoid follicles are more stretched

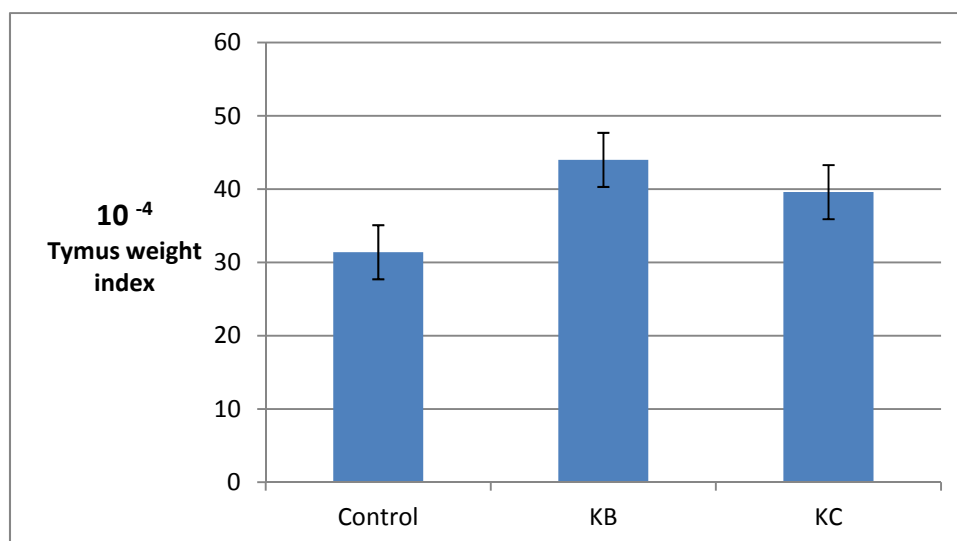


Fig. 1 The graph of weight index of chicken thymus after 35 days treatment.

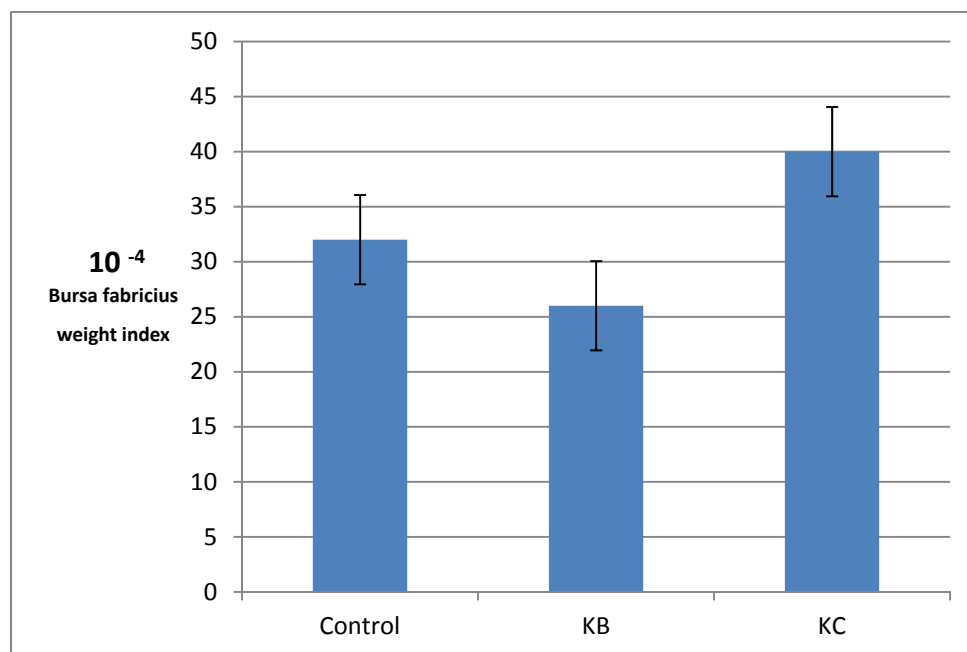


Fig. 2 The graph of chicken weight index of bursa of Fabricius after 35 days treatment.

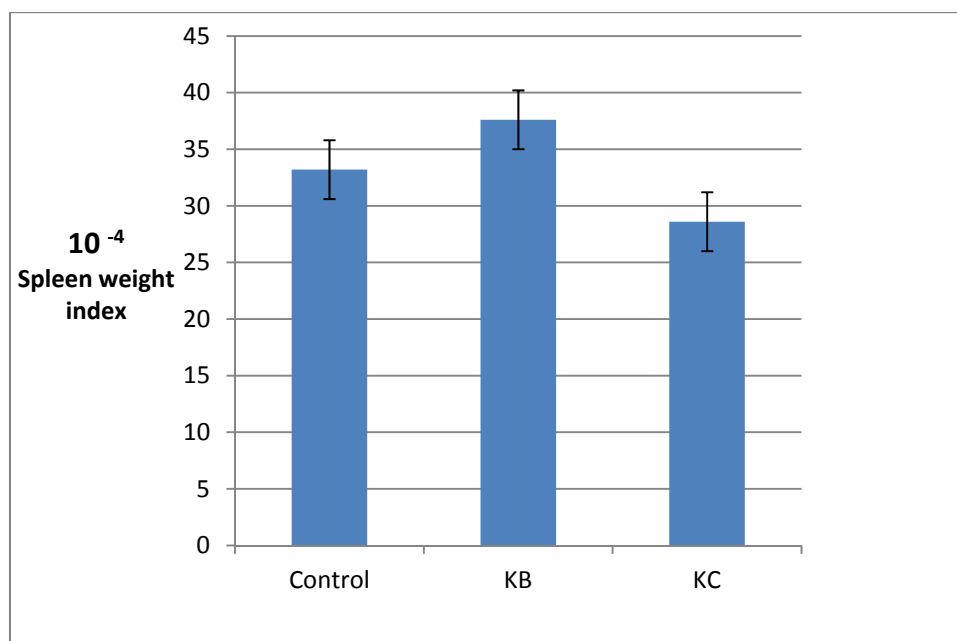


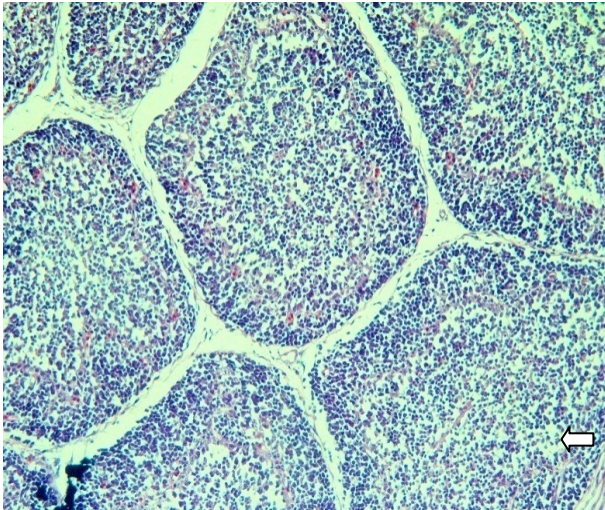
Fig. 3 The graph of weight index of chicken spleen after 35 days treatment.

and lymphocyte cells begin to condense; it showed the increase and proliferation of lymphocytes that are within lymphoid follicles in chicken bursa of Fabricius in KC group as shown in Fig. 6.

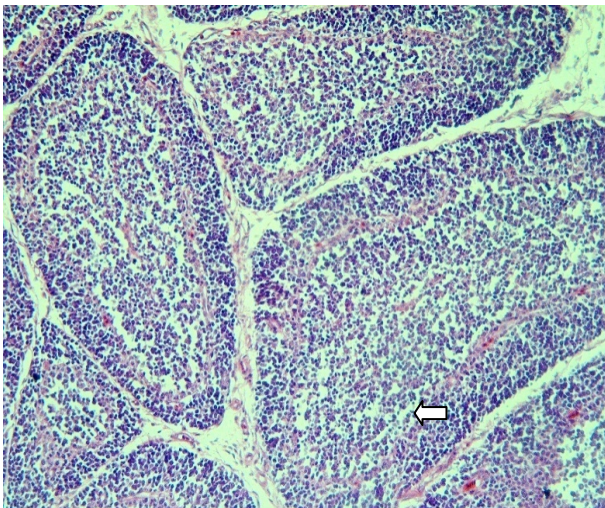
Microscopic examination of the thymus organ shows compliance with the examination of the thymus weight index; thymus of the chickens in the control group KA have a wider part of the medulla and

narrower in the cortex, meaning that the content of lymphocytes is less because the medulla has more number of reticular cells than lymphocytes as shown in Fig. 7. While in the group KB (turmeric + phyllanthus) and group KC (herbals formula Viranur + phyllanthus), the cortex of thymus tends to be wider and has more the number of lymphocytes than the medulla as shown in Figs. 8 and 9.

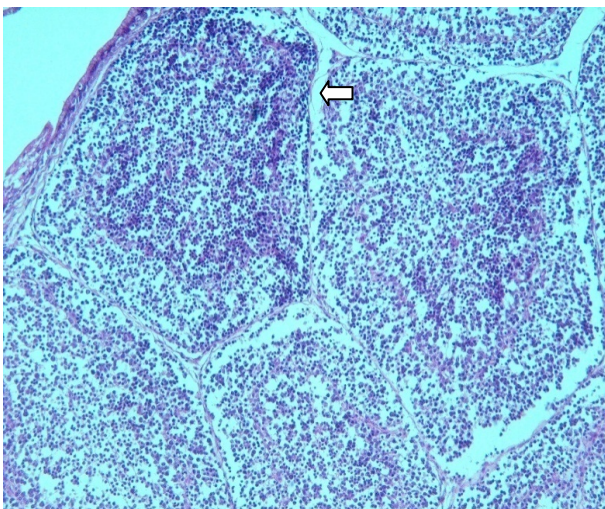




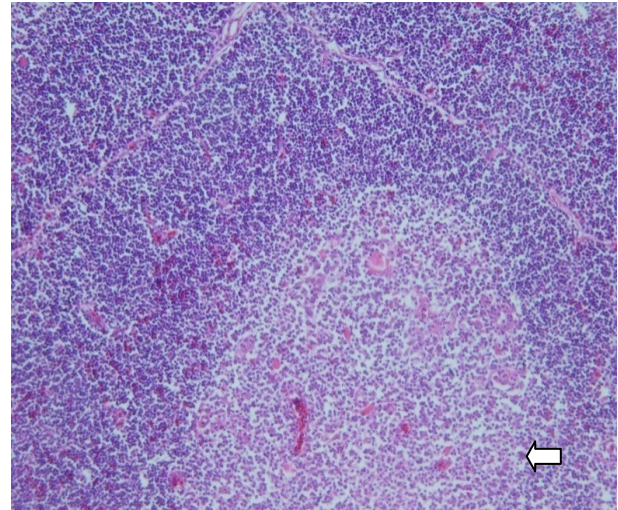
**Fig. 4** The histopathology of bursa of Fabricius (KA). In the medulla follicle, lymphocytes slightly reduced (↔).



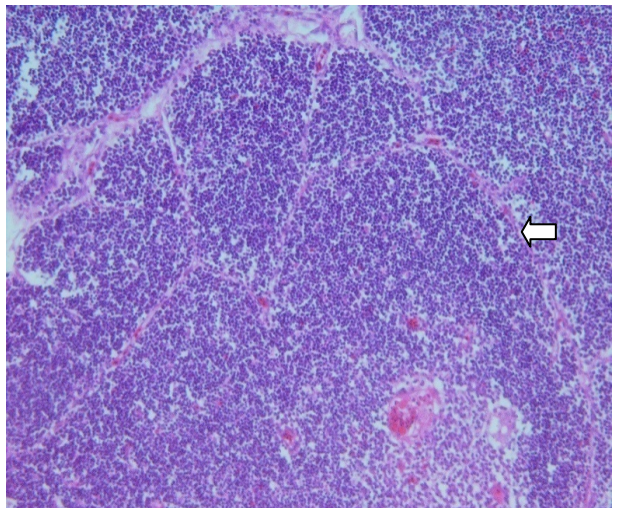
**Fig. 5** The histopathology of bursa of Fabricius (KB). In the medulla follicle, lymphocytes slightly reduced (↔).



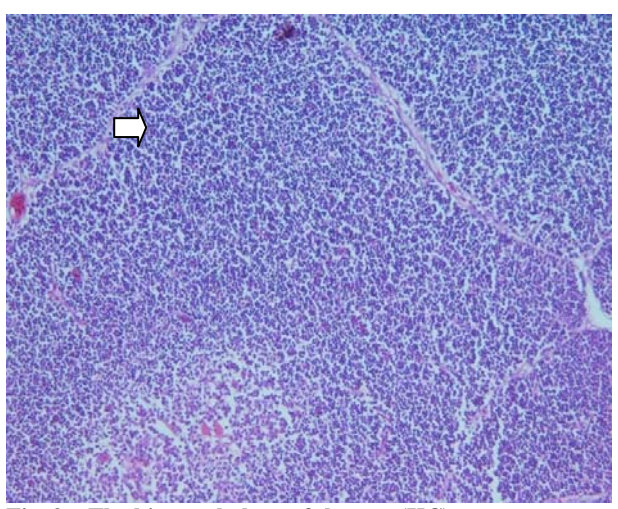
**Fig. 6** The histopathology of bursa of Fabricius (KC). A part of medullary follicles begin increase (↔).



**Fig. 7** The histopathology of thymus (KA). Part cortex is reduced (↔) and parts of medullary widening.

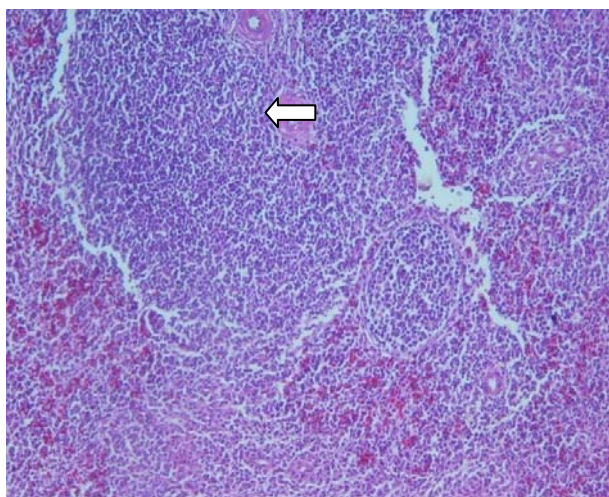


**Fig. 8** The histopathology of thymus (KB). Part cortex is wider, medulla is not widened (↔).

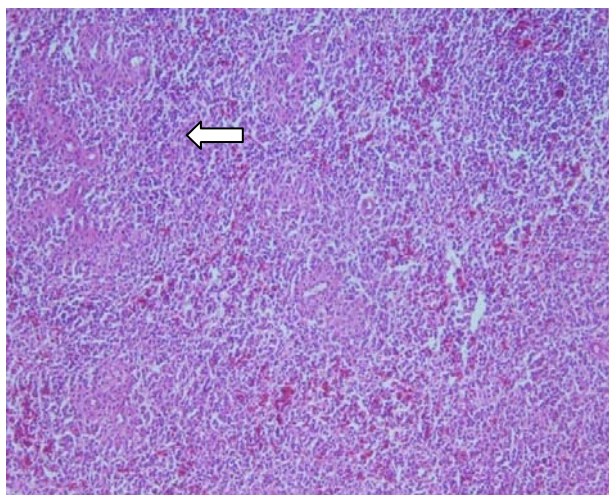


**Fig. 9** The histopathology of thymus (KC). Part cortex is wider (↔), the medulla is not widened.

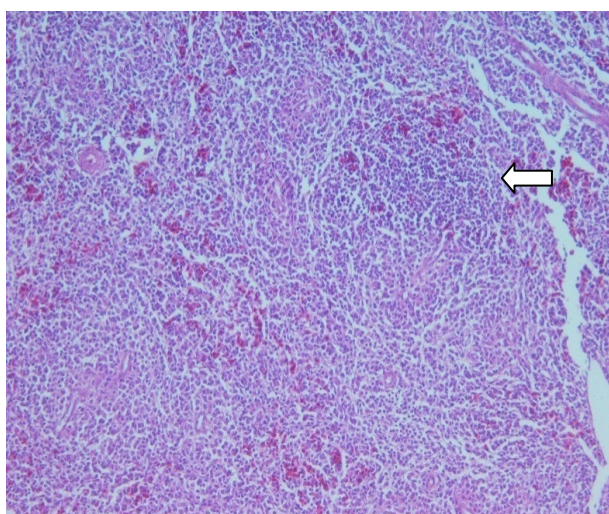




**Fig. 10** The histopathology of spleen in KA group. Lymphocytes in white pulp look much (↔).



**Fig. 11** The histopathology of spleen in KB group. Lymphocytes in white pulp reduced (↔).



**Fig. 12** The histopathology of spleen in KC group. Lymphocytes in white pulp reduce (↔).

In microscopic examination of the chicken spleen in the control group KA, it has the increased number of lymphocytes in the white pulp as shown in Fig. 10, while the group KB and KC did not yet showed an increased number of lymphocytes in the white pulp (Figs. 11 and 12). This shows the increase in the number of lymphocytes in primary lymphoid organs with a relatively faster than the secondary lymphoid organs, such as the spleen.

#### 4. Discussion

When seen from the graph of organ weights index and histopathological examination results, the administration of turmeric, phyllanthus and herbals formula Viranur was enough to give the effect of increased lymphocytes although not significant. The increased lymphocytes were not significant, because the provision of extracts were mixed into the drinking water so that the maximum possible dose was not achieved, although the dose has been calculated according to the dosage use in humans by Reagent-Shaw et al. [10].

Results of testing the weight index of bursa of Fabricius showed an upward trend in chickens of group KC (herbals formula Viranur + phyllanthus), and this result was supported by microscopic examination of bursa of Fabricius in the group KC, showing that part medullary lymphoid follicles undergo widening and were filled by lymphocytes relatively dense when compared with bursa of Fabricius follicles in the control group KA and group KB. This shows that the administration of medicinal plants can increase cell density of lymphocytes in bursa of Fabricius, so it is possible for the increase in hemagglutination inhibition (HI) titers. The relationship of lymphoid follicles in Bursa of Fabricius with antibody titers has also been proved by Qureshi et al. [11], that bursa of Fabricius damage due to infection with infectious bursal disease virus caused depletion of lymphocytes in medulla lymphoid follicles and the fall of antibody titers in the blood of

chicken. The KC group that was given herbals formula Viranur, is fairly complete herbal formula, which consists of a mixture turmeric (*Curcuma heyneana*) 100 g, fennel (*Foeniculi fructus*) 40 g, cubeb (*Piper cubeca*) 30 g and 500 g of sugar. The flavonoids contents of fennel and cubeb in the group KB are possible to greatly influence the change of lymphocytes that are in bursa of Fabricius follicle. Fennel (*Foeniculi fructus*) contains flavonoids, like quercetin, rutin and isoquercetin, was shown to have immunomodulatory activity [12]. Other studies showed that extracts of alcohol fennel can increase the average red blood cells and white blood cells in the rat at a dose of 250 mg/kg and can increase clotting time (CT) at a dose of 500 mg/kg [13].

Results of testing the weight index of thymus and microscopic examination of thymus also showed similar changes with bursa of Fabricius of chicken in the treatment groups that there was an increased tendency of weight index. Microscopic examination of the thymus of chickens in control showed that the part of cortex is narrower, while the medulla has a wider tendency; while in the thymus of the chickens in both the treatment groups (KB and KC), the part of cortex tends to be wider than the medulla. Widening section of the thymus cortex indicated an increased content of lymphocytes in it.

Examination weight index of spleen and microscopic examine does not show significant differences. The weight index of spleen is more varied among chickens fed with herbs. In chicken group KB, there is a tendency to have weight index higher than that in chicken group KC, although the microscopic results showed both equally reduced lymphocyte cells constituent in white pulp. Decreased lymphocyte cells in the functional spleen will be less profitable for chicken due to decreased immunity. The other research conducted by Setiyono and Barmawie [14] showed that the administration of herbals medicine in chickens is able to defend against challenge with avian influenza virus H5N1, so that the chicken was

still able to survive (46%), although the depletion of lymphocytes in the spleen.

The extract of turmeric and phyllanthus as shown in the KB group also had the effect of increasing the index of thymus and spleen weights, and histopathology confirmed the results of the wider cortex than the medulla in thymus organ. Turmeric (*Curcuma heyneana*) containing curcumin has been shown to have the effect of immunomodulator to increase the number of hemocyte population and the expression of antimicrobial peptides (AMPs) on prawns infected with *Vibrio alginolyticus* [15]. The extract of phyllanthus (*Phyllanthus niruri*) in rats at a dose of 400 mg/kg were able to increase the number of red blood cells and packed cell volume (PCV), and even with a dose of 100 mg/kg have been able to significantly increase the total number of leukocytes circulating and neutrophils [16].

## 5. Conclusions

The combination of herbal medicine administration of both turmeric (*Curcuma heyneana* Val.) + phyllanthus (*Phyllanthus niruri* L.) and herbals formula Viranur + phyllanthus (*Phyllanthus niruri* L.) in chickens can stimulate lymphocyte activity.

## Acknowledgments

This work was supported by Higher Education of Indonesia Research Grant in the year of 2015.

## References

- [1] Ade, F., Aznam, N., and Sulistyani, N. 2010. "Power Test Antivirus Hexane Fraction of Methanol Extract Temugiring Rhizome (*Curcuma heyneana* Val.), against Newcastle Disease Virus in Embryonated Chicken Eggs." M.Sc. thesis, Yogyakarta State University, Yogyakarta, Indonesia.
- [2] Rai, D., Yadav, D., Balzarini, J., De Clercq, E., and Singh, R. K. 2008. "Design and Development of Curcumin Bioconjugates as Antiviral Agent." *Nucleic Acids Symp. Ser.* 52 (1): 599-600.
- [3] Jafari, R. A., Ghorbanpoor, M., and Diarjan, S. H. 2009. "Study on Immunomodulatory Activity of Dietary Garlic in Chickens Vaccinated against Avian Influenza Virus

- (Subtype H9N2)." *Int. J. Poult. Sci.* 8 (4): 401-3.
- [4] Yuniwanti, E. Y. W. 2012. "Virgin Coconutoil as an Immunomodulator in Broilers Infected Avian Influenza Virus." Ph.D. thesis, Veterinary Science Doctoral Program, UGM, Yogyakarta.
- [5] Fourchier, R. A., Munster, V., Walltensen, A., Besterbroer, T. M., Herfst, S., Smith, D., Rimmelzwaan, G. F., Olsen, B., and Osterhaus, A. D. 2005. "Characterization of a Novel Influenza A Virus Hemagglutinin Subtype (H16) Obtained from Black-Headed Gulls." *J. Virol.* 79 (5): 2814-22.
- [6] Webster, R. G., and Hulse, D. J. 2004. "Microbial Adaptation and Change Avian Influenza." *Rev. Sci. Tech.* 23 (2): 453-65.
- [7] Easterday, B. B., Hinshaw, V. S., and Halvorson, D. A. 1997. "Influenza." In *Disease of Poultry*, 10th ed., edited by Calnek, B. W. Ames, Iowa: Iowa State University Press, 583-605.
- [8] Songsern, T., Alongkorn, A., Rungroj, J., Namdee, S. H., Noppodal, M., Nuananong, P., Sunchai, P., Apiradee, T., and Yong, P. 2006. "Avian Influenza H5N1 in Naturally Infected Domestic Cat." *Emerging Infectious Diseases* 12 (4): 681-3.
- [9] Thiry, E., Zicola, A., Addie, D., Egberink, H., Hartmann, K., Lutz, H., Paulet, H., and Horzinek, M. C. 2007. "Highly Pathogenic Avian Influenza H5N1 Virus in Cat and Others Carnivores." *Veterinary Microbiology* 122 (1-2): 25-31.
- [10] Reagen-Shaw, S., Nihal, M., and Ahmad, N. 2008. "Dose Translation from Animal to Human Studies Revisited." *The FASEB J.* 22 (3): 659-61.
- [11] Qureshi, M. A., Hussain, I., and Heggen, C. L. 1998. "Understanding Immunology in Disease Development and Control." *Poultry Science* 77 (8): 1126-9.
- [12] Cherg, J. M., Chiang, W., and Chiang, L. C. 2008. "Immunomodulatory Activities of Common Vegetables and Spices of Umbelliferae and Its Related Coumarins and Flavonoids." *Food Chem.* 106 (3): 944-50.
- [13] Mansouri, E., Kooti, W., Bazvand, M., Boroon, M. G., Amirzargar, A., Afrisham, R., Afzalzadeh, M. R., Astary-Larky, D., and Jalali, N. 2015. "The Effect of Hydro-Alcoholic Extract of *Foeniculum vulgare* Mill. on Leucocytes and Hematological Tests in Male Rats." *Jundishapur J. Nat. Pharm. Prod.* 10 (1): e18396.
- [14] Setiyono, A., and Barmawie, N. 2014. "Overview Histopathologic and Clinical Chicken Herbal after Tested Challenge with Avian Influenza Virus H5N1." *Jurnal Kedokteran Hewan* 8 (1): 30-4.
- [15] Alambra, J. R., Alenton, R. R. R., Gulpeo, P. C. R., Mecenas, C. L., Miranda, A. P., Thomas, R. C., Velando, M. K. S., Vitug, L. D., and Maningas, M. B. B. 2012. "Immunomodulatory Effect of Turmeric *Curcuma longa* (Magnoliophyta, Zingiberaceae) on *Macrobrachium rosenbergii* (Crustacea, Palaemonidae) against *Vibrio alginolyticus* (Proteobacteria, Vibrionaceae)." *AACL Bioflux* 5 (1): 13-7.
- [16] Taiwo, I. A., Oboh, B. O., and Francis-Garuba, P. N. 2009. "Haematological Properties of Aqueous Extract of *Phyllanthus amarus* (Schum and Thonn.) and *Xylopi aethiopica* (Dunal) A. Rich in Albino Rats." *Ethno-Med.* 3 (2): 99-103.