The Level Difference of Survival Rates and Prevalence of Carp (Cyprinus carpio) Seeds Which is Infected

by Citra Panigoro

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The Level Difference of Survival Rates and Prevalence of Carp (*Cyprinus carpio*) Seeds Which is Infected by *Aeromonas hydrophila*by Addingof Binahong (*Anredera Cordifolia*)Leaf Flour in the Feed

Juliana [1], Ira Panigoro [2], Yuniarti Koniyo[3]
[1,3]Aquaculture, Faculty of Fisheries and Marine Science
State University of Gorontalo
[2]Management of Aquatic Resources, Faculty of Fisheries and Marine Science
State University of Gorontalo

Email: juliana@ung.ac.id

ABSTRACT

Aeromonas hydrophila bacteria is a type of bacteria found in freshwater and can infect freshwater fish that are cultivated. Prevention of bacterial infections in freshwater fish farming can be done by administering antibacteria derived from active compounds from artificial and natural chemicals. One of the plants that contain anti-bacteria is the leaves of binahong (*Anredera Cordifolia*). Therefore it can be used as an antibacteria in freshwater fish farming. This study aims to analyze differences in the survival rate of seeds and the prevalence of Carp seeds infected with Aeromonas hydophila by adding binahong leaf flour to the feed. The treatment in this study is the difference in the percentage of the addition of Binanong leaf flour (*Anredera Cordifolia*) in the manufacturing of feed. It consists of four treatments namely treatment A (5%), treatment B (7%), treatment C (9%), and treatment D (control). Samples in the form of carp seeds measuring ± 5 cm which has been infected with *Aeromonas hydrophila* and has shown symptoms of infection, maintained with a density of 2 fish seeds per litre. Data analysis used is Anova one-factor analysis to determine differences in survival rates and prevalence of carp seeds based on the treatment given. The results showed that the highest survival rate of carp seeds was in C treatment, while the lowest survival in treatment A. The lowest prevalence was obtained in treatment C, while the highest prevalence in treatment D.

Keywords: *Aeromonas*, Binahong, Prevalence, Survival Rate.

Introduction

The disease is one of the factors that greatly influence the success of aquaculture in addition to feeding. Diseases in fish are generally caused by bacterial, viral, parasitic attacks and poor environmental quality. The fish disease is anything that can cause both physical and physiological disorders in fish.

Bacterial infections in fish can be caused by disharmony that occurs in the environment or culture media where the fish are cultivated. Inadequate or unsuitable environmental conditions will cause stress on the farmed fish, causing disruption. Conditions and consequences of fish are easily infected by bacteria which reduce the quality and quantity of production in freshwater fish farming activities.

Aeromonas hydrophila is a type of pathogenic bacteria that infects much freshwater fish whichcauses health problems. Fish infected with *Aeromonas hydrophila* can reduce the quality and quantity of freshwater fish farming. Control and treatment of *Aeromonas hydrophila* bacterial infections are still being developed. Prevention and treatment carried out so far are using antibiotics derived from chemicalsbut are considered to be less effective because they can cause residues on the culture media that result in a decrease in environmental quality. The use of active compounds derived from plants is one alternative that is very well developed because it does not cause residues that can reduce the quality of cultivation media.

One way that is considered quite good in dealing with and preventing the attack of *Aeromonas hydrophila* is to use a variety of herbs that have active compounds that can function as antibacteria. One of the studies that have been done is analyzing the healing potential of medicinal plant extracts in fish infected by *Aeromonas hydrophila*(Thiyagarajan, Bhavani, Ebbie, & Chandra, 2014)..

Research conducted by Ali, *et al* (2014) states that one of the ectoparasites that often attacks freshwater fish is *Aeromonas hydrophila*. In connection with these problems, there need to be alternative safer drug ingredients that can be used in controlling fish diseases. One alternative is to use traditional medicinal plants that are anti-parasitic, anti-fungal, anti-bacterial and anti-viral. Some of the advantages of using traditional medicinal plants include being relatively safer, easily obtained, inexpensive, does not cause resistance and is relatively harmless to the surrounding environment.

Several studies have also been carried out on the use of plants to overcome bacterial attacks on freshwater fish farming. Based on the results of the study, it is

stated that the content of active compounds possessed by some plants in the form of flavonoid compounds, steroids and tannins can function as antibacterial in aquaculture. Dragon fruit plants also contain antibacterial compounds derived from dragon fruit peel extract (*Hylocereus costaricensis*) and it has been tested to determine the effect of giving dragon fruit skin extracts to the histopathology of tilapia liver (*Oreochromis niloticus*) infected with *A. hydrophila*(Masfia Ifatul, *et al*. 2018.). Other studies that have been carried out also are the effect of the roots of *Withania somniferaL. Dunal* as a food additive on immunological parameters and disease resistance to *Aeromonas hydrophila* on the seeds of Labeo rohita fish (Sharma, Deo, Tandel Riteshkumar, Chanu, & Das, 2010).

Binahong leaves are one of the natural ingredients that can be used to treat infections in fish caused by *A. caviae* bacteria because binahong leaf extracts (*A. cordifolia*) contain flavonoid compounds that can function as antioxidants in plants, alkaloids, and polyphenol compounds. This study aims to determine the effect of the administration of binahong (*A. cordifolia*) leaves in feed on the survival of carp (*C. carpio*) seeds infected with *Aeromonas hydrophila* bacteria.

Research Method

The research method used is the experimental method by conducting maintenance of carp seeds infected with *Aeromonas hydrophila* bacteria. The study design used a randomized complete design with four treatments and three replications. The treatment consists of different dosages of leaves of binahong (A. *cordifolia*) added to the manufacture of feed. Feed is made by adding binahong leaf flour with different doses, namely treatment A (5% per kg of feed), B (7% per kg of feed), C (9% per kg of feed) and D (control without the addition of binahong leaves) . Feeding is carried out three times with a dose of 10% of the total body weight per day.

The carp seeds (*C. carpio*) used are ± 5 cm in size and are kept in an aquarium with a volume of 18 liters and filled with as much as 12 liters of water with a density of two fish seeds per liter. Carp seeds (*C. carpio*) are first infected by injecting *Aeromonas hydrophila* bacteria obtained from pure cultures that have

been diluted. Fish seeds that have been infected with *Aeromonas hydrophila* bacteria are left for 24 hours and observations are made on the morphological changes in carp seeds. Seed that has been infected with *Aeromonas hydrophila* will show symptoms in the form of slow motion, loose scales, woundson the surface of the skin. The seeds will be used as test animals in research. Carp seeds that have shown symptoms of infection are kept for eight weeks and given food according to treatment as much as 10% of total body weight with frequency of feeding three times a day.

Data to be analyzed in this study are the survival rate and prevalence obtained from the maintenance of test animals for eight weeks. The data obtained were then analyzed using an analysis of one-factor variants to determine differences in survival rates and the prevalence of Carp seeds in each treatment. The equation used to calculate survival and prevalence is as follows (Kabata, 1985):

$$SurvivalRate(\%) = \frac{amountoffishmaintained}{theamountofdeadfish} x100$$

$$Prevalence(\%) = \frac{amountoffishinfectedbybacteria}{amountoffishanalysed} x100$$

Result and Discussion

Survival Rate

Carp seeds that have been infected with *Aeromonas hydrophila* are maintained for eight weeks to calculate the survival rate of carp seeds (*C. Carpio*). The results showed that the survival rate of carp seeds that had been treated with the addition of binahong leaf flour with different percentages in the feed showed differences in the average value of the survival rate of carp seeds (Table 1).

Table 1. Descriptive Analysis of the Survival Rate of Carp Fish

Descriptives

Survival Rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Treatment A	8	68.75	17.474	6.178	54.14	83.36	50	100
Treatment B	8	89.38	7.289	2.577	83.28	95.47	80	100
Treatment C	8	98.75	3.536	1.250	95.79	101.71	90	100
Treatment D	8	87.50	11.339	4.009	78.02	96.98	75	100
Total	32	86.09	15.332	2.710	80.57	91.62	50	100

The average survival value of carp seeds in each treatment was obtained in the range of 68.75 - 98.75%. Descriptive analysis results showed that the highest average value was found in treatment C, while the lowest survival was found in treatment A. The difference in the percentage of binahong leaf flour given in feed that was given descriptively showed different values. The average survival rate obtained during maintenance was eight weeks, then analyzed using a one-factor variance analysis to determine differences in survival between treatments (Table 2).

Tabel 2. Homogeneity Test Results for Survival Rate Carp (*C. Carpio*) Seeds

Test of Homogeneity of Variances

Survival Rate

Levene Statistic	df1	df2	Sig.
6.392	3	28	.002

The analysis shows that the value of Levene Statistics is 6.39 with a significance of 0.002. The significance value obtained is smaller than 0.05, it can be concluded that the treatment variants compared to show a difference between treatments. Data analysis was continued using one-factor ANOVA to test

statistically, to find out the difference in mean survival between treatments (Table 3).

Tabel 3. ANOVA Results from One Factor for Survival Rates of Carp (*C. Carpio*) Seeds.

ANOVA

Survival Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3789.844	3	1263.281	10.115	.000
Within Groups	3496.875	28	124.888		
Total	7286.719	31			

The analysis showed that the significance value obtained was 0.000 <0.05.So it can be concluded that the average survival rate for the four treatments given was significantly different. The analysis results were continued using the Tukey test to find out which treatment I differed in (Table 4).

Tabel 4. Tukey Test Results for Survival Rate of Carp (*C. Carpio*) Seeds.

Survival Rate

Tukey HSD

		Subset for alpha = 0.05	
Treatment	N	1	2
Treatment A	8	68.75	
Treatment D	8		87.50
Treatment B	8		89.38
Treatment C	8		98.75
Sig.		1.000	.207

Means for groups in homogeneous subsets are displayed.

The analysis shows that treatment A is in subset 1, while treatments B, C and D are in subset 2, it can be concluded that treatment A shows a significant difference to the other three treatments. The results also showed that treatments B, C and D did not show any significant difference between the three treatments.

Prevalence

Prevalence was analyzed to determine the level of attack of bacteria or parasites that infect aquaculture organisms. The results of the analysis of the average prevalence in this study indicate descriptively thatgives a different average value for each treatment (Table 5). The lowest prevalence value was obtained in treatment C, while the highest value was obtained in treatment D. The prevalence value indicates the level of Aeromonas hydrophila bacteria found after treatment. So from this value, it can be concluded that treatment A is the best treatment used for the handling of carp seeds that are infected with *Aeromonas hydrophila*.

Table 5. Results of Descriptive Analysis of Fish Carp (*C. carpio*) SeedsPrevalence

Descriptives

Prevalence

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
Treatment A	8	37.50	33.166	11.726	9.77	65.23	0	100
Treatment B	8	20.62	35.096	12.408	-8.72	49.97	0	100
Treatment C	8	17.50	34.949	12.356	-11.72	46.72	0	100
Treatment D	8	48.12	38.908	13.756	15.60	80.65	0	100
Total	32	30.94	36.132	6.387	17.91	43.96	0	100

Prevalence level analysis results obtained, then analyzed using a homogeneity test to determine differences in the average value of prevalence at each treatment (Table 6). The results of the analysis show that the value of the statistical Levene is 0.345 with a significance value of 0.739. The resulting significance value is greater than 0.05. It can be interpreted that the four treatments being compared are different. To find out the difference in the mean values of the four treatments given, then proceed with Anova (Table 7).

Table 6. Homogeneity Test Results Prevalence of Carp (*C. Carpio*) Seeds

Test of Homogeneity of Variances

Prevalence			
Levene Statistic	df1	df2	Sig.
.345	3	28	.793

Table 7. ANOVA Results of One Factor in Prevalence of Carp (*C. Carpio*) Seeds

ANOVA

Prevalence					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5003.125	3	1667.708	1.317	.289
Within Groups	35468.750	28	1266.741		
Total	40471.875	31			

The results of one factor ANOVA show that the significance value produced is 0.289 greater than 0.05, so it can be concluded that the average prevalence does not show a significant difference in the four treatments compared. The results of the analysis were also proven through the Tukey test to find out the differences between the treatments compared (Table 8). The results of Tukey's analysis prove that the average prevalence of the four treatments did not show any difference, all treatments were in subset 1.

Table 8. Tukey Test Results Prevalence of Carp (*C. Carpio*) Seeds.

Prevalence

Tukey HSD		
		Subset for alpha = 0.05
Treatment	N	1
Treatment C	8	17.50
Treatment B	8	20.62
Treatment A	8	37.50
Treatment D	8	48.12
Sig.		.332

The results showed that the effect of the addition of binahong leaf flour to feed manufacturing on carp seeds infected with *Aeromonas hydrophila* descriptively gave different values. Binahong leaves contain active compounds that can function as antioxidants against bacteria infected with fish. The results of research on the minimum bacterial concentration test showed that the higher the concentration of a given binahong leaf, the higher the inhibitory power produced was based on the test. This is due to the greater concentration of binahong leaf extract.The higher the content of anti-bacterial compounds that can help inhibit bacteria (Raka, Kartika, & Andayani, 2016).

Some studies also show that the content of active compounds that can inhibit bacterial infections is owned by several types of plants. Research on the use of extracts of *Andrographis paniculata* and *Azardirachta indica* as alternative ingredients in the process of healing diseases caused by bacteria is also proven (Thiyagarajan *et al.*, 2014). The chemical compounds in the form of terpenoids, steroids, flavonoids and alkaloids contained in extracts of *Avicennia sp* bark extract are also potential to be used as alternative materials to inhibit the attack of pathogenic bacteria that can attack freshwater fish (Ali Alimuddin *et al,* 2018). Meniran leaf has good potential as a natural ingredient that can function as a substitute for antibiotics, besides meniran leaf extract can be used as an immunostimulant material for disease prevention efforts due to bacterial and viral infections in aquaculture (Supriyadi & Iftitah, 2009).

Bacteria-infected carp seeds have decreased quality and have an impact on the growth and survival rate of fish. In addition to fish survival, the prevalence also needs to be known to determine the inhibition or speed of healing from the use of natural ingredients that contain active compounds given to fish seeds infected with bacteria, parasites or viruses. Fish infected with the bacterium *Aeromonas hydrophila* will show clinical symptoms, such as bleeding on the base of the fins or on the skin, distended abdomen, protruding eyes, usually referred to as hemorrhagic septicemia caused by Aeromonas sp. The results of the study conducted by Wassif, 2018, stated that *Oreochromis niloticus* and *Ictalurus punctatus* were naturally infected with bacteria showing degenerative changes,

inflammatory reactions and blockages, especially in hemobiotic organs (liver, spleen, and kidneys) and gills. Skin and muscle can be associated with bacterial pathogenesis and virulence factors. The pathogenesis experienced by fish that is attacked by bacteria is influenced by extracellular toxins (enterotoxins, hemolysines and proteases), structural features (*Pili*, *S coating, lipopolysaccharides*), adhesion and invasion (Wassif, 2018).

Factors that influence the inhibition of bacteria on the use of plant extracts are influenced by the dose given or the amount of extract used in infected fish either by soaking or adding to the feed. The results showed that the best survival rate and prevalence were treatment C (9%) with the highest percentage adding binahong leaf extract to fish feed. The results of this study are also proven by research on the use of ketapang peel extract (*Terminalia catappa*) on the survival rate and histipatology of carp (*C. carpio*) that have shown that the effective dose to reduce the damage caused by A. hydrophila infection is 770 ppm . The survival rate of gourami fish will decrease after higher concentration of ketapang skin. Ketapang skin concentration is able to maintain the liver of carp but in some cases will trigger the death of carp, therefore the dose of ketapang extract should be given optimally (Fenn, Mavis L; Koppedrayer, 2008).

Other studies conducted by (Hardi, *et a*l, 2019) on the use of combined extracts of *Boesenbergia pandurata*, *Solanum ferox*, and *Zingimber zerumbet* with different concentrations have potential immunostimulant properties for tilapia to inhibit bacterial infection of *A. hydrophila* and *Pseudomonas fluorescens* infections . *Tilapia* infected with *A. hydrophila* and *P. fluerescens* show abnormalities in irregular swimming patterns, weakened movements and are aggressive to the touch. The results showed that at 4 weeks the infected fish still experienced physiological abnormalities but the results of the study also showed that 60% of the fish had recovered. As many as 40% of fish return to normal from aggressiveness if touched (Hardi, *et al*, 2019).

Higher survival rates with very low mortality and increased levels of immunological parameters were recorded in the adjuvant group vaccinated with H. fossilis for testing of pathogenic bacteria. The results showed that the natural

herbal extract used was very influential on vaccination given to fish for controlling bacterial pathogens (Kavitha, *et al*., 2018).Fish infected with bacteria, parasites or viruses will show different clinical symptoms. *Aeromonas caviae* infection in test fish causes behavioral and morphological changes. Changes in behavior indicate a decrease in the response to eating and swimming is not normal which is characterized by vertical and slow swimming. Decreased response to feed is thought to be related to disruption of the body's metabolism of African catfish due to *A. caviae* infection.

Irianto (2015) states that one of the target organs of *A caviae* is the liver, which is the center of the body's metabolism, when the liver process is disrupted due to exposure to pathogenic toxins, it affects the body's metabolic processes (Kurniawan *et al*., 2014). Other research also on the challenge test for bacterial infection A. hydrophila states that the highest death (100%) occurs in the use of *Psidium guajava* extract in feed given to infected fish can increase growth, provide nutritional benefits, immune system and increase survival *O. niloticus* seeds (Omitoyin *et al*., 2019).

Based on the results of research and studies that have been done previously relating to the prevention and control of bacterial infections in freshwater aquaculture activities, it can be concluded that the cause of bacterial infections in fish is influenced by several factors. Factors that influence bacterial infections that can cause disease include environmental conditions, fish seed quality, feed management and the quality and quantity of facilities and infrastructure that support sustainable aquaculture activities (Jasminandar, Y., 2011).

Conclusion

The addition of binahong (*Anredera cordifolia*) leaf flour with different dosages in the manufacture of fish feed given to carp (*C. carpio*) infected with Aeromonas hydrophila bacteria showed significant differences in survival rates. While the average prevalence of the four treatments used did not show a significant difference. The survival and prevalence of carp seeds infected with

A.hydrophila bacteria are also influenced by several factors, including the environment and health of the cultivated seeds.

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