Original Research Article

Effectiveness Of Adding Garlic Extract (*Allium sativum*) In Commercial Feed To The Resistance Of Nilem Fish (*Osteochillus hasselti*) Infected With *Aeromonas hydrophila*

ABSTRACT

Determining the concentration of garlic extract addition in commercial feeds that are effective for increasing the body's resistance and survival of nilem fish to the attack of *Aeromonas hydrophila* bacteria. This study was carried out at the Aquatic animal physiology laboratory of building 2 and the biotechnology laboratory, faculty of fisheries and marine sciences, Universitas Padjadjaran, between November and December 2021. This study used the Complete Randomized Design (CRD) method with five treatments and three tests. The treatment used is the addition of garlic extract to commercial feed at doses: 0 g / kg of feed (A), 10 g / kg of feed (B), 20 g / kg of feed (C), 30 g / kg of feed (D), and 40 g / kg of feed (E). The treatment period is carried out for 21 days. Furthermore, nilem fish were tested with *Aeromonas hydrophila* bacteria with a density of 10^8 CFU / ml by intramuscular injection. The results showed that the addition of garlic extract to the feed did not produce a noticeable influence on the survival value of fish. However, the administration of garlic extract into the feed of 20 g / kg affects the health of the fish body in the form of a white blood cell count of 12800 cells / mm^3.

Keywords: Nilem carp; Aeromonas hydrophila; extract garlic; immunostimulants.

1. INTRODUCTION

Nilem carp (Osteochilus hasselti) is adored by most people. However, the cultivation of nilem carp is almost abandoned and has not been carried out intensively. The decreased cultivation of nilem carp is caused by the high mortality rate caused by diseases from bacteria and parasites [1].

MAS (Motile Aeromonas Septicemia) or red spot disease caused by the pathogenic bacterium Aeromonas hydrophila. This disease is one of the obstacles that hinder fish cultivation. The percentage of mortality due to *Aeromonas hydrophila* bacteria ranges from 80-100% [2].

One of the disease control attempts is prevention. Immunostimulant is a substance that can increase the body's immune system. The natural substance could be used as an immunostimulant. Garlic is one of the natural immunostimulants.

The use of garlic as the immunostimulant is based on the contained compounds in garlic named allicin. Allicin in garlic can significantly improve the fish’s immune system [3].

2. MATERIALS AND METHODS

2.1 Time and Place

This research was carried out at the Aquatic animal physiology laboratory of building 2 and the biotechnology laboratory, faculty of fisheries and marine sciences, Universitas Padjadjaran. Data collection for this research was conducted from November – December 2021.

2.2 Material and Tools

The tools used in this research are an aquarium, aerator, heater, spray bottle, microscope, hemocytometer, and hand counter. The materials used in this study were nilem carp seeds with a size of 7-9 cm, commercial feed PF-1000, aquadest, Hayem's solution, Turk's solution, and garlic.

2.3 Research Procedure

The first thing to do in this research is feed test preparation, and garlic extract was weighed according to each treatment, then the extract was thawed with distilled water. Afterward, the thawed garlic extract was placed through the spray bottle. The next step is spraying the garlic extract into the feed distributed evenly, then drying the feed that has been sprayed with the garlic extract at room temperature. Dried feed mixed with the garlic extract is given to the fish as much as 5% of the fish's body weight and given 2 times a day at 08.00 am and 4.00 pm Indonesian Time Zone. Fish were reared with feed mixed with garlic extract for 21 days.

After 21 days of rearing, a challenge test with Aeromonas hydrophila with 10^8 CFU/ml density was carried out. The challenge test was carried out by injecting 0.1 ml of bacteria per fish. Fish were injected intramuscularly [4].

Observations of clinical symptoms were carried out for 14 days after the challenge test. The visible clinical symptoms were damage to the fish's body, feed response, and shock response.

White blood cells number were observed by slashing the caudal vein of the fish until the blood came out. The blood was sucked with a Sahli pipette to 0,5 scale, followed by sucking the Turk's solution to 11 scales, then shaking it until homogeneous. Furthermore, it was dropped on a hemocytometer and observed under a microscope with ten times magnification while counting the number of white blood cells.

Red blood cells number were observed by slashing the caudal vein of the fish until the blood came out. The blood was sucked with a Sahli pipette to 0,5 scale, followed by sucking the hayem solution to 101 scales, then shaking it until homogeneous. Furthermore, it was dropped on a hemocytometer and observed under a microscope with ten times magnification while counting the number of red blood cells.

2.4 Method

The method used in this research is an experimental method using a completely randomized design (CRD) with five treatments and three replications, i.e., A (Control), B (10g/kg), C (20g/kg), D (30g/kg), and E (40g/kg). The used treatment is the garlic extract used in the mixed feed as the immunostimulant.

The general model of a completely randomized design is the linear model [5] as follows:

$$Y_{ij} = \mu_i + \tau_i + \varepsilon_i \text{ or } Y_{ij} = \mu_i + \varepsilon_i$$

Description:
i = 1,2, ..., t
j = 1, 2,..., r
Y_{ij} = observations on the i treatment and j repetition
μ = general average
τ_i = effect of the i treatment
ε_i = random effect on the i treatment and j repetition

2.5 DATA ANALYSIS

An increasing number of white blood cell and red blood cell data of nilem carp were analyzed by the F test, and if there was a difference between the treatments, it was analyzed by Duncan's test with a 95% confidence level. Clinical symptoms, water quality, and survival rate data were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 white blood cell number

Based on the results and examination of the health condition of the fish using white blood cells during rearing, it was seen that there were differences after 21 days of rearing and post-challenge test. The performance of white blood cells is shown in Figures 1 and 2.

Figure 1. White blood cell numbers before and after the treatment

Figure 2. White blood cell number after the challenge test

The number of white blood cells during the 21 days rearing period with garlic extract feed shows that the white blood cells were in the normal range. The number of white blood cells after adding garlic extract to the feed is shown in figure 1 in the range of 86833 – 12800 cells/mm^3, while the average number of white blood cells is 3390 – 14200 cells/mm^3 [6]. The increasing white blood cells can be an indicator of an increasing nonspecific immune system [7].

After the challenge test, the number of white blood cells in each treatment increased, especially in treatment A (control) (figure 2). An increase in white blood cells after a challenge test with Aeromonas hydrophila bacteria indicates a response to the body's resistance to disease-causing antigens. The number of white blood cells will increase when the fish is experiencing an infection because white blood cells are an active unit in the body's defense system. The white blood cells have a role in fighting against foreign objects in the body, one of which is bacteria (Yanto et al., 2015)[8].

There was an increase in the number of white blood cells at the B, C, D, and E treatment, which was higher than the control. When infected with Aeromonas hydrophila, White blood cells cannot fight the attack of bacteria, so to fight it by producing white blood cells. This caused the white blood cell count in treatment A after being challenged to be higher than in other treatments (Nursatia et al., 2017) [9]. The primary function of white blood cells is to fight and protect the body from infection by phagocytosing foreign organisms and producing and distributing antibodies throughout the body. Therefore, animals with low white blood cells have a high risk of attack by infectious diseases, while animals with high white blood cells can generate antibodies in the phagocytosis process and have high resistance to disease (Soetan et al., 2013; Isaac et al., 2013) [10][11].

3.2 Red blood cell number

Based on the results and examination of the health condition of the fish using red blood cells during rearing, it was seen that there was a difference in values after 21 days of rearing and post-challenge testing. The performance of red blood cells is shown in Figures 3 and 4.

Figure 3. The number of red blood cells before and after treatment

Figure 4. The number of red blood cells before and after treatment

The number of red blood cells in the 21-day rearing period with adding garlic extract to the feed showed that it was in the normal range. The value shown after adding garlic extract to the feed in Figure 3 is in the range (2.14 – 2.60)x106 cells/mm3, while the normal range of red blood cells for teleost fish is 1.05×106 ± 3.0× 106 cells/mm3 (Irianto 2005). This shows that feeding containing phytopharmaca does not interfere with the health of the test fish (Fauziah and Wahjuningrum, 2015) [12].

After the challenge test, the number of red blood cells in Figure 2 seems to have decreased. This happens because the bacterial attack resulted in the rupture of the fish's blood vessels, decreasing the number of red blood cells. This decrease in red blood cells was caused by bleeding after being infected with A. hydrophila bacteria causing the fish to experience anemia (Dianti et al., 2013) [13]. In addition, it is also suspected that red blood cells undergo lysis caused by A. hydrophila bacteria. These bacteria have a toxic, namely hemolysin. Hemolysin in A. hydrophila bacteria can cause red blood cells to undergo lysis Haditomo (2017) [14]. While the decrease in the number of red blood cells

of fish given the addition of garlic extract was relatively low, this value indicated that the fish were undergoing healing. This is evidenced by the damage to the fish body that is not too severe. According to Feldberg et al. (1988) [15], allicin compounds are antibacterial so that they can fight pathogenic bacteria. In addition to garlic, allicin contains the enzyme gramanium. The enzyme gramanium is a substance that can prevent the destruction of red blood cells, so when the challenge test was carried out on treated nilem fish, there was no significant decrease in the number of red blood cells.

3.3 Macroscopic clinical symptoms

3.3.1 Damage to the body of the nilem fish

Clinical symptoms on the fish's body began to appear in the 5th hour after the challenge with Aeromonas hydrophila bacteria. Then during the 24-hour observation after the challenge test, the symptoms that appeared were peeling scales (Figure 5a), abdominal bulge (Figure 5b), bleeding (Figure 5c), and inflammation (Figure 5d). There is damage to the body of fish infected with Aeromonas hydrophila bacteria in the form of wounds and bleeding caused by extracellular toxins that work in collaboration to damage the tissues in the fish body. Hemocillin produced by these bacteria works to break down and lyse red blood cells (Mangunwardoyo 2016) [16].

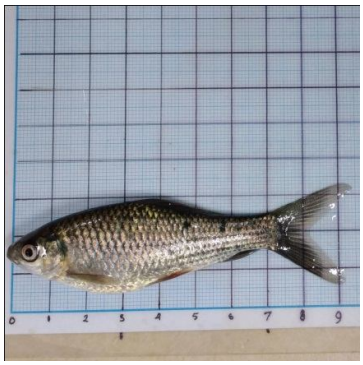

figure 5a. Healthy nilem fish

Figure 5b. peeling scales

figure 5c. *dropsy*

Figure 5d. *hemorrhagic*

figure 5e. inflammation

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3.3.2 Shock response

Observation of fish response to shock was carried out 14 days after the challenge of A. hydrophila bacteria. The goal is to determine the reflexes of fish movement to stimuli in the form of surprise. One of the clinical symptoms of fish infected with A. hydrophila bacteria is behavioral changes, such as a decreased response to shock. Fish are more often clustered around aeration and become weak. Prayitno (2014)(b) [17] . Based on the results of observations, the response of the test fish to shock varied for each treatment (Table 1).

Table 1. Results of Observation of Responses to Surprises

Days	Treatment														
	A			B			C			D			E		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+
3	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	M	+	+	+	+	+	+	+	+	+	M	M	+	M
7	+	M	+	+	+	+	+	+	+	+	M	M	M	M	M
8	+	M	M	+	+	++	+	++	M	M	M	M	M	M	M
9	+	M	M	+	+	++	+	++	M	M	M	M	M	M	M
10	M	M	M	M	M	++	++	++	M	M	M	M	M	M	M
11	M	M	M	M	M	++	++	++	M	M	M	M	M	M	M
12	M	M	M	M	M	++	++	++	M	M	M	M	M	M	M
13	M	M	M	M	M	++	++	++	M	M	M	M	M	M	M
14	M	M	M	M	M	++	++	++	M	M	M	M	M	M	M

Description: (-) No response; (+) Response to shock is low; (++) Response to shock is normal; (M) Dead

Based on Table 1, it can be seen that on the first day of observation, the test fish in all treatments did not give a surprise response. The decrease in response occurred after the test fish were infected with A. hydrophila bacteria, which fish indicated at the bottom of the aquarium and clustered close to aeration. The fish swam abnormally, namely, swimming sideways.

3.4 LIFE SUSTAINABILITY

Observation of the survival of the test fish was carried out for 14 days after the challenge test with A. hydrophila bacteria. The results of the analysis of variance showed that the administration of garlic extract in the feed was not significantly different. Because the results showed no significant difference, it could not be continued with Duncan's follow-up test. However, it resulted in varying survival in each treatment (Figure 6).

Figure 6. Graph of Nilem Fish Survival After Challenge Test

Based on Figure 6, treatments A (control) and E (40 g/kg feed) showed a low survival rate of 0%. Treatment A gave the lowest yield because it proved that the fish's natural body resistance was low. This can happen because treatment A's immune system is not stimulated by the stimulant ingredients found in garlic extract. As a result, the fish cannot resist attacks from the bacteria A. hydrophila. Antibacterial compounds found in garlic extract are thought to affect the survival of nilem fish infected with A. hydrophila bacteria. The active compounds contained among them are saponins, flavonoids, quinones, alkaloids, terpenoids, steroids, and phenols. Flavonoids function as antimicrobials by inhibiting the formation of bacterial energy through respiration so that bacterial growth stops, and saponins reduce the surface tension of bacterial cell walls and damage membrane permeability (Andriani et al., 2017) [18]. Treatment D (30 g/kg) and E (40 g/kg) gave low survival rates of 2.22% and 0%, respectively. This is because the higher the dose, the suppression of the defense mechanism will occur, becoming a suppressor. Because of this, the fish's body defense system is less than optimal and does not function properly. According to Johnny et al. (2017) [19].

Treatment C presented a high survival rate compared to other treatments, namely 6.67%. When viewed from the parameters of damage to the body of nilem fish, treatment C showed less severe damage and healing that was gradually improving compared to other treatments. This is due to the allicin compounds acting as immunostimulants to increase the immune system of fish infected with A. hydrophila bacteria. The increase in fish's immune system in the treatment given garlic extract is known from the survival value of fish at the end of the study compared to the treatment not given garlic extract, which has a low survival value [9].

3.5 water quality

Observation of water quality was carried out for 21 days of maintenance period of feeding added with garlic extract. The measured water quality samples included temperature measurements, dissolved oxygen (DO), and pH. Water quality measurement data was used as a supporting parameter during the research. The results of water quality observations during the maintenance period are presented in Table 2.

Table 2. Results of Observation of Water Quality During Research

treatment (mL/kg)	Water quality parameter		
	temperature (^{o}C)	DO (mg/L)	pH
A (control)	26 – 27,20	5,70 – 7	6,70 – 6,80
B (10)	25,30 – 27,30	5,60 – 7,10	6,80 – 7
C (20)	27 – 29,50	5,10– 7,20	6,70 – 7,10
D (30)	26,20 – 27,40	5,40 – 7,90	6,80 – 7
E (40)	26,90 – 27,60	5,60 – 7	5,60 – 6
Quality Standard Parameters *	28 – 32^{o}C	$\geq$ 5 mg/L	6,8 – 8,5

* Government Regulation No. 82 (2001) for freshwater fish farming activities (class II)

Table 2 shows that the results of water quality measurements in the form of dissolved oxygen (DO) have an average value in the range of 5 – 7 mg/L, pH ranges from 6 – 7, and an average temperature range of 25 – 29°C. Based on measurements and observations of water quality during the study, adding garlic extract to feed did not adversely affect water quality. This can be seen from the value of the water quality range, which does not exceed the quality standard limits according to PP No. 82 of 2001 [20].

4. CONCLUSION

Based on the research that has been carried out, it can be concluded that:

1. Addition of garlic extract to commercial feed is practical to increase fish's immune system in the form of white blood cells, which is 12800 cells/mm^3.

2. After the challenge test, the fish experienced high mortality, in this case, due to insufficient white blood cells to fight the bacterial attack. The energy generated from adding garlic extract was still used for adaptation, so the survival value produced was only 6,67%.

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