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The effect of green betel leave extract (*Piper betlel*) in feed as prevention of *Edwardsiella ictaluri* bacterial infection in catfish (*Pangasianodon hypopthalmus*)

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ABSTRACT

This study aimed to determine the effect of adding green betel leaf extract to feed on the prevention of Edwardsiella ictaluri bacterial infection in catfish. Betel leaf extract was sprayed into the feed according to the research dose, namely Treatment A pelleted feed + 0.3 mL betel leaf extract / 100 g of feed + injected with E.ictaluri bacteria. Treatment B pelleted feed + 0.6 mL of betel leaf extract/ 100 g of feed + injected with E.ictaluri bacteria. Treatment C pelleted feed + 0, 9 mL betel leaf extract / 100 g feed + injected E.ictaluri bacteria Control (+) feed pellet + injected E.ictaluri bacteria Control (-) pelleted feed + injected with physiological solution of NaCl 0, 9%. Feeding was carried out for 14 days, the challenge test was carried out on the 16th day, for observation of clinical symptoms, and survival rates were observed for 10 days after the challenge test. The results of the study showed that the best survival rate was in treatment C (66.66%) and the lowest was in treatment A (53.33%) with fewer external and internal clinical symptoms among other treatments.

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1. Introduction

Catfish (*Pangasionodon hypophthalmus*) is a species of freshwater fish of the Pangasidae type and is one of the introduced fish species that has economic value for cultivation. According to Tahapari *et al.*, (2008) that with many advantages of catfish, aquaculture commodities have a high economic value, both in the hatchery, consumption, and breeding business segments. In the cultivation of catfish (*P. hypophthalmus*) often encountered bacterial disease Enteric septicemia of catfish (ESC) caused by bacterial infection *Edwardsiella ictaluri*. ESC can cause catfish mortality up

to more than 50%. Disease control generally uses antibiotics, but the use of antibiotics has a bad impact because it can cause residues in fish and can endanger the health of consumers who consume fish with antibiotic residues (Wahjuningrum *et al.*, 2012).

Therefore, an environmentally friendly alternative is needed. One of them is to use plants that have antibacterial active ingredients, namely betel leaf. Betel leaf can be used as an anti-bacterial because it contains 0.9-1.2% essential oil (Sulianti & Chairul, 2002). Betel leaf contains phenol, which has a role as a poison for microbes by inhibiting their enzyme activity. Catechol, pyrogalol, quinone, eugenol, flavones and flavonoids are included in the phenol group and have the ability as antimicrobial agents (Suliantari *et al.*, 2008). The active ingredient content can be used to treat infectious diseases caused by Aeromonas hydrophila bacteria in catfish (Ferdinandus *et al.*, 2019). According to Reveny, (2011), the propanoid phenolic compounds contained in betel leaf have strong antimicrobial and antifungal properties and can inhibit the growth of several types of bacteria, including *Salmonella* sp, Klebsiella, Pasteurella. In this study, we wanted to examine the effectiveness of betel leaf extract in feed to prevent *E. ictaluri* bacterial infection in catfish.

2. Material and methods

The fish used in the research were catfish fry (*P. hypophthalmus*) with an average weight of ±11.32 g/ind. Fish were obtained from BPBAT (Freshwater Cultivation Fisheries Center) Sungai Gelam. The number of fish used in the test was 10 fish/aquarium. The test containers used in this study were 15 aquariums measuring 60 × 30 × 30 cm with 20 L of water. The feed used was PF 1000 feed with the addition of green betel leaf extract according to the treatment dose. For treatment A, pellet feed was added with betel leaf extract 0.3 mL kg^{-1} of feed and 1.7 mL of distilled water was added to kg^{-1} feed. For treatment B, pelleted feed was added to 0.6 mL of betel leaf extract kg^{-1} of feed and 1.4 mL of distilled water was added to feed, in treatment C pelleted feed was given betel leaf extract 0.9 mL kg^{-1} of feed and 1.1 mL of distilled water was added. 2 mL of aquadest kg^{-1} was added to feed for feed control. In order for the extract to stick to the feed, each treatment was added with 2 ml of egg white for 1 kg of feed. Before spraying, the feed is first leveled so that the extract is sprayed evenly, after being sprayed the feed is air-dried. The test bacteria came from the Jambi BPBAT collection which had gone through the identification process and had been in Koch's Postulates.

The lethal dose (LD_{50}) test was calculated using the Reed and Muench method in Safratilofa (2015) and the results were obtained with a bacterial density of 107 CFU mL^{-1}. The test fish were fed the test feed satisfactorily twice a day for 14 days, the challenge test was carried out on the 16^{th} day with a bacterial density of 0.1 mL. For negative control, the test fish were injected with physiological solution. Observation of the test parameters was carried out for 10 days after the challenge test. After challenge test fish are fed commercial feed.

Survival Rate (SR)

Observation of survival rate was calculated using the formula Effendi (2003). The percentage ratio of live fish at the end of the study to the number of fish at the beginning of the study.

Clinical Symptoms

Observations of clinical symptoms observed were external (fins and skin) and internal (liver and kidney).

Data Analysis

Parameters of clinical symptoms were analyzed descriptively, for survival parameters were analyzed using variance (ANOVA) at the 5% level, if significantly different, further test was carried out by DNMRT SPSS 20Water quality checked every 6 hours include the temperature, pH and dyssolved oxygen, ammonia, nitrate. Observed until 10^{th} day since bacterial infection and the rest of the fish checked for bacterial re-isolated. Survival rate each treatment calculated.

3. Results and Discussion

Survival Rate (SR)

The results of observations during the study of the survival/SR of catfish (*P. hypopthalmus*) given green betel leaf extract (*Piper betlel*) can be seen in Table 1.

Table 1 Survival Rate of Catfish After 14 Days of Maintenance and 10 Days of Observation After Challenge Test

Treatment	Average	Notation
K +	43.33	a
A (0,3 ml)	53.33	b
B (0,6 ml)	56.66	b
C (0,9 ml)	66.66	c
K -	100	d

From Table 1, the highest SR value is in treatment C (0.9 mL kg^{-1}) of 66.66%, and significantly different between treatments A and B. This is because the amount of betel leaf extract added is more than treatment According to Suliantari *et al*. (2008) flavonoids are included in the phenol group and have the ability of some antimicrobial ingredients. In addition, flavonoid phenol compounds have strong antimicrobial and antifungal properties and can inhibit the growth of several types of bacteria, including *Salmonella* sp., Klebsiella, Pasteurella, and can kill *Candida albicans* (Reveny, 2011). Betel leaf also contains essential oils that can inhibit several types of bacteria. According to Sujono et al (2019), Green betel leaf (*Piper betle* L.) essential oil can inhibit Streptococcus pyogenes and *Staphylococcus aureus*.

In the K+ treatment, the further test results showed a significant difference to the A, B, C, and K- treatments, in the K+ treatment the SR value was lower than the A, B, C and K- treatments, the low survival rate in the positive control treatment was due to feed that given does not contain betel leaf extract, so it is unable to inhibit the growth of *E. ictaluri* which results in the low SR produced. According to Wahjuningrum *et al*. (2010) that the percentage value of mortality in the positive control treatment was higher than the treatment given phytopharmaca, indicating that the administration of phytopharmaca extract mixed in feed can inhibit infection from bacteria and can increase the fish's immune system.

Clinical Symptoms

The results of observing clinical symptoms externally and internally can be seen in Table 2 and Table 3.

Table 2. External clinical symptoms of each treatment catfish after injection of *E. ictalurid*

Treatment	Fin	Abdomen	Information
Negative control			(A) fins do not see red spots. (B) Red and flaky fins. (C) Red and flaky fins. (D) The fins are red and not flaky. (E) The fins are slightly red and not flaky. (F) Abdomen doesn't swell. (G) Swollen abdomen. (H) Swollen abdomen. (I) Slightly enlarged abdomen. Slightly enlarged abdomen
Positive control			
A 0,3 ml			
B 0,6 ml			
C 0,9 ml			

Table 2 above, it can be seen that external clinical symptoms that began to appear after *E. ictaluri* bacterial infection were red spots on the body and fins of the fish, as well as swelling of the fish's abdomen. In treatments A and B, there were many red spots on the fins, almost every fin appeared red and clearly visible, in addition to red spots, the fins were also flaky or the fins seemed to fall out, while the abdomen of the fish looked swollen. Whereas in treatment C, the external clinical symptoms on the fins of the red spots were not very visible, the fins of the fish did not look flaky, and the abdomen of the fish was not too swollen. Sakai et al., (2008) stated that some clinical signs of ESC disease are the presence of red patches on the skin under the jaw, operculum, around the abdomen, and anus and the bottom of the fins.

Table 3. Internal clinical symptoms of each experimental catfish after injection of *E.ictaluri*

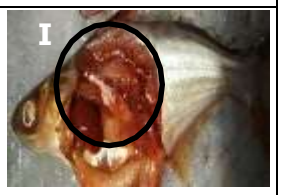
Treatment	Fin	Abdomen	Information
Negative control			(A) No visible white spots on the liver and no swelling. (B) Clearly visible white spots on the liver and swelling occurs (C) Clearly visible white spots on the liver and swelling occurs. (D) There are white spots on the liver and there is swelling. (E) Looks a little white spots on the liver and a little swelling. Kidney color is still normal. (G) Black kidney. (H) Black kidney (I) Kidney color is a little black
Positive control			
A 0,3 ml			
B 0,6 ml			
C 0,9 ml			

Table 3, changes in anatomical pathology in test fish infected with *E. ictaluri* bacteria are damage to internal organs (internal). In treatments A and B, it was very visible, including pale liver, enlarged and white spots on the liver, kidneys turning blackish red, presence of ascites, or accumulation of yellow, cloudy or clear fluid, or blood. An enlarged liver and the presence of ascites result in abdominal swelling. Whereas in treatment C, the internal clinical symptoms were not very visible compared to treatments A and B, in the liver the white spots were not too visible, in the kidneys the color was not too black, the fluid accumulation was not too large. As reported by Hassan *et al*. (2012) that found the presence of ascites in fish that died due to *E. ictaluri* infection. While in the positive control treatment, internal clinical symptoms were very visible compared to treatments A, B and C.

Water quality during the study was within the normal water quality threshold for aquaculture. The results of temperature measurements in each fish rearing container ranged from 26.3 to 26.9 ºC, pH ranged from 6.5 to 6.9 and dissolved oxygen ranged from 8.0 to 8.1 mg/L.

Conclusion

Feeding with the addition of betel leaf extract of 0.9 ml/100 g of feed was the most effective treatment with a survival rate of 66.66% and fewer external and internal clinical symptoms among other treatments

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