

## **The used of chopped banana *Musa paradisiaca* stem for stimulating immune responses and streptococcosis resistance of Nile tilapia *Oreochromis niloticus***

### **Cacahan batang pisang ambon *Musa paradisiaca* untuk meningkatkan respons imun dan daya tahan ikan nila *Oreochromis niloticus* terhadap streptococcosis**

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#### **ABSTRACT**

Streptococcosis is caused by *S. agalactiae* and often found in Nile tilapia farming. This study was performed to determine the effectiveness of the concentration and frequency of giving chopped banana stem as the immunostimulant to stimulate the non-specific immune system of tilapia against *S. agalactiae*. This study used factorial completely randomized design, consisted of two factors: concentration and changing frequency (replacement time interval) of banana stem on Nile tilapia rearing media with 11 treatments and three replications each treatment. The concentration of 5 g/L, 10 g/L, and 15 g/L and the three days, seven days and no replacement time interval were used in this study. The immersion treatment with the chopped banana stem was done for 14 days, then the challenge test with *S. agalactiae* was on the 15<sup>th</sup> day for 14 days observation. The result showed that chopped banana stem contained active compounds of alkaloids (17.63% flavonoids, 0.02% tannin and 0.24% saponins). Those active compounds increased the non-specific immune system including respiratory burst, lysozyme activity, phagocyte activity, erythrocyte, leucocyte, and haemoglobin. Treatment with a concentration of 5 g/L and no replacement of the chopped banana stem was the best treatment with 75% relative percent survival. In addition, it also had highest phagocytes (36.3%), respiratory burst (0.58 at O.D. 630), and lysozyme activity (72.7 unit/mL) after four days challenged with *S. agalactiae*.

Keywords: Banana stem, immunostimulant, Nile tilapia, non-specific immune system, *Streptococcus agalactiae*

#### **ABSTRAK**

Penyakit streptococcosis disebabkan oleh *S. agalactiae* yang ditemukan pada ikan nila. Penelitian ini bertujuan menentukan konsentrasi dan frekuensi pergantian cacahan batang pisang sebagai imunostimulan untuk meningkatkan daya tahan tubuh ikan nila terhadap serangan penyakit streptococcosis. Penelitian ini menggunakan rancangan acak lengkap faktorial dengan dua faktor yaitu konsentrasi dan frekuensi pergantian cacahan batang pisang pada media pemeliharaan ikan dengan 11 perlakuan dan 3 ulangan. Konsentrasi batang pisang yang digunakan yaitu 5 g/L, 10 g/L, dan 15 g/L, sedangkan frekuensi pergantian batang pisang dalam penelitian ini yaitu tiga hari, tujuh hari dan tanpa pergantian batang pisang. Perlakuan perendaman dengan cacahan batang pisang dilakukan selama 14 hari, kemudian dilakukan uji tantang dengan bakteri *S. agalactiae* pada hari ke-15 selama 14 hari pengamatan. Hasil penelitian menunjukkan bahwa cacahan batang pisang ambon mengandung senyawa aktif berupa alkaloid (flavonoid 17,63%, tanin 0,02%. dan saponin 0,24%). Senyawa aktif tersebut mampu meningkatkan respons imun non-spesifik pada ikan nila yaitu *respiratory burst*, aktivitas lisozim, aktivitas fagositik, total leukosit, total eritrosit, dan hemoglobin. Perlakuan dengan konsentrasi 5 g/L dan tanpa pergantian cacahan batang pisang merupakan perlakuan terbaik dengan nilai *relative percent survival* (RPS) mencapai 75%, lebih besar dari perlakuan lainnya. Selain itu, perlakuan ini memiliki nilai tertinggi pada aktivitas fagositik (36,6%), *respiratory burst* (0,58 pada O.D. 630 nm) dan aktivitas lisozim (72,7 unit/mL) setelah hari ke-4 uji tantang dengan bakteri *S. agalactiae*.

Kata kunci: Batang pisang, ikan nila, imunostimulan, respons imun, *Streptococcus agalactiae*

## INTRODUCTION

Nirwana tilapia strain with high growth performance has low disease resistance (Alimuddin *et al.*, 2015). *Streptococcosis* caused by the bacteria *Streptococcus agalactiae* is a type of disease that is often found in Nile tilapia farming in Indonesia, especially in West Java (Taukhid, 2014). It has high mortality rates up to 70% (Huang *et al.*, 2013). *S. agalactiae* is a gram positive group bacteria, not motile, fermentatively positive and negative catalase (Hardi *et al.*, 2008) which can infect freshwater and sea water fish, both wild and cultivated species (Yi *et al.*, 2014).

Immunostimulants, vaccines, and probiotics are considered to be ideal and effective prevention in disease control strategies that support sustainability in aquaculture. Immunostimulant such as levamisole (Bedasso, 2017),  $\beta$ -glucan (Meena *et al.*, 2013; Pilarski *et al.*, 2017), peptidoglycans (Gan *et al.*, 2016), chitin, chitosan (Harikrishnan *et al.*, 2012; Abu-Elala *et al.*, 2015), yeast, vitamin combinations, and phytopharmaca are biological components that potentially enhance the non-specific cellular and humoral defense mechanisms, the first line of defense against pathogens (Harikrishnan *et al.*, 2011; Selim *et al.*, 2014; Selim & Rasha, 2015). Immunostimulant can be applied by injection, immersion, and oral (Harikrishnan *et al.*, 2012). It depends on the time of application, dosage, the method used, and the physiological conditions of the fish (Rattanavichai *et al.*, 2015).

Immunity is a resistance to infectious diseases, classified into non-specific and specific immune responses. The non-specific immune response is the first line of defense against pathogens (Hariskrishnan *et al.*, 2012). The non-specific immune system consists of cellular defense mechanisms (macrophages, monocytes, granulocytes, non-specific cytotoxic cells and cell lines) and humoral defense mechanisms (lysozyme, complement, interferon, C-reactive protein, transferrin, and lectin) (Magnadottir, 2006). Immunostimulant, vaccines, and probiotics are believed to be ideal and effective disease control strategies that foster sustainability in aquaculture. The popularity of these alternatives was brought forth when the demand of antibiotics reduction arose because of uncontrolled use potentially lead to antibiotic resistance in bacteria, causing risks to the consumers and environment and for the development of an eco-friendly industry arose (Calbello, 2006; Abarike *et al.*, 2018)

Banana stem (*Musa paradisiaca*) is one of the phytopharmaca that can be used as an immunostimulant for preventing pathogen infection. The survival rate after challenge tested by *Aeromonas hydrophila* in catfish through immersion of “kepok” banana stem (75.0%) was higher than the control treatment (25.0%) (Siregar *et al.*, 2016). There is no previous study yet on the application of banana stem by immersion for Nile tilapia farming and the influence of it in the water. The dosage and replacement time interval is expected to determine the effectiveness and the efficiency of the chopped banana stem. Therefore, this study performed to determine the effective concentration and replacement time interval of the chopped banana stem as an immunostimulant to stimulate the non-specific immune system of Nile tilapia against *S. agalactiae* infection.

## MATERIALS AND METHODS

### Experimental design

This research was conducted using factorial completely randomized design. The treatments consisted of two factors; the concentration and the changing frequency (replacement time interval) of banana stem on Nile tilapia rearing media, then each treatment was given three replications (Table 1).

### Preparation of the chopped banana stem

Ambon banana stem (*M. paradisiaca*) obtained from the experimental pond in Babakan, Faculty of Fisheries and Marine Science, Bogor Agricultural University. The banana stem was selected from the harvested plant and it was chopped into 1–2 cm of its length, height, and width.

### Preparation of container and fish

This study used 44 aquarium sizing of 65×30×35 cm<sup>3</sup> with 45 L of water. Nirwana tilapia strain with body length of 8.67 ± 0.18 cm with body weight of: 12.10 ± 0.95 gram was derived from the study conducted by Alimuddin *et al.*, (2015). Each aquarium filled with 20 fishes per unit treatment and fish fed on commercial diet with protein content of 28% twice a day to an apparently satiation.

### Preparation of bacterial suspension of *Streptococcus agalactiae* and challenge test

*S. agalactiae* was obtained from Center for Research and Development of Freshwater

Table 1. The treatment of chopped banana stem with different concentration and the frequency of replacement banana stem

The treatment	Materials	Concentration of banana stem (g/L)	Replacement time interval (days)
Negative control (C-)	Without banana stem	0	No replacement
Positive control (C+)	Without banana stem	0	No replacement
A 3	Chopped of banana stem	5	3
B 3		10	
C 3		15	
A 7	Chopped of banana stem	5	7
B 7		10	
C 7		15	
A 14	Chopped of banana stem	5	No replacment
B 14		10	
C 14		15	

Note : (A 3), (B 3), (C 3)= treatment of 5 g/L, 10 g/L, 15 g/L concentrations with three days replacement time interval, and at the time of challenge test fish was injected with *S. agalactiae*; (A 7), (B 7), (C 7)= treatment of 5 g/L, 10 g/L, 15 g/L concentrations with seven days replacement time interval, and at the time of challenge test fish was injected with *S. agalactiae*; (A 14), (B 14), (C 14)= treatment of 5 g/L, 10 g/L, 15 g/L concentrations with no replacement of chopped banana, and at the time of challenge test fish was injected with *S. agalactiae*; (C+)= no banana stem added to rearing media and at the time of challenge test fish was injected with *S. agalactiae*; (C-)= no banana stem added to rearing media and at the time of challenge test fish was injected with PBS.

Aquaculture (BPPBIH), Depok. Virulence bacteria tested using Koch's postulates. The LD<sub>50</sub> was determined before challenge test performed. Challenge test conducted by injecting *S. agalactiae* at a density of 10<sup>7</sup> CFU/mL with a dose of 0.1 mL/fish through intraperitoneal injection. Changes occurred were observed and recorded for 14 days.

### Parameter of observation

#### *Qualitative and quantitative analysis of phytochemistry*

The qualitative and quantitative analysis was conducted at Research Institute for Medicinal Plants and Spices, Bogor. Qualitative analysis of phytochemical was performed by following the procedure of Harborne (2006). Quantitative analysis for flavonoid and tannin were done through the method of spectrophotometry, while saponin was by thin-layer of chromatography scanner to calculate the amount of each active compound expressed as a percentage (%).

#### *Survival rate*

The survival rate of tilapia was computed at day-14 after immersion and at day-14 after challenge test with *S. agalactiae*. The survival rate of tilapia was calculated according to Effendi (2004).

$$SR (\%) = \frac{N_t}{N_o} \times 100$$

#### *Hematology and immune response*

Fish blood collection for hematology and immune response parameters conducted at day-14 after immersion and at day-14 after challenge test with *S. agalactiae*. The observed hematology and immune response observed were erythrocyte and leucocyte (Blaxhall & Daisley, 1973), phagocytic activity (Anderson & Siwicki, 1993), respiratory burst, and lysozyme activity (Wu *et al.*, 2013).

#### *Clinical signs*

The observed clinical signs consisted of changes in fish behavior included a change in swimming habit, feeding response, and a change in external and internal anatomy (melanosis, exophthalmia, corneal opacity, purulence, scoliosis, and dropsi).

#### *Water quality*

Temperature and dissolved oxygen were measured daily by thermo scientific submersible dissolved oxygen probe DO 300 and pH were measured by Waterproof pH Testr® 30. Measurement of hardness (titration) and total ammonia (spectrophotometer) was done every seven days, and the level of potassium and calcium (atomic absorption spectrophotometer) were measured at the beginning and the end of the study.

## Data analysis

Survival rate, immune responses, and hematology parameters were analyzed quantitatively by using Microsoft Excel 2010 and SPSS version 16.0. If the result of the ANOVA test was significantly different, post hoc test of Duncan was performed at  $P=0.05$ . The data of clinical signs and water quality parameter were analyzed descriptively.

## RESULT AND DISCUSSION

### Active compound content of ambon banana stem

The result of the qualitative and quantitative analysis is shown in Table 2. The chopped banana stem contained active compounds of flavonoid (17.63%), tannin (0.02%), saponin (0.24%), and alkaloid (Table 2).

Table 2. Qualitative and quantitative analysis of chopped banana stem

Active compound	Analysis method	
	Qualitative	Quantitative
Alkaloid	+	×
Saponin	+	0.24
Tannin	+	0.02
Phenolic	–	×
Flavonoid	+	17.63
Terpenoids	–	×
Steroid	–	×
Glycoside	–	×

(+)= detected; (–)= not detected; (×)= quantitative analysis are not performed

The previous study on phytochemical test of chopped ambon banana stem active compound in water showed that water added with the chopped banana stem of 5 g/L, 10 g/L, and 15 g/L contained flavonoid, respectively of 0.049%, 0.098%, and 0.147%, and phenolic in each concentration at day–7 of immersion. The result of the qualitative analysis in water added with chopped banana stem and fish indicated that only alkaloid was detected at the day–14 of immersion. It is expected that the amount of alkaloid compound in the water was higher compared to other active compounds, hence it was absorbed by the fish body, and the amount of alkaloid was still abundant in the water and was still able to be detected at day-14 of immersion. This finding was in line with the study conducted by Onyema *et al.*, (2016) that alkaloid percentage in the banana stem (8.16%)

was higher than other active compounds such as flavonoid (4.02%) and saponin (3.5%). Another possibility is the fact that alkaloid is not easily dissolved in the water (Cushnie *et al.*, 2014), while other active compounds (flavonoid, saponin, and tannin) is able to dissolve in the water (Cazzarolli *et al.*, 2008) thus alkaloid durability in the water has a longer period of time compared to other active compounds.

### Survival rate of fish and water quality during immersion treatment

The addition of chopped banana stem in the water did not affect the survival rate of tilapia during immersion, all fish survived in all treatments (SR 100%). A similar result was also reported in catfish (Siregar *et al.*, 2016). Thus, water quality during the immersion treatment in this study, namely: temperature (26.00–27.28°C); pH (6.57–8.26); DO (4.16–7.44 mg/L); hardness (44.04–116.12 mg/L); and TAN (0.095–0.659 mg/L) was still within the ideal range for tilapia (Pandit & Nakamura, 2010; El-Sherif & Elfeky, 2009; Colt *et al.*, 2011; Covalcante *et al.*, 2009).

### The content of calcium and potassium in rearing media of tilapia

Banana stem, besides containing active compounds, also has high mineral content, namely potassium and calcium. Potassium and calcium content of banana stem according to Poyyamozhi and Kadirvel (1986) are 95.34 g/kg and 4.98 g/kg of the fresh banana stem, respectively. Potassium functions as an antibacterial and anti-inflammatory agent, improving metabolism and body organ function (Insana & Wahyu, 2015) as well as help to maintain osmotic pressure and acid–base balance. Moreover, the addition of potassium together with hormone (insulin and thyroxine) is able to stimulate lymphocyte proliferation and against the pathogen (Hill *et al.*, 2005). Furthermore, calcium is one of the components of the skeleton and cofactor of several types of enzymes such as  $\text{Na}^+/\text{K}^+$  ATP-ase and also plays in the process of osmoregulation and nerve activity. In addition, its administration with phorbol ester simultaneously can stimulate T cell and B cell in catfish (Lin *et al.*, 1992). In this research, potassium content in the water at day-14 after banana stem immersion ranged from 11.2 to 61.5 mg/L. Moreover, calcium content in the water at day-14 after banana stem immersion had a range from 5.0 to 9.2 mg/L. Treatment with a concentration of 5 g/L and no replacement of

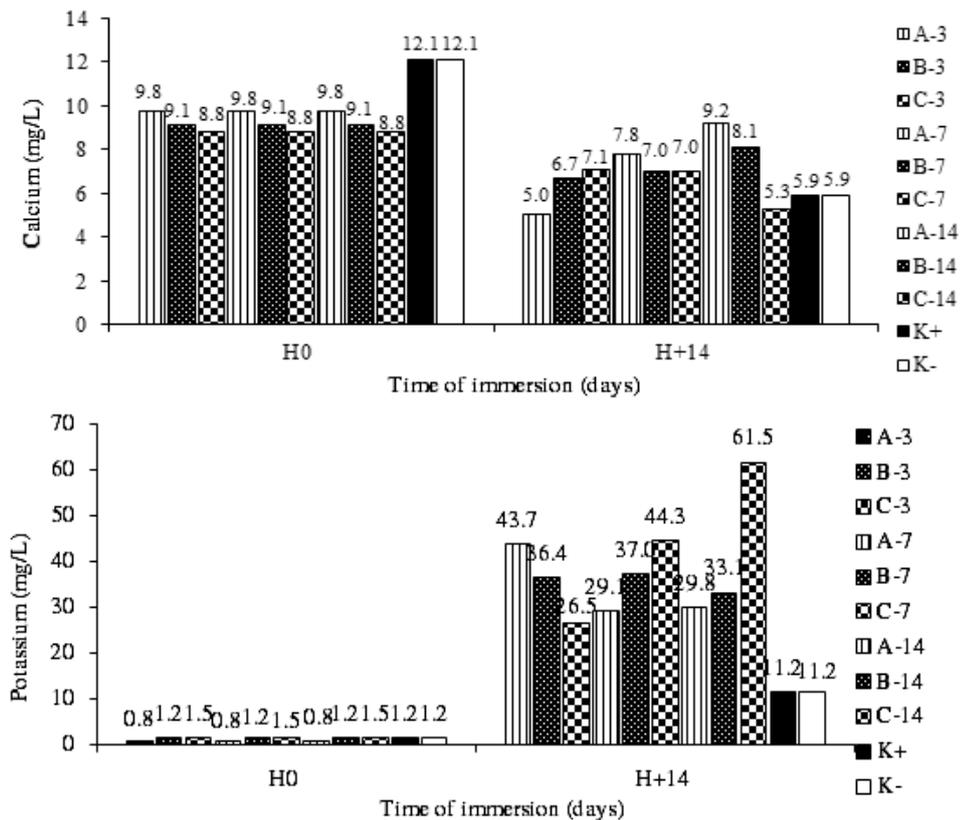


Figure 1. Calcium and potassium levels in water during the immersion of banana stem. (A)= concentration 5 g/L; (B)= concentration 10 g/L; (C)= concentration 15 g/L; (3)= 3 days replacement interval; (7)= 7 days replacement interval; (14)= no replacement of chopped banana; (C-)= negative control, no banana stem added to rearing media; (C+)= positive, control no banana stem added to rearing media.

chopped banana stem (A 14) generated the highest calcium content (9.2 mg/L).

### Survival rate of fish after challenge test with *Streptococcus agalactiae*

In this study, the effect of the chopped banana stem on fish survival after challenging test with *S. agalactiae* was significant. The highest SR was obtained in treatment A 3 (banana stem concentration was 5 g/L with three days replacement interval of banana stem: was  $81.67 \pm 2.89\%$ ) and A 14 (banana stem concentration was 5 g/L with no replacement of chopped banana stem: was  $85.00 \pm 0.00\%$ ) and significantly different from other treatments, including treatment C+ ( $40.00 \pm 5.00\%$ ) ( $P < 0.05$ ; Figure 2). The addition of banana stem at the highest concentration (15 g/L) resulted in lower fish survival compared to other concentrations (5 g/L and 10 g/L), except for the positive control, while the replacement time interval of was not affected. It is likely that alkaloid in high amount is toxic to fish. It means that alkaloid in a certain amount is not always toxic to fish, and may work synergically with other compounds (Queiroz *et al.*, 2013). Survival rate after challenge test with

*Aeromonas hydrophila* in catfish with “kepok” banana stem through immersion (75.0%) has also been reported to be higher than the control treatment (25.0%) (Siregar *et al.*, 2016).

### Hematology and immune responses

#### Total erythrocyte and hemoglobin

Total erythrocyte of tilapia after immersion with the chopped banana stem in each treatment at day-14 increased up to 13% compared to the control (Table 2). This increase was still within the normal range according to Bittencourt *et al.* (2003). Furthermore, the level of fish hemoglobin increased at day-14 after immersion with the chopped banana stem of 18% (Table 3). Total erythrocyte of tilapia after challenge test with *S. agalactiae* at day-4 had an average decline amounted to 20% of total erythrocyte after immersion. However, this was still within the normal range according to Bittencourt *et al.* (2003). The decrease of total erythrocyte in tilapia after challenge test with *S. agalactiae* followed with the declining level of tilapia hemoglobin to 33.3% from total hemoglobin level of fish after immersion (Table 3). The decrease in total erythrocyte count and hemoglobin after challenge

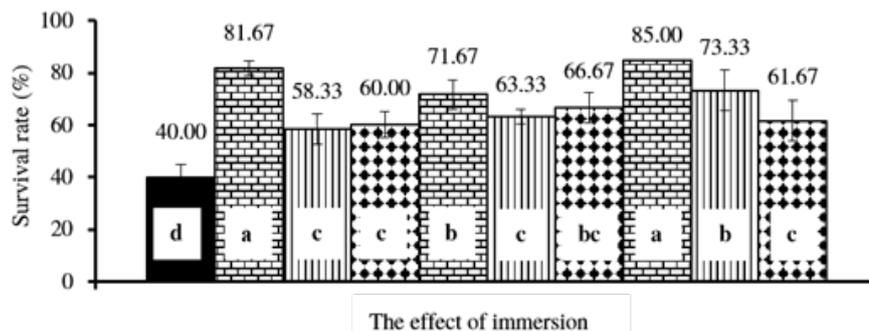


Figure 2. Survival rate (%) of tilapia after challenge test with *Streptococcus agalactiae*. (A)= concentration 5 g/L; (B)= concentration 10 g/L; (C)= concentration 15 g/L; (3)= 3 days replacement interval; (7)= 7 days replacement interval; (14)= 14 days replacement interval; no banana stem added to rearing media; (C+)= positive, control no banana stem added to rearing media. Different superscript letters in the bar graphic bar show significantly different values ( $P < 0.05$ ).

test that indicate anemia in fish was showed by hemorrhage on fish kidney (Hardi *et al.*, 2011).

At day-12 after challenge test, the total erythrocyte and hemoglobin, respectively increased up to 21.7% and 50.0% of total fish erythrocyte and hemoglobin at day-8 after challenge test (Table 3). Based on Hardi *et al.* (2011), the increase of total erythrocyte indicated a homeostatic effort of fish to produce more blood cell, and replace erythrocyte lysis due to infection. Yunita *et al.* (2016) stated that flavonoid and other active compounds in banana stem might help in improving and activating

damaged of hemoglobin. Moreover, An *et al.* (2015) mentioned that flavonoid might inhibit the occurrence of hemolysis in erythrocyte.

#### Total leucocyte and phagocytic activity (PA)

Total leucocyte increased of 32% compared to the control treatment at day-14 after immersion (Table 4). Based on Nugroho *et al.* (2016), flavonoid can have a function as biocatalysator for leucocyte production and stimulate leucocyte as non-specific cellular immunity. Increased total leucocyte has an impact on phagocytic activity. In this study, the phagocytic activity value had

Table 3. Total erythrocyte ( $\times 10^6$  cell/mm<sup>3</sup>) and hemoglobin (g/dL) in tilapia after immersion (H+14) dan post-challenge with *S. agalactiae* (H+4, H+8, and H+12)

Treatment	After immersion		After challenge test with <i>S. agalactiae</i>					
	H+14		H+4		H+8		H+12	
	Hb	Erythrocyte	Hb	Erythrocyte	Hb	Erythrocyte	Hb	Erythrocyte
C-	6.7±0.1 <sup>d</sup>	2.3±0.2 <sup>efg</sup>	5.0±0.0 <sup>cd</sup>	3.4±0.0 <sup>a</sup>	6.2±0.5 <sup>b</sup>	3.2±0.0 <sup>a</sup>	9.1±0.2 <sup>a</sup>	3.1±0.0 <sup>a</sup>
C+	6.3±0.2 <sup>d</sup>	2.3±0.2 <sup>efg</sup>	4.3±0.1 <sup>ef</sup>	1.1±0.1 <sup>f</sup>	4.7±0.3 <sup>c</sup>	1.0±0.0 <sup>g</sup>	5.7±0.9 <sup>a</sup>	2.2±0.1 <sup>cd</sup>
A3	7.6±0.2 <sup>bc</sup>	2.1±0.2 <sup>fg</sup>	6.0±0.3 <sup>a</sup>	1.7±0.2 <sup>cd</sup>	7.9±0.1 <sup>a</sup>	1.7±0.1 <sup>d</sup>	7.6±0.3 <sup>a</sup>	1.2±0.0 <sup>g</sup>
B3	7.5±0.2 <sup>bc</sup>	2.4±0.2 <sup>ef</sup>	4.9±0.6 <sup>cd</sup>	2.5±0.2 <sup>b</sup>	4.4±0.3 <sup>cd</sup>	1.5±0.0 <sup>e</sup>	7.7±0.9 <sup>a</sup>	1.8±0.1 <sup>f</sup>
C3	9.4±0.3 <sup>a</sup>	3.8±0.0 <sup>a</sup>	5.3±0.1 <sup>bc</sup>	1.3±0.2 <sup>ef</sup>	3.5±0.4 <sup>ef</sup>	1.1±0.1 <sup>g</sup>	6.0±0.2 <sup>a</sup>	2.0±0.1 <sup>e</sup>
A7	7.4±0.3 <sup>c</sup>	2.1±0.1 <sup>fg</sup>	4.1±0.1 <sup>ef</sup>	1.5±0.3 <sup>de</sup>	4.3±0.4 <sup>cd</sup>	1.4±0.2 <sup>f</sup>	8.7±0.3 <sup>a</sup>	2.6±0.3 <sup>b</sup>
B7	7.6±0.2 <sup>bc</sup>	2.4±0.1 <sup>de</sup>	4.1±0.1 <sup>ef</sup>	2.3±0.2 <sup>b</sup>	4.0±0.0 <sup>de</sup>	1.7±0.2 <sup>d</sup>	7.9±0.1 <sup>a</sup>	2.1±0.2 <sup>de</sup>
C7	8.1±0.9 <sup>b</sup>	3.2±0.3 <sup>b</sup>	4.1±0.2 <sup>f</sup>	1.8±0.2 <sup>c</sup>	3.4±0.0 <sup>f</sup>	2.4±0.0 <sup>c</sup>	6.5±0.3 <sup>a</sup>	2.2±0.1 <sup>cde</sup>
A14	7.9±0.1 <sup>bc</sup>	3.1±0.2 <sup>bc</sup>	5.8±0.4 <sup>ab</sup>	2.4±0.2 <sup>b</sup>	4.7±0.1 <sup>c</sup>	2.5±0.1 <sup>b</sup>	8.8±0.5 <sup>a</sup>	2.4±0.1 <sup>c</sup>
B14	7.5±0.5 <sup>bc</sup>	2.9±0.2 <sup>cd</sup>	4.7±0.4 <sup>de</sup>	1.1±0.1 <sup>f</sup>	4.4±0.2 <sup>cd</sup>	1.5±0.0 <sup>ef</sup>	7.1±0.6 <sup>a</sup>	1.7±0.1 <sup>f</sup>
C14	6.4±0.4 <sup>d</sup>	1.9±0.0 <sup>g</sup>	5.1±0.7 <sup>cd</sup>	1.6±0.2 <sup>cde</sup>	4.6±0.1 <sup>cd</sup>	1.2±0.0 <sup>g</sup>	5.9±0.1 <sup>a</sup>	1.6±0.1 <sup>f</sup>

References:

0.7–28 ( $\times 10^6$  cell/mm<sup>3</sup>) erythrocyte (Bittencourt *et al.*, 2003)

6.58–15.98 g/dL Hb (Bittencourt *et al.*, 2003)

Data were expressed as mean  $\pm$  standard deviation. Different superscript on the same coloum showed significantly different ( $P < 0.05$ ). (A)= concentration 5 g/L; (B)= concentration 10 g/L; (C)= concentration 15 g/L; (3)= 3 days replacement interval; (7)= 7 days replacement interval; (14)= no replacement of chopped banana stem; (C+)= positive control (no banana stem added to rearing media). (C-)= negative control (no banana stem added to rearing media).

Table 4. Total leucocyte ( $\times 10^4$  cell/mm<sup>3</sup>) and phagocytic activity (%) of tilapia after immersion (H+14) and post-challenge with *S. agalactiae* (H+4, H+8, and H+12)

Treatment	After immersion				After challenge test with <i>S. agalactiae</i>			
	H+14		H+4		H+8		H+12	
	Leucocyte	PA	Leucocyte	PA	Leucocyte	PA	Leucocyte	PA
C-	6.1±0.2 <sup>g</sup>	24.2±0.9 <sup>a</sup>	8.4±0.3 <sup>d</sup>	25.0±1.8 <sup>f</sup>	4.2±2.1 <sup>g</sup>	24.8±1.3 <sup>a</sup>	5.9±0.0 <sup>g</sup>	26.5±1.3 <sup>a</sup>
C+	6.8±0.2 <sup>f</sup>	24.3±0.9 <sup>a</sup>	7.2±0.6 <sup>e</sup>	28.0±0.8 <sup>e</sup>	6.6±2.5 <sup>f</sup>	27.0±1.4 <sup>a</sup>	5.5±0.1 <sup>g</sup>	27.5±1.3 <sup>a</sup>
A 3	8.5±0.6 <sup>cb</sup>	26.3±2.2 <sup>a</sup>	10.2±0.2 <sup>c</sup>	31.0±1.8 <sup>cd</sup>	11.6±0.3 <sup>a</sup>	33.3±1.3 <sup>a</sup>	10.4±0.3 <sup>a</sup>	36.0±1.8 <sup>a</sup>
B 3	7.4±0.2 <sup>de</sup>	28.8±0.9 <sup>a</sup>	11.2±0.1 <sup>ab</sup>	32.0±2.2 <sup>bcd</sup>	10.6±0.3 <sup>b</sup>	34.3±1.5 <sup>a</sup>	9.3±0.3 <sup>cd</sup>	34.0±1.2 <sup>a</sup>
C 3	9.7±0.5 <sup>a</sup>	28.3±2.1 <sup>a</sup>	11.1±0.9 <sup>ab</sup>	33.8±1.5 <sup>abc</sup>	11.5±0.3 <sup>a</sup>	34.8±0.5 <sup>a</sup>	6.4±0.3 <sup>f</sup>	34.3±0.9 <sup>a</sup>
A 7	7.6±0.3 <sup>d</sup>	30.3±2.4 <sup>a</sup>	11.3±0.3 <sup>ab</sup>	35.8±2.9 <sup>a</sup>	10.3±0.4 <sup>b</sup>	34.8±2.1 <sup>a</sup>	9.7±0.2 <sup>bc</sup>	37.0±0.8 <sup>a</sup>
B 7	9.7±0.1 <sup>a</sup>	31.0±0.8 <sup>a</sup>	11.5±0.3 <sup>a</sup>	32.8±2.1 <sup>bcd</sup>	9.1±0.7 <sup>c</sup>	36.3±0.9 <sup>a</sup>	5.9±0.8 <sup>g</sup>	35.5±2.1 <sup>a</sup>
C 7	7.0±0.3 <sup>ef</sup>	30.8±1.7 <sup>a</sup>	10.6±0.3 <sup>bc</sup>	30.5±1.0 <sup>de</sup>	9.5±0.1 <sup>c</sup>	35.3±0.5 <sup>a</sup>	8.9±0.1 <sup>de</sup>	35.3±0.9 <sup>a</sup>
A 14	9.7±0.1 <sup>a</sup>	30.5±1.7 <sup>a</sup>	11.4±0.2 <sup>ab</sup>	36.3±2.2 <sup>a</sup>	8.4±0.4 <sup>d</sup>	34.8±2.1 <sup>a</sup>	9.7±0.1 <sup>b</sup>	38.3±2.6 <sup>a</sup>
B 14	8.9±0.4 <sup>b</sup>	29.8±1.5 <sup>a</sup>	10.6±0.7 <sup>bc</sup>	34.5±2.7 <sup>ab</sup>	8.0±0.8 <sup>de</sup>	36.5±2.4 <sup>a</sup>	8.8±0.1 <sup>e</sup>	35.8±2.2 <sup>a</sup>
C 14	8.1±0.2 <sup>c</sup>	30.3±0.9 <sup>a</sup>	11.5±0.3 <sup>a</sup>	32.0±1.8 <sup>bcd</sup>	7.5±0.2 <sup>e</sup>	33.5±1.3 <sup>a</sup>	10.3±0.3 <sup>a</sup>	33.3±0.9 <sup>a</sup>

Reference: 2–15 ( $\times 10^4$  cell/mm<sup>3</sup>) Leucocyte (Svobodova & Vyukusova, 1991)

Data were expressed as mean  $\pm$  standard deviation. Different superscript on the same coloum showed significantly different ( $P < 0.05$ ). (A)= concentration 5 g/L; (B)= concentration 10 g/L; (C)= concentration 15 g/L; (3)= 3 days replacement interval; (7)= 7 days replacement interval; (14)= no replacement of chopped banana stem; (C+)= positive control (no banana stem added to rearing media). (C-)= negative control (no banana stem added to rearing media).

Table 5. Respiratory burst (O.D at 630 nm) and lysozyme activity (unit/mL) of tilapia after immersion (H+14) and post-challenge with *S. agalactiae* (H+4, H+8, and H+12)

Treatment	After immersion				After challenge test with <i>S. agalactiae</i>			
	H+14		H+4		H+8		H+12	
	RB	Lysozyme	RB	Lysozyme	RB	Lysozyme	RB	Lysozyme
C-	0.14±0.01 <sup>f</sup>	18.1±0.8 <sup>g</sup>	0.40±0.02 <sup>de</sup>	16.9±0.7 <sup>h</sup>	0.23±0.01 <sup>bc</sup>	20.5±2.9 <sup>g</sup>	0.19±0.00 <sup>de</sup>	22.0±1.9 <sup>b</sup>
C+	0.13±0.00 <sup>f</sup>	18.1±0.8 <sup>g</sup>	0.22±0.02 <sup>f</sup>	21.8±2.3 <sup>g</sup>	0.23±0.02 <sup>bc</sup>	22.8±0.2 <sup>fg</sup>	0.22±0.02 <sup>bc</sup>	31.6±0.9 <sup>g</sup>
A3	0.19±0.01 <sup>cd</sup>	45.8±1.9 <sup>c</sup>	0.25±0.03 <sup>f</sup>	31.7±2.4 <sup>de</sup>	0.21±0.01 <sup>de</sup>	31.6±2.2 <sup>cd</sup>	0.17±0.01 <sup>fg</sup>	44.9±3.2 <sup>b</sup>
B3	0.18±0.00 <sup>d</sup>	37.8±0.7 <sup>e</sup>	0.44±0.02 <sup>b</sup>	32.3±3.5 <sup>de</sup>	0.22±0.00 <sup>cd</sup>	26.9±1.7 <sup>ef</sup>	0.15±0.00 <sup>g</sup>	43.2±3.3 <sup>bc</sup>
C3	0.18±0.00 <sup>d</sup>	36.0±2.8 <sup>e</sup>	0.46±0.06 <sup>b</sup>	33.8±1.4 <sup>de</sup>	0.25±0.01 <sup>ab</sup>	28.4±2.5 <sup>de</sup>	0.16±0.01 <sup>fg</sup>	33.6±0.8 <sup>fg</sup>
A7	0.24±0.03 <sup>a</sup>	66.1±2.1 <sup>a</sup>	0.43±0.05 <sup>bc</sup>	59.5±2.1 <sup>b</sup>	0.20±0.00 <sup>e</sup>	32.9±2.8 <sup>c</sup>	0.21±0.01 <sup>cd</sup>	38.4±1.5 <sup>de</sup>
B7	0.22±0.01 <sup>b</sup>	53.2±1.8 <sup>b</sup>	0.24±0.01 <sup>f</sup>	35.2±2.7 <sup>d</sup>	0.21±0.01 <sup>de</sup>	37.3±3.0 <sup>b</sup>	0.19±0.00 <sup>de</sup>	37.2±1.0 <sup>def</sup>
C7	0.20±0.00 <sup>b</sup>	31.6±1.6 <sup>f</sup>	0.35±0.06 <sup>cde</sup>	44.2±3.3 <sup>c</sup>	0.22±0.01 <sup>cd</sup>	32.7±2.2 <sup>c</sup>	0.18±0.02 <sup>ef</sup>	35.1±1.8 <sup>efg</sup>
A14	0.16±0.00 <sup>e</sup>	37.5±0.9 <sup>e</sup>	0.58±0.02 <sup>a</sup>	72.7±1.4 <sup>a</sup>	0.25±0.00 <sup>ab</sup>	41.8±1.7 <sup>a</sup>	0.26±0.01 <sup>a</sup>	51.4±3.9 <sup>a</sup>
B14	0.18±0.01 <sup>d</sup>	32.2±1.3 <sup>f</sup>	0.28±0.01 <sup>ef</sup>	29.8±3.1 <sup>ef</sup>	0.26±0.01 <sup>a</sup>	35.1±3.5 <sup>bc</sup>	0.24±0.01 <sup>b</sup>	40.8±3.2 <sup>cd</sup>
C14	0.18±0.00 <sup>cd</sup>	42.9±2.7 <sup>d</sup>	0.41±0.01 <sup>bcd</sup>	27.4±1.8 <sup>f</sup>	0.26±0.01 <sup>a</sup>	34.7±2.9 <sup>bc</sup>	0.22±0.02 <sup>bc</sup>	36.6±2.5 <sup>ef</sup>

Data were expressed as mean  $\pm$  standard deviation. Different superscript on the same coloum showed significantly different ( $P < 0.05$ ). (A)= concentration 5 g/L; (B)= concentration 10 g/L; (C)= concentration 15 g/L; (3)= 3 days replacement interval; (7)= 7 days replacement interval; (14)= no replacement of chopped banana stem; (C+)= positive control (no banana stem added to rearing media). (C-)= negative control (no banana stem added to rearing media).

an increase up to 21% compared to the control at day-14 after immersion (Table 4).

Total leucocyte of tilapia at day-4 post-challenge with *S. agalactiae* experienced an average increase amounted to 27.3% of total leucocyte after immersion (Table 4). According to Hardi *et al.* (2011), infection of *S. agalactiae*

can cause fish sending more leucocyte cell to the area of infection as a defensive effort. The value of phagocytic activity in tilapia at day-4 after challenge test increased to 11.8% of phagocytic activity percentage after immersion. This increase occurred continuously to occur at day-12 after challenge test.

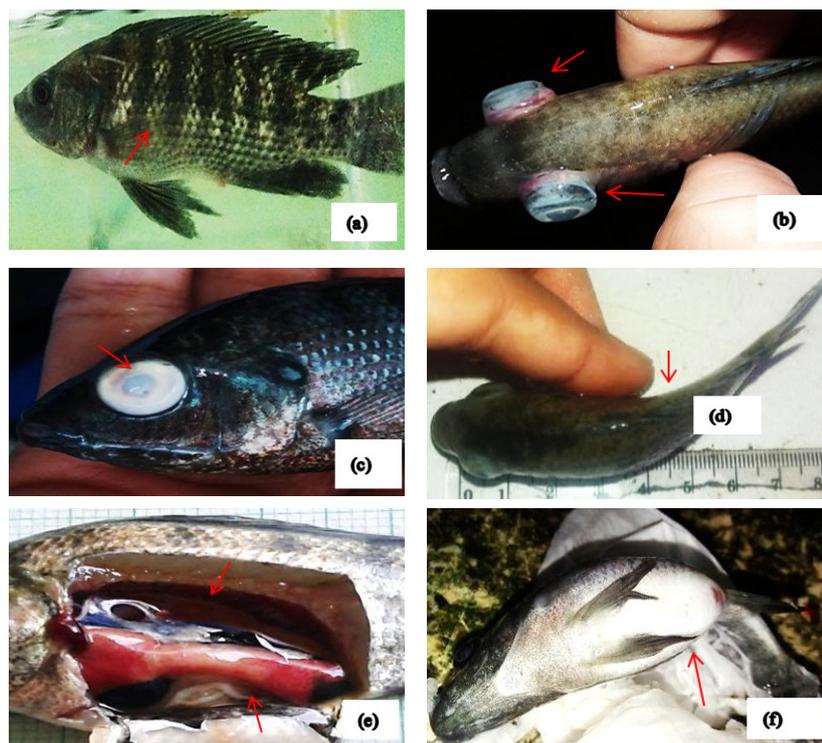


Figure 3. Clinical signs. (a)= melanosis; (b)= exophthalmia and corneal opacity; (c)= purulens; (d)= scoliosis; (e)= haemorrhage in the liver and gallbladder; (f)= dropsy.

### Respiratory burst and (RB) and lysozyme activity

The RB value increased to 43% compared to control at day-14 after immersion. Furthermore, lysozyme activity also had an increase to 57% compared to control at day-14 after immersion (Table 5). The RB value post-challenge at day-4 had an increase to 100% of the previous RB value, with treatment A-14 ( $0.58 \pm 0.02\%$ ) as treatment with the highest RB value ( $P < 0.05$ ). The increase of lysozyme activity also occurred in the highest activity was found at day-4, 8, and 12 after challenge test with *S. agalactiae*, that was A-14 treatment (concentration of 5 g/L and no replacement of chopped banana stem) which was  $72.7 \pm 1.4$ ,  $41.8 \pm 1.7$ , and  $51.4 \pm 3.9$ , respectively (Table 4). The addition of banana stem in the water may increase proliferation of leucocyte cell and the amount of leucocyte cell in secreting lysozyme, so the level of lysozyme will further increase (Nugroho *et al.*, 2016).

### Clinical signs

In this research, clinical signs observed in tilapia after challenge test with *S. agalactiae* were the same as reported by Li *et al.* (2013); and Jantrakajorn *et al.*, (2014). Those clinical symptoms were: whirling and fish did not actively swim, melanosis, exophthalmia, corneal opacity,

purulent, clear operculum, scoliosis, and dropsy.

High level of survival rate and immune response that was achieved after challenge test with *S. agalactiae* in each immersion treatment proved that there were an optimal concentration and frequency of chopped banana stem replacement obtained from the active compound in the banana stem. It was expected *S. agalactiae* that entered the fish body was failed to be virulence due to the active compound of alkaloid can have a function as antibacterial agent, able to disrupt the virulence gene regulation, inhibit sorase, disrupt fimbriae and other adhesions, inhibit bacterial defenses against the host immune system, inhibit secretion system of bacteria, inhibit the destructive enzyme-mediated effect, and inhibit exotoxin-mediated effect (Cushnie *et al.*, 2014). Antibacterial property is also found in the phenolic compound and it is able to inhibit the growth of bacteria through the secretion of inhibitor enzyme and oxidation of pathogen (Santangelo *et al.*, 2007). In addition, flavonoid plays a role as an anti-inflammatory agent by inhibiting the release of histamine from mast cell, inhibit biosynthesis of prostanoid, inhibit phosphodiesterases, inhibit protein kinases, and activation of transcription (Rathee *et al.*, 2009). Therefore, tilapia at day-12 after the challenge test was already in normal condition based on the data of total erythrocyte and hemoglobin (Table

3).

Concentration treatment of 5 g/L with no replacement of chopped banana stem (A 14) was the best treatment in this study. It was because treatment A 14 produced the higher survival rate and immune response, and also considered to be more efficient using the banana stem, it was 213.875 g as well as required less effort to provide the banana stem. In further study, it is required to test whether lower level of banana stem than 5 g/L allows higher immune response and fish survival, apply the results of this study in pond until fish reach a marketable size (about 200 g), to assess the durability period from the effect of banana stem active compound after immersion in the fish body and to assess the mechanism of active compound durability in the water, particularly the fish rearing media.

### CONCLUSION

The addition of the chopped banana stem into rearing media was able to affect immune response and survival of tilapia. Moreover, the concentration treatment of 5 g/L and no replacement of chopped banana stem was the best treatment.

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