

The Potential Of Mixed Epibiotic (Binahong Leaves, Anrederacordifolia, And Garlic, Allium Sativum, Extracts) As A Feed Additive To Combat Aeromonashydrophilainfection On Catfish (Clariasgariepinus)

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The potential of mixed epibiotic (binahong leaves, *anrederacordifolia*, and garlic, *allium sativum*, extracts) as a feed additive to combat *aeromonashydrophilainfection* on catfish (*clariasgaripepinus*)

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Abstract. Catfish are one of the most commercially important fish cultured and consumed in Indonesia. Catfish (*Clarias garipienus*) is one of the most commercially important freshwater fish cultured and consumed in Indonesia. However, Motile Aeromonas Septicemia (MAS) disease was caused by *Aeromonas hydrophila* is one of the obstacles which are often to be found during the culture. This study aimed was to determine the epibiotic extracts of binahong leaves (BLE) and garlic (GE) as a feed additive on the growth performance and survival rate of catfish against *Aeromonas hydrophila* infection. This research was conducted with Completely Randomized Design (6 treatments and 3 replications) in the aquaculture department laboratory, Fisheries and Marine Science Faculty of Diponegoro University. The treatments were various dosages of the mixture of epibiotic extracts, namely A (2.500 ppm BLE+0 ppm GE), B (1.875 ppm BLE+250 ppm GE), C (1.250 ppm BLE+500 ppm GE), D(625 ppm BLE+750 ppm GE), E (0 ppm BLE+1.000 ppm GE) and K (0 ppm BLE+ 0 ppm GE). The result of the research indicated that a mixture of epibiotic was able to increase the survival rate but not in the growth rate. This treatment also was affected by the blood profile of cat fish ie. Erythrocyte, leucocyte, and hemoglobin These results showed that the best dosage of a mixture of epibiotic extracts (1.250 BLE+500 ppm GE) resulted in 86,67% of survival rate.

1. Introduction

Catfish (*Clariasgaripepinus*) are one of the most commercially important fish cultured and consumed in Indonesia. This species also becomes a major in leading the country for fisheries commodity. Catfish, however, are vulnerable to a variety of diseases, most particularly caused by the bacterial species from the genus *Aeromonas*. *Aeromonas hydrophila* has widely known a major freshwater pathogen, which also causes MAS (Motile Aeromonas Septicemia). This outbreak not only leads to severe levels of economic loss but also has had devastating on survival rates and production of this fish. *A. hydrophila* is a gram-negative that was widely distributed in aquatic water worldwide leading to several diseases in fish[4].*A. hydrophila* is an opportunistic bacteria, a Gram-negative, and lead an outbreak. The outbreak can cause high mortalities reaching about 80% or even 100% occasionally. When *A. hydrophila* infects, catfish develop swim above the surface of the water, unresponsive and loss of appetite [10], haemorrhagic septicaemia also causes lesions that lead to wound infections and ulcers [21].



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Antibiotics are the main approach in the treatment of infections caused by bacterium [23]. However, the abuse of antibiotics in aquaculture leads to the emergence of antibiotic resistance strain and environmental pollution. The spread of antibiotic-resistant bacteria strains can also reduce the effectiveness of antibiotics in the treatment of *A. hydrophila* infection and is a potential threat to human health. Therefore, there is an urgent need for new approaches against *A. hydrophila* infections in catfish. Moreover, both binahong leaves (*Andrographis difolia*) and garlic (*Allium sativum*) extract could protect catfish against *A. hydrophila* infection. Garlic is one of the herbs that sparks great interests since it possesses antibacterial and antifungal activities. Garlic has a broad-spectrum role and contains an active compound, allicin, which affects various enzymes that can affect the metabolism of virulent bacteria [18]. It also contains organosulphur compounds (S-allylcysteine, S-allylcysteininesulfoxide, S-methylcysteine, and S-ethylcysteine) and several phenolic compounds [13]. The antibacterial compound can inhibit bacterial growth and is especially used to cure the infection. Garlic can be used as an antibacterial agent because it contains allicin. Allicin is the main compound that plays an important role in producing a garlic flavor and one of the active compounds which could eliminate germs by impairing the cell walls and inhibiting protein synthesis. Binahong leaves contain active compounds (flavonoids, tannin, saponin, and alkaloids). Binahong leaves ethyl acetate extract quantitatively found 9,614% of flavonoid compounds, as an antioxidant in plants, alkaloid compounds and polyphenol compounds [6]. *A. cordifolia* leaves extract can stimulate potential responses of African catfish (*C. gariepinus*) egg cell membranes which the water has had contaminated with hydrogen peroxide compounds [11].

This study aimed to determine the mixture epibiotic extracts of binahong leaves (BLE) and garlic (GE) as a diet additive on the growth performance and survival rate of catfish against *A. hydrophila* infection.

2. Material and methods

This research was conducted from February – June 2019 in aquaculture laboratories, Fisheries and marine science Diponegoro University. A total of 180 healthy catfish with average weight and size of 7-8 g and ± 9 cm and were obtained from a fish breeding farm in Demak, Central Java. The catfish were randomly divided into three replicates for each treatment in aquariums with 20 L water. Binahong leaves and garlic were taken from Semarang. *A. hydrophila* was obtained from Quarantine Station, Quality Fish Control and Safety of Fishery Products, Yogyakarta. Commercial pellets were added by BLE and GE respectively according to various treatments. The experimental method with Completely Randomized Design (CDR) was used with 6 treatments and 3 replications. The treatments were various dosages of the mixture of epibiotic extracts namely A (2.500 ppm BLE+0 ppm GE), B (1.875 ppm BLE+250 ppm GE), C (1.250 ppm BLE+500 ppm GE), D (625 ppm BLE+750 ppm GE), E (0 ppm BLE+1.000 ppm GE) and K (0 ppm BLE+ 0 ppm GE). The extracts were added by spraying to pellet diets.

Catfish were fed with additive diets with a frequency of 3 times a day (07.00 am, 12.00 pm and 05.00 pm) for two weeks. Challenge was done by intramuscular injection with $0,1 \text{ mL}^{-1}$ suspensions of *A. hydrophila* containing 10^6 CFU/mL. The parameters measured included blood profiles, survival rate, and relative growth rate. Blood profiles included erythrocytes, leukocytes, and hemoglobin. Blood Profiles were recorded from day 0 of research, after catfish were fed with BLE and GE diets in 7th day, before injected by *A. hydrophila* on day 14 and after the 24th day of treatment. Relative growth rate and survival rate were recorded for 7 days due to *A. hydrophila* were injected. During running the trial, RGR was recorder 3 times a day on day 7, day 14 and day 24. Siphon was done every day to maintain water quality. Temperature, pH, dissolved oxygen (DO) were also recorded 1 time a week.

The blood diluting fluid as prepared as described by [3]. The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope [24]:

$$\text{RBC} = \sum L \times 10^6 \text{ cell/mm}^3$$

$$WBC = \sum L \times 10^4 \text{ cell/mm}^3$$

Post-infection *A. hydrophila*, catfish were counted to determine the survival percentage according to the following formula :

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

SR = Survival rate (%)

Nt = Number of fish at the end of the experiment

No = Number of fish at the beginning of the experiment

Fish body weights per gram were recorded at the beginning (day 1) and the end of the feeding experiment (day 24) for all fish of each treatment to determine the difference between the initial and final weights of fish at 24 days of the experimental period. The following formula was used to estimate the growth parameters according to [17]:

$$RGR = \frac{W_t - W_o}{W_o \times T} \times 100\%$$

RGR = Relative growth rate (%/day)

Wt = Final mean weight of fish at the end of the experiment (g)

Wo = Initial mean weight of fish at the beginning of the experiment (g)

T = Length of feeding trial (days)

3. Result and discussion

3.1 Result

3.1.1. Clinical symptoms. The clinical signs of catfish infected by *A. hydrophila* could be seen in table 1.

Table 1. Catfish (*Clarias* sp.) infected by virulent *Aeromonas hydrophila*

Days-	Treatment A (2500ppm/0 ppm)			Treatment B (1.875ppm/250 ppm)			Treatment C (1.250ppm/500 ppm)			Treatment D (625ppm/750 ppm)			Treatment E (0ppm/1.000 ppm)			
	Repeating	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
14	A/G	A	A/G	A	A/G	A	A/G	A	A	A/G	A	A/G	A/G	6 G	A/G	A/G
15	B	B	B	B	B	B	B	B	B	B	C	6	C	C	C	C
16	B	B	B	B	B	B	B	B	B	B	C	C	C	C	D	D
17	C	C	C	D	C	D	C/D	C	C	D	C	D	D	D	D	D
18	C	C	C	D	C	D	C/E	C	C	D	C	D	D	D	D	D
19	D	D	D	D	D	D	E	D	C/D	E	D	D	D	D	D	D
20	D	D	E	E	D	E	E	E	E	E	D	E	E	E	D	E
21	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
22	E	E	F	E	E	F	E	E	E	F	F	F	F	F	-	-
23	F	-	F	-	F	-	-	F	F	-	-	-	-	-	-	-

Inf. : A. swelling at the injection site; B. Inflammation at the injection; C. Bulging stomach; D. Injury/bomb at the injection area; E. Injury of the injection sores; F. Re-formation of the tire muscle; G. Fin rot; and -. No abnormalities occur.

Table 1 showed that the clinical symptoms of catfish were seen after 24 hours challenge test conducted. These symptoms were swelling and inflammation at the injection site, haemorrhage and wound. Recovery of clinical symptoms begins on day 18 the wound begins to shrink and new muscle tissue forms on day 22.

3.1.2. *Survival rate.* The results indicated that the survival rate of treatment A, B, C, D, E and K was $76,67 \pm 5,77\%$, $76,67 \pm 5,77\%$, $86,67 \pm 5,77\%$, $80 \pm 10\%$, $83,33 \pm 11,55\%$ and $70 \pm 17,32\%$. The highest survival was found in treatment C ($86,67 \pm 5,77\%$), whereas the lowest is on treatment K, ($60 \pm 17,32\%$).

3.1.3. *Blood profile.* Observation of catfish blood profile includes erythrocytes, leukocytes, and hemoglobin in the 0th day, 7th day, 14th day, and 21st day was shown in figure 2. The mean number of erythrocytes during the study is about 1,47 – 2,18 ($\times 10^6 \text{ sel/mm}^3$). The mean of the observations result number of erythrocytes was presented in Figure 2.

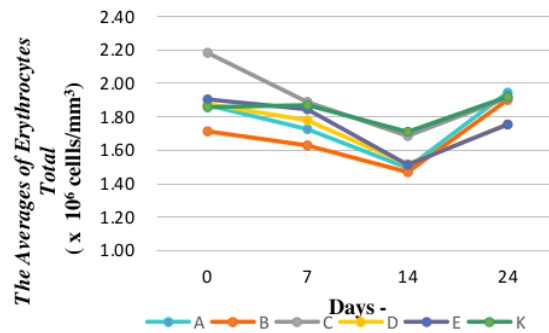


Figure 1. Average erythrocytes total number of catfish during study

Observation of average leukocyte levels during the study was presented in figure 2.

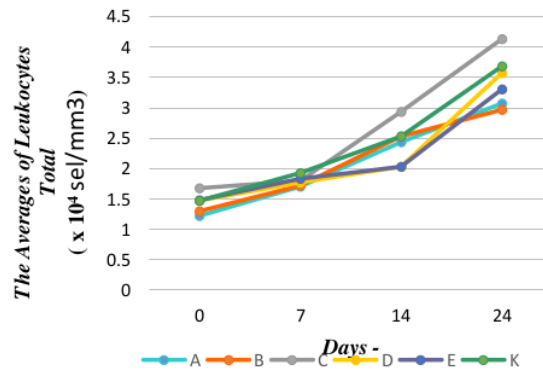


Figure 2. Average leukocytes total number of catfish during study

Based on Figure 2 shown that leukocytes were ranging from 1,22 – 4,13 ($\times 10^4 \text{ cells/mm}^3$). The mean leukocytes showed an increase for each treatment. The increasing of leucocyte in treatment A, B, C, D, E, and K was 60.26%, 56.23%, 59.32%, 58.82%, 55.15%, and 60.05 % respectively.

The hemoglobin levels in this experiment were presented in figure 3.

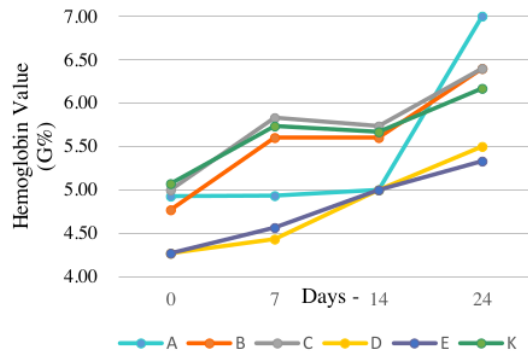


Figure 3. Average hemoglobin total number of catfish during study

Based on observations the average hemoglobin level ranges of 4.27-7 (G%). The levels of hemoglobin in catfish which were added with BLE and GE showed higher values compared to the treatment without extract mixture. The added mixed epibiotic also has a significant effect on and also had a significant difference ($P < 0.05$).

3.1.4. *Relative Growth Rate.* The research result indicated that the relative growth rate of catfish was a range between 2.82 - 4.63 %/day (figure 4).

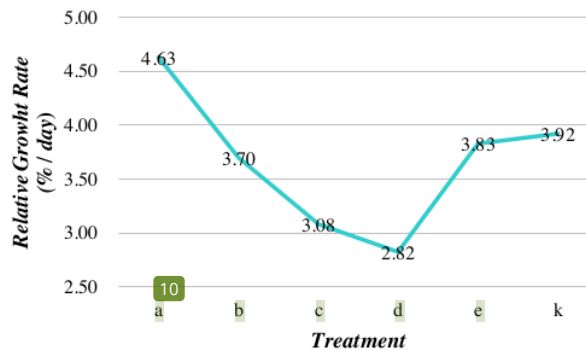


Figure 4. Relative growth rate of catfish during study

Based on the results of the relative growth rate (RGR) it experienced a significant increase and decrease. In figure 4. It can be seen the highest growth results to the lowest in treatment A, treatment K, treatment E, treatment B, Treatment C and Treatment K. The added of mixed epibiotic did not affect significantly the growth rate of catfish. Even though, The highest relative growth rate was shown in treatment A (4.63%)

3.1.5. *Water Quality.* Water quality parameters observed include DO, temperature, and pH. Water quality measurements are carried out once 7 week using a water quality checker. Water quality measurements during the study are presented in table 2.

Table 2. Measurement of water quality during the study

Parameter	Treatments					Range
	A	B	C	D	E	
DO (mg/l)	3-4,05	3,40-4,20	3,70-4	3,20-3,65	3,25-4,20	> 3 ^a
Temperature (°C)	26,5-27,8	26,6-27,2	26,1-27,5	25,7-28	25,5-28	25-30 ^a
pH	7,23-7,58	7,01-8,01	6,77-8,05	6,89-7,84	7,13-7,89	6,5-8,5 ^b

Based on the results of catfish water quality measurements during the study, showed that the water quality is in the optimum range and is still suitable for use during maintenance. Meanwhile, catfish mortality during treatments is not thought to be caused by water quality. Catfish mortality that occurs indicated due to *A. hydrophila* infection.

3.2. Discussion

Clinical symptoms of catfish after bacterial infection *A. hydrophila* can be seen less than 24 hours. These changes are behavior and morphology. Behavior changes of catfish during observation such as swimming lethargically and reduce feeding. Meanwhile in morphology results are swelling at the injection site, reddish wounds around the mouth, inflammation, abdominal dropsy, ulcer and fins were torn. The results of clinical symptoms in catfish appeared on the 15th-day post-infection in each treatment. It is suspected because the bacteria that injected in catfish have a high density so it made changes clinical symptoms less than 24 hours. The samples in the field show symptoms of septicemia. The symptoms generally seen were in the form of hemorrhage, wounds such as sores, reddish wounds around the mouth, and exophthalmia eyes[22]. *A. hydrophila* infection is acute when marked by the very quick mortality of the fish with the appearance of signs of clinical infection like exophthalmia, red patches on the skin, and accumulation of fluid in the abdominal pouch, flatulence, bleeding gill and injury to the dermis, and separated scales[2].

Based on the observations of survival rate, it is shown that the treatment C obtained the highest result (86,67%) and the lowest survival rate was obtained in treatment (K) without epibiotic (60%) This result also showed that the treatment C can increase the survival rate of the fish test. The increase in survival rate may due to the addition of mixed epibiotic extracts, especially garlic[1] and binahong leaf [22]. The mixed epibiotic contains a bioactive compound that was able to inhibit bacterial infections and increase the immune system of catfish. Therefore, the bioactive materials in this mixed epibiotic such as antimicrobial, anti-inflammatory and anti-oxidant have a role to combat *A. hydrophila*[22].

The highest result of the relative growth rate of catfish was shown in treatment A (4,63% per). This showed that the addition of 100% extract binahong leaf to feed was able to stimulate the growth of catfish. It was related to bioactive materials ie. antimicrobial, anti-inflammatory, anti-oxidants, this extract also contains ingredients as growth stimulant^[22]. Besides that, binahong is also rich in protein with large molecular weight. Then, these proteins able to become antigens that stimulate an antibody for growth. So this protein was able to stimulate nitric oxidase production so that it can increase the bloodstream contains nutrients to every cell tissue. As a result, this extract was able to stimulate the production of growth hormones[8].

The observation of the blood profile includes is erythrocytes, leukocytes, and hemoglobin. Based on observations, the total erythrocytes ranged from 1,47 to 2,18 ($\times 10^6$ cells/ mm³). Total erythrocytes during the study increased or decreased. The decrease occurred post-infection on the 14th day and then increase during 24 days of treatments. Total erythrocytes decreased in the measurement one day after the challenge in each treatment [19]. It was caused by stress at the time of injection and infection of the bacteria. Erythrocytes normal in Teleostei fish are $1,05 \times 10^6 - 3 \times 10^6$ cells/ mm³[14].

The average total of leukocytes showed improvement during 24 days of treatments. An increase in leukocyte cells is a reflection of the success of the fish's immune system in developing cellular (non-

specific) immune responses as triggers of the immune response. This is consistent with the opinion of White blood cells (leukocytes) function as the body's defenses, which react quickly to the entry of antigens into the body of the fish^[16]. The number of white blood cells (leukocytes) in fish ranges from > 20,000 mm³ [12].

Hemoglobin is an oxygen and carbon dioxide transport device found in erythrocytes[12]. Based on observations of catfish hemoglobin levels obtained during the study ranged from 4.27-7. The range is below the normal levels of fish Hb. Decreased hemoglobin levels because fish undergo lysis in the red blood cells. Normal Hb levels in fish were 12.0 g / dl - 14 g / dl. The decrease in the value of hemoglobin in the blood is related to the low value of the erythrocyte which is suspected because the fish undergo lysis in the blood. Lysis is caused by the rupture of red blood cells due to the presence of bacterial toxins in the blood called hemolysin. This toxin will lyse hemoglobin and release hemoglobin. Low hemoglobin levels can be an indication in fish for the occurrence of infection, in this case, are bacteria[9]. Based on this result can be found that mixture epibiotic (extract of BLE and GE) was potentially used to combat *A. hydrophyla* with added in the feed. The mixture epibiotic not only potentially to recovery the clinical sign, but it was also could be used to prevent bacterial diseases.

4. Conclusion

The conclusions obtained in this study found that a mixture of epibiotic extract of BLE and GE was potentially used to combat *A. hydrophyla* in catfish, treatment C (1,250 ppm BLE+500 ppm GE) in feed is the best concentration in preventing *Aeromonas hydrophila* with the survival rate of 86.67%. and plays a role in enhancing the immune system, accelerating wound healing and increasing fish growth before infection or post-infection.

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