Effectivity of *Polyscias obtusa* Simplicia as Immunomodulator on CaecaTonsil of Broiler Post Infection of *Salmonella typhimurium*

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**ABSTRACT**

Antibiotics usually used to protect the animals from pathogens, but antibiotics have residues which have negative side effect. Antibiotics can substituted with *Polyscias obtusa* simplicia because this plant contains a chemical compound called Flavonoid which roles in immune system as immunomodulator. This study aimed to determine the mobilization and development of CD4+, CD8+ and B220+ T-Cells lymphocytes from thymus of broiler that have been given additional feed *Polyscias obtusa* after infected by *Salmonella typhimurium* and determine the optimum dose of the addition of simplicia’s *Polyscias obtusa* which can influence the mobilization of T-Cells lymphocyte in Thymus. The procedure are confirmation of the *Salmonella typhimurium* isolates, prepare the simplicia’s *Polyscias obtusa* and *Salmonella typhimurium* bacterial culture, orally infections in 500 μl, prepare supplemented feeds by three doses (i.e. dose 1 (0.08%), dose 2 (0.16%) and dose 3 (0.26%)), thymus and T-Cells lymphocytes isolation and Flowcytometry analysis. The results showed that feeding with antibacterial feed + *Salmonella* infection and natural feed with additional *Polyscias obtusa* simplicia’s dose 2 (0.16%) have increased the relative number of CD4+, CD8+ and B220+. T-Cells lymphocytes of thymus with different time feeding period have significant differences for relative number of CD8+. This result gave proof that *Polyscias obtusa* simplicia have the ability and influence to increase the body's immune system.

**Keywords**: Broiler, *Polyscias obtusa*, *Salmonella typhimurium*, T-Cells lymphocyte, Thymus

**INTRODUCTION**

Recently, drugs usage for the prevention and treatment of animal diseases is becoming increasingly important. This is due to the increasing demand of livestock products, such as meat, eggs and milk. Presence of antibiotic residues in livestock products and the development of resistant microbes consumed either by animal or human caused by less attention at sub-therapeutic levels to the antibiotics usage [1]. Therefore, to maintain production efficiency on safe consumption of broiler farms, it is necessary to arrange the use of alternative feed supplement that can boost the immune system of the chickens.

Various kinds of medicinal herbs widely used for humans and animals as a substitute for antibiotics. One such plant is *Kedondong Laut* (*Polyscias obtusa*) that contain flavonoids and new polyscosida called polyscosida AH on its leaves and root [2]. Flavonoid compound known as agent of immunomodulator, antiviral and antioxidant [3]. Flavonoid also estimated to promote lymphocytes proliferation, increasing the number of T cell and improving IL-2 activities [4,5]. Francis et al. [3] mentioned that saponin is an important nutrition compound for human and animals. Saponin also known acted on membrane permeability, as immunostimulant, agent of anticarsinogen and hypcholesterolaemic.

Based on previous research, leaves extract of *Polyscias obtusa* can mobilize haematopoetic stem cells (CD34*, T CD4*, CD8*, CD25* and CD62L) in *S. thypii* infected mice [6]. Empirical fact also found in Sleman, Yogyakarta, where this herb widely used as an animal feed and known that animals that consume these plants able to avoid the attack of avian flu outbreaks.

Based on these descriptions, scientific experiment needs to be done to determine the modulation and development of chicks cellular immunity after additional food feeding of leaves simplicia of *Polyscias obtusa* which has been infected before with *Salmonella typhimurium*, by observing the alteration cell number of CD4* and CD8* cell in caeca tonsil organ.

**METHODS**

This research was conducted in March-September 2012 in Laboratory of Microbiology, Laboratory of Animal Physiology, Department of...
Effectivity of Polyscias obtusa as immunomodulator on Broiler Chicken
(Farsely, M., et al.)

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Material and Equipments
Equipment of this research are cages, lights, place chicken feed containers, chaff, reaction tubes, petri dish, oose, Bunsen, voluume pipettes, suction ball, Erlenmeyer, micropipettes, blue tip, wire, syringe, propylene tubes, ice box, centrifuge, and flow cytometer. The used materials are media of XLD, KIA, LIA, NB, NA, stain gram of A, B, C and D, H2O2 solution, aquades, mill feed of BR1, artificial feed (yellow corn, DL Metionin, fish meal, premix, copra, soybean meal and salt), mineral water, caeca tonsil, PBS, and antibody of Rat anti-CD4+ PE anti Rat anti-CD8+ FITC.

Confirmation Test
XLD Media Test
Subcultured colonies inoculated in XLD (Xylose Lysine Deoxycholate) media and then incubated for 24 hours at temperature of 37°C. Positive result on XLD media showed by black colored colonies.

Catalase Test
Subcultured colonies smeared on glass object which treacled with one drop of hydrogen peroxide (H2O2) 3%. Positive result showed when there were gas bubbles.

Gram Staining Test
Positive colonies on XLD smeared up on sterilised glass object, then fixated on flame and treacled with gram staining A (Hucker’s crystal violet), B (Lugol'siodine), C (alcohol), and D (safranin) respectively for every 1 minutes and washed in running water, then dried and conducted morphology observation. The result showed that the bacteria is gram negative by its red colored cells.

KIA LIA Test
Positive colonies taken with graft and putted on KIA and LIA slant media then lines drawn to form lineage on slant. And then incubated in 37°C for 24-48 hours. Black colored colonies on media indicated positive test results.

Standard Curve
Isolates suspension cultured in Nutrien Broth media in different ratio between the concentration (dillution) explained in Table 1. Each suspension absorbance was assessed with spectrophotometer on 600 nm wave length and cell number was counted with haemocytometer.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Culture Stock (ml)</th>
<th>Steril Media (ml)</th>
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<tbody>
<tr>
<td>8:0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>7:1</td>
<td>0.5</td>
<td>3.5</td>
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<tr>
<td>6:2</td>
<td>1</td>
<td>3</td>
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<tr>
<td>5:3</td>
<td>1.5</td>
<td>2.5</td>
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<tr>
<td>4:4</td>
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<td>2</td>
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<tr>
<td>3:5</td>
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<td>1.5</td>
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<tr>
<td>2:6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1:7</td>
<td>3.5</td>
<td>0.5</td>
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<tr>
<td>0:8</td>
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Growth Curve
Bacteria culture were taken for 1 oose to grown in Nutrien Broth 10 ml media then incubated in 37°C for 24 hours. A total of 6 ml of culture mixed with 54 ml of NB medium. Sampling were done in every 1 hours by taking 4 ml and the addition of 500 μl formalin. Subsequently, absorbance were calculated with a spectrophotometer at a wavelength of 600 nm and the number of cells measured with haemocytometer.

Feed Manufacture
Leaves of P. obtusa which has been separated from the stalks washed, wind dried and smoothed with a dry blender, then weighed to the artificial feed additives according to treatment, i.e. dose of 0.08%, 0.16% and dosis 0.26% of the total feed. Feed manufactured in order of feed percentage composition, from lowest to the highest dose.

Oral Infection of Salmonella typhimurium
Bakteri Salmonella typhimurium dalam Nutrient Broth disentrifuse dengan kecepatan 10.000 rpm selama 10 menit suhu 25°C. Obtained pellet resuspended in physiological saline solution 0.9% NaCl, then taken 500 mL and fed orally (through the mouth) to infected chickens.

Isolation Lymphocytes Cell
Caeca tonsil organ taken from chickens that have been dissected. Caeca tonsils crushed clockwise with syringe base on wire as filter.
Obtained results putted in propylene tubes and added 12 ml PBS, and centrifuged at 2500 rpm, temperature of 4°C, for 5 minutes. Supernatant were removed and remained pellet resuspended with 1 ml PBS. Pipetting applied then to get homogenates. 50 µl homogenates moved into another new microtube and added 500 µl PBS. Then centrifuged at 2500 rpm, temperature 4°C for 5 minutes. Supernatant was discarded and pellet containing lymphocytes was obtained.

**Flowcytometry Analysis**

Isolated cells from caeca tonsil incubated in 50µL Rat anti-CD4+ PE (R-Phycoerythin) and Rat anti-CD8+ FITC (Fluoresence Isothiocyanat) antibodies for 15 minutes. Sample then added with 1 ml PBS moved into flowcytometer cuvette. Furthermore, flowcytometer calculated the relative number of total cells and the number of cells detected by antibody labeling. Obtained result processed further with BD cellquest Pro™ program.

**Statistic Analysis**

Data were analyzed with factorial complete randomized design with SPSS 16.0 program. Subsequently, data were analyzed using multiple analysis of variance. If the obtained results are significant then processed further with Tukey test.

**RESULT AND DISCUSSION**

**Comparison of Relative Cell Number of CD4+ on Caeca Tonsil Organ of Starter and Finisher Group**

Results in Fig. 3 showed that there is no significant difference between treatment groups in all starter group. The results were not significant presumably caused by the time treatment is still in the adaptation stage, so that the antibody response in chicks confined to a particular antigen epitopes. Immunologic respond in young animals still at the lowest levels of immunological [7].

*S. typhimurium* that infect 1-2 weeks aged chicks only caused low antibody response. This condition may caused by the nature of hiporesponsive cells in the immune system which has not maturely functioned yet, or dissemination of lymphoid tissue is still too early and antibody formation was rudimentary [8].

While in finisher group, the relative number of CD4+ significantly difference in the treatment group 0.08 % dose compared with the treatment of mill feed. However, changes in the relative number of CD4+ occurred in the treatment group

P. *obtusa* dose of 0.08 %, 0.16 %, and 0.26 %, which shows the increase of relative number of CD4+ in group finisher than starter, i.e. 12.22 % enhancement in treatment group of 0.08% dose P. *obtusa*. Thus it is presumably that the addition of P. *Obtusa* simplicia in the artificial feed increased the relative number of CD4+ caeca tonsil of Broiler with optimum doses of 0.08 %.

The real difference between the relative number of CD4+ starter and finisher group allegedly because flavonoids as immunomodulatory properties that can be turned into immunosupressan to the average index of phagocytic macrophages, when given in large doses and for long periods of time.

![Cell Number of T-CD4+ (%) Caeca Tonsil](image)

**Comparison of Relative Cell Number of CD8+ in Caeca Tonsil Organ of Starter and Finisher Group**

The results in Fig. 4 shows that there is significantly no difference in the relative number of CD8+ in all treatment groups in both starter and finisher group (p> 0.05). However, based on the treatment period, the relative number of CD8+ in the P. *obtusa* treatment finisher group increased more than starter group, with the highest increase of 7.58 %, at 0.08 % dose administration of P. *obtusa*.

Mechanisms of flavonoid and alkaloid contained in P. *obtusa* simplicia as immunomodulator, i.e. by increasing the activity of IL-2 and proliferation of lymphocytes. It is known that IL-2 is a cytokine that functions as one of the factor differentiated lymphocytes. Susceptibility and resistance of animals against microbial infection depends on the activation of T-CD4+ cells that differentiate into two groups.
based on the pattern of cytokine secretion, the response patterns of Th1 and Th2 [9,10].

**Fig 4.** Number of T-CD8\(^+\) Cell (%) Caeca Tonsil (p<0.05)

1 = mill feed  
2 = mill feed & *S. typhymurium*  
3 = *P. obtusa* dose 1 (0.08 %)  
4 = *P. obtusa* dose 2 (0.16 %)  
5 = *P. obtusa* dose 3 (0.26 %)

Lymphocytes subset Th1 through its production, i.e. interferon-\(\gamma\) (IFN-\(\gamma\)), IL-2 and IL-12. IFN-\(\gamma\) involved in the creation and presentation of antigenic peptides on the cell surface, because IFN-\(\gamma\) has the ability as a factor in macrophage activation. Meanwhile, IL-12 is the main and the most important cytokine in induction to Th1 subset, either in *vitro* or in *vivo* [11]. Thus, T cell differentiation into T-CD4\(^+\) cells increased because the role of these cytokines after administration of *P. obtusa* simplicia with different doses.

Flavonoids contained in *P. obtusa* indirectly affect the activity of T-CD8\(^+\) cells, so that the relative number of CD8\(^+\) was not significantly different among all treatment groups. Mechanism of flavonoid and alkaloid of *P. obtusa* as immunomodulator, which increased the activity of IL-2, also indirectly increase the activity of IFN-\(\gamma\). IFN-\(\gamma\), in addition to having a unique ability to regulate the expression of MHC II molecules, so as to improve the response of T-CD4\(^+\) cells lymphocytes, also increased the expression of MHC molecules I. Consequently, IFN-\(\gamma\) helps to improve or optimize the cytolytic activity of cytotoxic T-lymphocyte (CD8\(^+\)) which only recognize antigen or target cell when antigen was presented with MHC molecules I. In other words, for the full differentiation, T-CD8\(^+\) cells need cytokines that produced by T-CD4\(^+\) cells or co-stimulator that expressed on infected cell [12,13,14,15,16,17].

**CONCLUSION**

There are significant differences between treatment groups *Polyscias obtusa* dose of 0.08% with mill feed in terms of the relative number of CD4\(^+\). The treatment duration lead to significant difference results on the relative number of CD4\(^+\) and CD8\(^+\) cells. The best treatment in increasing the number of CD4\(^+\) T lymphocytes and CD8\(^+\) cells were artificial feeding with dose of *Polyscias obtusa* 0.08%.

**REFERENCES**


[10] Rifa’i M., Shi Z., Zhang S.Y., Lee Y.H., Shiku H., Isobe K., Suzuki H. 2008. CD8\(^+\)CD122\(^+\) regulatory T cells recognize activated T cells via conventional MHC class I-\(\alpha\)BTCE interaction and become IL-10-producing


