

Post feeding protection from Bilimbi to motile *Aeromonas Septicemia* disease in African Catfish

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ABSTRACT

Bilimbi (*Averrhoa bilimbi*) is a medicinal plant used for the prevention and treatment of fish diseases. Therefore, this study aims to determine the protection period of bilimbi-fed with different duration to enhance fish immunity and protection from *Aeromonas hydrophila* infection. The Randomized Block Design Factorial (RBD) with 2 factors was used, where the first (A) was the period of feeding with bilimbi juice consisting of 3 treatments, namely 0 days (T0), 7 days (T1), and 14 days (T2). The second factor (B) was the protection period after the administration of bilimbi juice to those infected by *A. hydrophila* bacteria consisting of 3 treatments, 5 days (D5), 10 days (D10), and 15 days (D15). This research was conducted at the Laboratory of Aquaculture Experiment Pond, Faculty of Agriculture, Sriwijaya University in October-December 2019, and the parameters observed included prevalence of infection, growth, survival, feed efficiency, and water quality. The result showed that administration of 300 mL.kg⁻¹ of bilimbi juice with different periods significantly affected the period of fish protection for *A. hydrophila* infection. Furthermore, survival rate of fish before infection was not different significantly which was about 90-94%. However, post infection revealed that the best treatment was T2D5 with prevalence value and survival rate was 3.33% and 100% respectively. The water quality under experiment was 26-29.7°C for temperature, pH 6.8-7.7, dissolved oxygen 4.35-5.37 mg/L, and ammonia 0.09-0.21 mg/L.

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Introduction

Fish diseases are a major problem in aquaculture due to massive mortality and economic losses. A dead or moribund fish due to pathogen infection has a direct impact on market value. Commonly, fish diseases are caused by the interaction of three main factors which are fish immunity, pathogen, and environment. Therefore, the management of fish diseases in aquaculture activities should be applied as the implementation of good practices.

Hemorrhagic septicemia or *Motile Aeromonas Septicemia* (MAS) has been observed in many species of fish and is considered to be the main disease affecting freshwater. It is caused by a gram-negative bacterium, *Aeromonas hydrophila*, which has some virulence factors such as hemolysins, cytotoxins,

and proteases (Rasmussen-Ivey *et al.*, 2016). Mortality in fish varies from 60-100% in 48 hours until 2 weeks with different patterns depending on bacterial density and fish condition (Austin and Austin, 2016; Hayatgheib *et al.*, 2020; Zhang *et al.*, 2016). The mortality rate due to MAS infection can lead to economic losses in aquaculture, therefore, prevention and control of this disease should be implemented in fish production.

Moreover, the primary method in fish health management is prevention and treatment since the ethnomedicinal plant is an alternative to control disease in aquaculture. Furthermore, it can act as an immunomodulator and immunostimulant (Citarasu, 2010; Reverter *et al.*, 2017; Jana *et al.*, 2018). Bilimbi (*Averrhoa bilimbi*) is an example of herbs that can

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control MAS infection in several fish (Agustina et al., 2017; Khaerani et al., 2013; Mengkrin, 2019) since it is used as medicine and immunostimulant to control fish disease. Some bio-actives and vitamin C in bilimbi act as an antioxidant to protect fish from infection (Alhassan and Ahmed, 2016; Lim, 2012; Sonia, 2016), and continuous applications of herbs can also protect them. However, the protection value of herbs in fish is strongly influenced by doses, duration of exposure, and length of herbal attraction after exposure. As an immunostimulant, bilimbi stimulates non-specific immunity, the mode of action of which does not involve memory cells, and protection of the immune response occurs briefly (Mastan, 2015).

This study aims to determine the protection period of bilimbi after oral administration with different frequencies. Furthermore, it produces an effective and efficient usage of bilimbi in fish farming activities.

Materials and Methods

Location and time of research

This study was conducted in October-December 2019, located at Laboratory of Kolam Percobaan, Program study of Aquaculture, University of Sriwijaya.

The African catfish reared in circular tarps (2 m in diameter) about 8-10 cm in length, ripe bilimbi, commercial pellet, and *Aeromonas hydrophila* (collection from Laboratory of Aquaculture) were used. Other materials used include syringe 1 ml, juicer, spectrophotometer, pH meter, DO meter, and thermometer.

A Randomized Block Design Factorial (RBDF) with 2 factors was used, where the first (A) was the period of feeding with bilimbi juice consisting of 3 treatments, namely 0 days (T0), 7 days (T1), and 14 days (T2). The second factor (B) was the protection period after the administration of bilimbi juice to those infected by *A. hydrophila* bacteria consisting of 3 treatments, 5 days (D5), 10 days (D10), and 15 days (D15). All pellets were added with bilimbi juice 300 mL for 1 kg pellet, and the illustration of the study design is shown in Figure 1

Procedures

Production of Bilimbi pelleted

Pelleted bilimbi was made from commercial pellet then re-pelleting with bilimbi juice. First, the commercial pellet was crushed into a powder then mixed using 300 ml bilimbi juice for 1 kg. It was re-formed by using a meat grinder after making the dough, and the pellet was dried under sunlight.

Finally, it was stored in a jar until the day of the experiment (Agustina et al., 2017).

Analysis of vitamin C content, antioxidant activity (AA%) and phytochemical properties in bilimbi

Harborne (1987) showed the measurement of phytochemical properties including tannin, saponin, flavonoid, alkaloid, terpenoid, and steroid. Furthermore, antioxidant activity used the DPPH method (Molyneux, 2004) while vitamin C content used the Farmakope Indonesia method.

Fish rearing and challenge test

The stocking density of African catfish was 1 ind/L and fed three times per day with the *ad satiation* method. Before rearing, the fish was adapted with a commercial pellet which was already re-pelleting for 7 days, and the challenge test started from 5, 10, and 15 days after treatment. It used 10^6 CFU/ml *Aeromonas hydrophila* and was administered by intramuscular injection about 0,1 ml before observing the symptoms of MAS for 2 days.

Data analysis

Data of prevalence and mortality post-infection were analyzed statistically with analysis of variance (ANOVA) with a confidence interval of 95%. When the result of treatment was significantly different, the Least Significant Difference (LSD) was then used. However, data of phytochemicals, antioxidant activity, vitamin C, water quality, survival rate, and feed efficiency were analyzed descriptively.

Results

Phytochemical content of bilimbi, antioxidant and vitamin C assay are shown in Table 1. Survival rates during the experiment before infection are shown in Table 2, while the prevalence value and mortality rate of fish after infection are shown in Table 3.

Table 1. Data of Phytochemical, vitamin C and antioxidant in bilimbi juice.

Phytochemical analysis	Result
Alkaloid	+ (orange)
Steroid	+ (green)
Flavonoid	+ (yellow)
Tannin	+ (yellow)
Terpenoid	+ (dark red)
Saponin	+ (foaming)
Vitamin C (%)	2,29
AntioxidantActivity (%)	75,8%

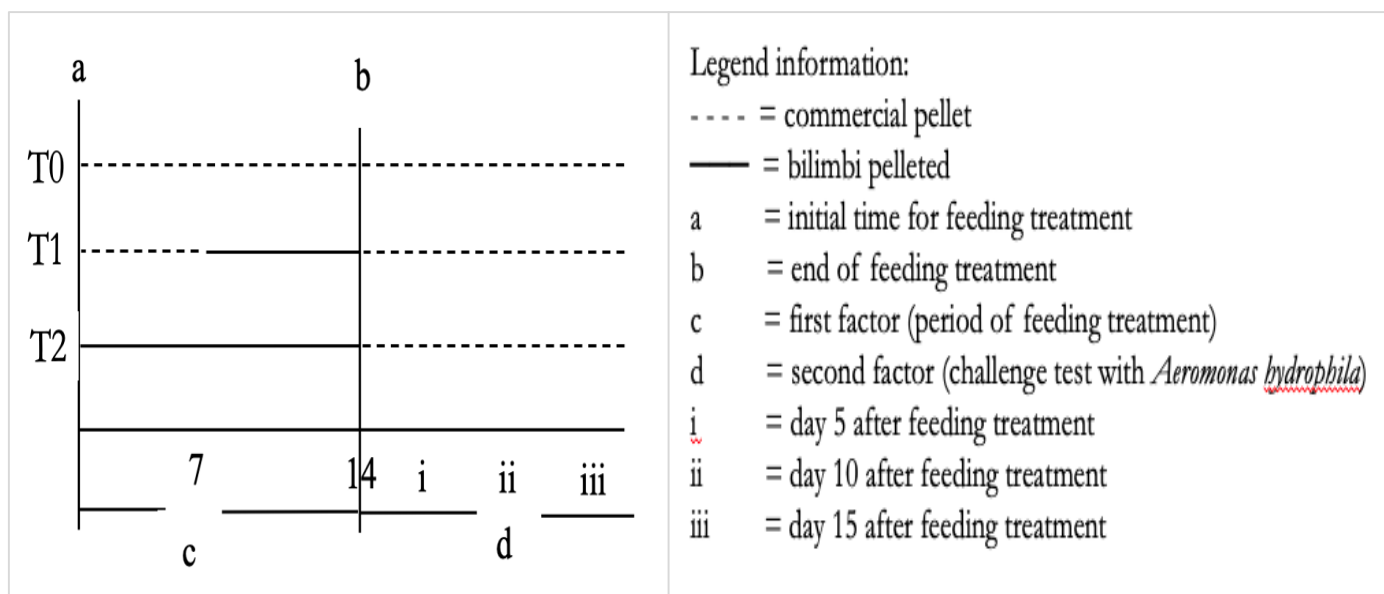


Figure 1. Experimental design of this research.

Water quality was monitored until a day before infection as shown in Table 4. The average value of the Feed Conversion Ratio (FCR) and Feed Efficiency (FE) are presented in Table 5. There was also a significant linear relationship between the duration of bilimbi-fed to fish and its protection. Furthermore, linear regression was used for forecasting the potency of duration protection from bilimbi for 7 days and 14 days (Figure 2).

Table 2. Survival rate (SR) of fish before infection.

Treatment	Initial	Final	SR (%)
T0	120	109	90.83
T1	120	111	92.50
T2	120	113	94.17

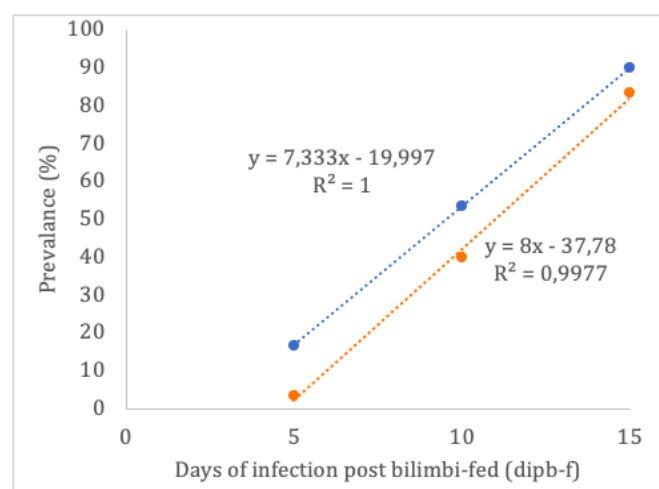


Figure 2. Regression linear forecasting for duration protection of bilimbi-fed.

Table 3. Prevalence number of fish post infection.

Factor A	Factor B	Prevalence (%) + SDEV
T0	D5	96.67 ^f ± 5.77
	D10	100.00 ^f ± 0.00
	D15	100.00 ^f ± 0.00
T1	D5	16.67 ^b ± 5.77
	D10	53.33 ^d ± 5.77
	D15	90.00 ^{ef} ± 10.00
T2	D5	3.33 ^a ± 5.77
	D10	40.00 ^c ± 10.00
	D15	83.33 ^e ± 5.77

Note: numbers are followed by difference letters on the same column are significantly different ($p \leq 0.05$)

Table 4. Water quality of fish rearing before infection.

Water quality	Treatments			Standard (SNI, 2014)
	T0	T1	T2	
Temp (°C)	26-29.6	26-29.7	26-29.3	25-30
pH	6.8-7.7	6.8-7.5	6.9-7.5	6.5-8
DO (mg/L)	4.48-4.56	4.35-4.83	4.96-5.37	> 3
Ammonia (mg/L)	0.13-0.19	0.09-0.17	0.1-0.21	≤ 0.1

Table 5. Feed Efficiency and Feed Conversion Ratio of bilimbi-fed and non-bilimbi-fed.

Parameter	Treatments					
	W0 (g)	D (g)	Wt (g)	F (g)	FE (%)	FC R
T0	851.6	96.3	1067.2	657.6	47.4	2.1
T1	845.9	84.1	1081.7	624.5	51.2	2
T2	850.2	72.5	1134.5	631.9	56.5	1.8

Discussion

The use of medicinal plants in aquaculture activities has been widely applied both when disease occurs and as a preventive measure (Reverter et al., 2017; Jana et al., 2018). However, long-term use has the potential to cause health problems in organisms. According to Fatima and Nayeem (2016), the opinion that medicinal plants do not have side effects is a myth. Therefore, its use for a certain period should be considered in the application. Likewise, when medicinal plants are no longer consumed, there is a breakdown time which affects the effect of these plants in the body lost. The use of medicinal plants should be consistent with the instructions for use, such as the duration and quantity allowed to be consumed.

Bilimbi (*Averrhoa bilimbi*) is an ethnomedicinal plant used as an immunomodulator and immunostimulant. Based on several studies, the compounds of bilimbi can be used as curative or preventive care on diseases control in fish and shrimp (Handayani et al., 2020; Prayogo et al., 2011). As an immunostimulant, bilimbi has vitamin C and antioxidant properties. The results showed that bilimbi juice consists of vitamin C about 2.29% and antioxidant about 75.8 %. Therefore, it can be used as a source of immunostimulants (Table 1). Antioxidant activity can be classified into three classes based on free radical value. The extracts that showed DPPH^{*} scavenging activity above 70% are considered to be effective, while those presenting activity between 60–70% is classified to have moderate action. Furthermore, the extract showing lower than 60% activity is considered to be of poor antioxidant activity (Boylan et al., 2015). The high antioxidant content of bilimbi was also reported in some studies (Chowdhury et al., 2012; Suluvoy and Berlin Grace, 2017). Vitamin C plays a role in maintaining the immune system of fish and has been widely used in aquaculture activities (Dwinanti and Fitriani, 2016; Dwinanti and Sasanti, 2019; Ngasainao et al., 2017). However, the use in inappropriate amounts can cause health problems for fish and increase production costs (Dawood and Koshio, 2016).

According to protection data generated (Table 3), pellet containing bilimbi juice provides better protection than those that are not given. This is due to the high content of vitamin C and the antioxidants of bilimbi. In addition, the length of time for feeding containing bilimbi influences protection value after it was stopped. The feeding of Bilimbi-fed fish for 7 days only protects about 50% of the population when infected by *Aeromonas*

hydrophila in 10 days post-feeding. Meanwhile, 15 days post-feeding was not significant with control and were not able to protect. Based on regression equation $y=7.333x-19.997$, the ability of bilimbi to protect fish 100% from infection of *A. hydrophila* can be assumed in three days post-feeding. Moreover, feeding bilimbi for 14 days can protect fish longer than 7 days. Based on the regression equation $y = 8x-37.78$, the best protection time is 5 days. Therefore, the duration of protection is strongly influenced by the length of time of exposure and the number of bilimbi in the fish body.

The effect of vitamin C and antioxidants of bilimbi for fish growth and feed efficiency is not significantly different between treatments. Therefore, the dose and duration of feeding containing bilimbi did not give negative effect to growth. Furthermore, the need for vitamin C in catfish is very dependent on its life stage and at the right dose. The growth will only be accelerated at the right dose, on the contrary, the health will be compromised (Dey et al., 2019; Gbadamosi et al., 2013; Okhionkpanwonyi and Edema, 2017).

Bilimbi feed does not affect the addition of organic matter (ammonia) in the water. This shows that bilimbi does not decrease water quality which can affect the health or growth of catfish. Similarly, Standar Nasional Indonesia's (2014) study showed that the water quality during catfish farming was in good condition.

Conclusion

The duration of feeding bilimbi to catfish has a significant effect on the length of time to protect catfish against *Aeromonas hydrophila* infection. Feeding with bilimbi for 14 days can protect fish from *A. hydrophila* infection until 5 days when it is stopped with a protection value of 96.7%. Therefore, feeding catfish with bilimbi is suggested for 14 days within an interval of 5 days. This can be used as a reference to protecting catfish from *A. hydrophila* infection.

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