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Effect of Addition of Betel Leaf Extract (*Piper betle*) on Feed for the Treatment of Tilapia (*Oreochromis niloticus*) Infected with *Aeromonas hydrophila*

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Abstract:
This study aims to determine the effect of adding betel leaf extract (Piper betle) to feed and the most effective doses for the treatment of tilapia (Oreochromis niloticus) infected with Aeromonas hydrophila. This study was an experimental study using a completely randomized design trial consisting of 4 treatments and 3 replications. The treatments tested consisted of the control treatment (without giving betel leaf extract), treatment A (giving betel leaf extract 0.40 g/100 g feed), treatment B (giving betel leaf extract 0.60 g/100 g feed), and treatment C (giving betel leaf extract 0.80 g/100 g feed). The data collected includes behavior, morphology, anatomy, growth rate, survival rate, and water quality, including temperature, pH, dissolved oxygen, and ammonia. The results showed that treatment A (giving betel leaf extract 0.40 g/100 g feed) was the best treatment with the fastest healing process of clinical symptoms including behavior and anatomy, positive effect on the internal organs of fish, producing the highest absolute weight with an average of 0.72 g, an average absolute length of 0.35 cm, an average specific weight of 0.55% and a survival percentage with an average of 60%.

Keywords: *Tilapia, bacteria, aeromonas hydrophila, betel leaf*

1. Introduction

Tilapia (*Oreochromis niloticus*) is one type of freshwater cultured fish that has good prospects for development. Tilapia is widely cultivated in Indonesia and is a cultivated fish that is one of the export commodities (Mulia, 2003). Tilapia is much favored by the people because the meat is quite thick and tastes delicious. Tilapia contains 43.76% of protein, 7.01% of fat, and 6.80% of ash content per gram of fish weight (Zainuddin *et al*., 2018).

Tilapia cultivation is inseparable from the problem of disease attacks, which can cause death and can cause economic losses. Disease attacks also cause consumer rejection of fish due to a decrease in fish quality. Disease infections in fish also affect human health if they contain *zoonotic parasites*.

The emergence of disease in fish is generally the result of an unequal interaction between three components in aquatic ecosystems, namely weak hosts (fish), pathogens, and deteriorating environmental quality. Causes of fish disease include disease-causing microbes (pathogens), which can be parasites, bacteria, viruses, or fungi (Kordi, 2004).

Diseases that are often encountered by aquaculture organisms are bacterial diseases. One of the bacteria that causes fish disease is Aeromonas hydrophila bacteria (Giyarti, 2000). This bacterium attacks various types of freshwater fish, one of which is tilapia. Aeromonas hydrophila bacterial infection can cause disease with symptoms including skin peeling easily, red spots all over the body, gills discolored or bluish, exophthalmia (eyeballs protruding), bleeding dorsal fins, pectoral fins, pelvic fins, and tail fins, also bleeding in the anus, and loss of appetite in fish (Mulia, 2003).

In an effort to control diseases in fish farming, antibiotics or chemicals can be used. However, some chemicals commonly used by fish cultivators are not easily decomposed naturally, so they are categorized as not environmentally friendly. The negative impact of using antibiotics is that they accumulate in tissues, especially in bones, so they can harm humans who consume them. Continuous use of antibiotics and chemicals can lead to bacterial resistance to antibiotics.

One of the medicinal plants that are thought to be used to treat *Aeoromonas hydrophila* bacterial infections that attack fish is betel leaf because, like antibiotics, betel leaf also has anti-bacterial properties. This ability is due to the various substances contained therein. The effectiveness of betel leaf extract as an anti-bacterial in tackling disease infections caused by *Aeromonas hydrophila* bacteria in gouramy (*Osphronemus gouramy*) has been carried out by Farisi (2020) and catfish by Mulia and Husin (2012). However, there has been no research on the effect of adding betel leaf extract (*Piper betle*) to feed for the treatment of tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Therefore, a test has been carried out to determine the effect of betel leaf extract (*Piper betle*) and the effective dose in treating tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila* bacteria.

2. Research Purposes

Tujuan dalam penelitian ini yaitu untuk menganalisis pengaruh dan dosis terbaik pada penambahan ekstrak daun sirih (*Piper betle*) pada pakan dalam pengobatan ikan nila (*Oreochromis niloticus*) yang terinfeksi *Aeromonas hydrophila*.

3. Method

This type of research is experimental research, with an experimental design using a completely randomized design with 4 treatments and 3 replications. The test fish used were tilapia seeds with an initial size of 7 cm. The treatments tested were:

- Treatment K (control), namely giving pellet feed without the addition of betel leaf extract,
- Treatment A, namely 100 g pellet feed + 0.40 g betel leaf extract,
- Treatment B, namely 100 g pellet feed + 0.60 g betel leaf extract, and
- Treatment C, namely 100 g pellet feed + 0.80 g betel leaf extract

Observation of fish clinical symptoms includes observation of fish behavior and morphology. Observation of fish behavior and morphology was carried out after the tilapia seeds were challenged until the 15th day after the challenge test, where observations were made every 6 hours. The observation time refers to previous research conducted by Patang (2012), while the observation of internal fish organs was carried out at the end of the research period. The following observations were made related to the growth rate of fish, including absolute weight growth, absolute length gain, and specific growth rate. Observations regarding the survival rate were carried out to determine the percentage of the number of fish that were alive at the beginning of the study with the number of fish that were alive at the end of the study.

Water quality parameters measured during the study were temperature, pH, dissolved oxygen, and ammonia (NH_3). Measurements of temperature, pH, and dissolved oxygen were carried out *in situ*. The tools used are thermometers, pH meters, and DO meters, carried out every day, namely in the morning and evening. On the other hand, the measurement of ammonia was carried out using the ammonia tetra test, which was carried out twice, namely at the beginning and end of the study. The data analysis technique used in this study was ANOVA analysis of variance and Kruskal-Wallis H analysis, and it was processed using the SPSS Version 24 program.

4. Results and Discussion

4.1. Effect of Betel Leaf Extract on Feed in the Treatment of Tilapia (Oreochromis Niloticus)

4.1.1. Observation of Fish Behavior

Based on observations for 15 days after infection, there was a difference in the percentage of behavior change in each treatment with betel leaf extract (*Piper betle*). On the 15th day, Treatments A and B showed the best level of change with an average of 5.00 on a behavior scale, followed by treatment C with an average change in behavior of 4.33 on a behavior scale. In contrast, the control treatment showed very low changes with an average value of 0.00 on the behavior scale.

Based on clinical symptoms of tilapia after the challenge test, fish infected with *A. hydrophila* bacteria showed changes in behavior at each treatment, such as abnormal swimming (swimming at the bottom of the aquarium and swimming slowly). This statement is in accordance with the opinion of Arindita *et al*. (2014) that fish infected with *A. hydrophila* bacteria will show changes in behavior, such as swimming that is not normal (slow and vertical). Fish that swim slowly are thought to be experiencing post-infection stress. It was further explained that the *A. hydrophila* bacteria that attacks fish can disrupt the balance of swimming so that the fish become abnormal and swim slowly.

The results of the Kruskal-Wallis H analysis showed that treatment with betel leaf extract had an effect on changes in fish behavior with a sig value ($P<0.05$). On the 15th day, changes in fish behavior showed significantly different values ($0.021 <0.05$); thus, the results obtained were that hypothesis H-1 was accepted, and there was an effect of adding betel leaf extract to the feed for the treatment of tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Then a follow-up test was carried out in the form of a PostHoc test, with the results of the control treatment being significantly different from treatment A and treatment B.

4.1.2. Observation of Fish Morphology

In this study, fish morphology was observed to determine the effect of betel leaf extract on fish feed on changes in the morphology of fish infected with *Aeromonas hydrophila* bacteria. Observations were made visually by observing the morphology of tilapia that appeared every day after the fish were challenged with *Aeromonas hydrophila* bacteria until the end of the 15-day rearing period.

Based on clinical symptoms of tilapia after the challenge test, fish infected with A. hydrophila bacteria showed morphological changes with each treatment, including loose fish scales, wounds/ulcers, cured fish scales, and purulent and blackening of the body color of the fish. Damage to the body surface of infected fish is caused by exotoxin enzymes from A. hydrophyla, such as protease and elastase because muscle tissue and blood vessels contain lots of protein (Kamaludin, 2011).

On the first day, the morphological conditions of the fish in all treatments were the same, where the color of the fish bodies was blackened; then, on the 8th day in treatment A, the color of the fish bodies was blackened, and in treatments B and C the color of the fish bodies was blackened and purulent, and in treatment K, the fish had scales flaking and purulent. On the 15th day, in treatments A and B, the fish were no longer injured, and in treatment C, the color of the fish bodies blackened, and in treatment K, not a single fish was alive.

The results of observations on the 15th day showed that the lowest changes in fish morphology were found in the control treatment (without betel leaf extract) with an average value of 0.00 on the morphological scale, while the best morphological treatment was found in treatment A and treatment B with an average morphological scale of 5.00, followed by treatment C with an average morphological scale of 4.67. The fish that healed the fastest were in treatment A, whereas on the 12th day, the fish were no longer injured, and then followed by treatment B, namely on the 15th day, for treatment C and Control, the fish were still injured until the end of the observation. Based on the results of the treatment of tilapia with betel leaf extract, the best results were obtained in treatment A with a concentration of 0.40 g/100 g of feed, seen from the relatively faster healing process compared to other treatments. Because of the content of betel leaf extract, 0.40 g/100 g feed works more optimally compared to other treatments.

The results of the Kruskal-Wallis H analysis showed that treatment with betel leaf extract had an effect on changes in fish morphology with sig. ($P<0.05$). On the 15th day, the changes in fish behavior showed significantly different values ($0.023 <0.05$); thus, the results were that hypothesis H1 was accepted, and there was an effect of adding betel leaf extract (Piper betle) to the feed for the treatment of tilapia (Oreochromis niloticus) infected with Aeromonas hydrophila). Furthermore, further tests were carried out in the form of a PostHoc test, with the results of the control treatment being significantly different from treatment A and treatment B.

4.1.3. Observation of Fish Anatomy

Observation of anatomical changes was carried out by dissecting the tilapia body at the end of the treatment (day 16 after infection with *A. hydrophila* bacteria). Observations made on the anatomy of the fish include the liver, kidney, and bile. The results of observations of tilapia anatomy at the end of the study were known, and the differences between the good treatments were control treatment, treatment A, treatment B, and treatment C.

The internal organs (anatomy) of fish observed were the liver, gall, and kidney organs. The results of the observations showed that the internal organs in treatments A and B had the same condition, namely the liver was brownish red, the bile was light green, and the kidneys were brownish red. This was not much different from treatment C which had brownish-red liver, dark green bile, and brownish-red kidneys. In contrast, the control treatment showed differences, namely internal organ abnormalities such as pale red liver, greenish-black bile, and pale red kidneys. At the end of the study, it was discovered that there were differences between the treatments where the observations in each treatment showed that treatment A, with a dose of 0.40 g and 0.60 g, was close to the cure rate marked by the color of the internal organs getting better again after treatment.

4.1.4. Tilapia Fish Growth

4.1.4.1. Absolute Weight Growth

The results showed that there were differences between each treatment where treatment A was the treatment that showed the best results among the other treatments with an average yield of 0.72 g, followed by treatment B with an average of 0.41 g, treatment C with an average value of 0.19 g and treatment K (Control) showed the lowest results with an average of -0.50 g. The results of observing absolute weight growth parameters can be seen in figure 1.

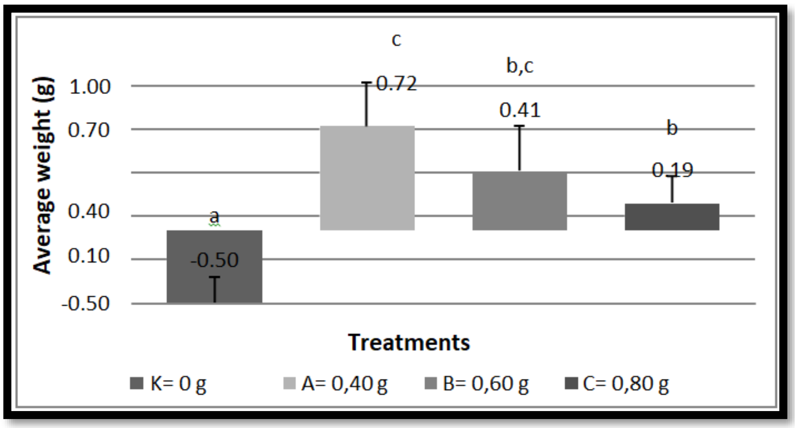

Figure 1: Average Absolute Weight of Fish

Based on the results of the analysis of variance (ANOVA) showed that the treatment had an effect on the absolute weight growth rate with a significant value (0.002<0.05). Furthermore, further tests were carried out in the form of Duncan's test, with the results of the control treatment significantly different from other treatments. According to Annisa *et al*. (2015), low growth is caused by damage to body tissues, so fish experience stress, and the utilization of feed nutrients that should be used for growth is replaced by damaged body cells. So the parturition of fish plants occurs very slowly.

4.1.4.2. Absolute Length Growth of Fish

The results showed that there were differences between each treatment where treatment A showed a higher increase in length compared to the other treatments with an average of 0.35 cm, followed by treatment B with an average of 0.28 cm, treatment C with an average of 0.21 and treatment K (Control) showed the lowest results with an average of 0.05 cm. The results of observing the absolute length gain parameter can be seen in figure 2.

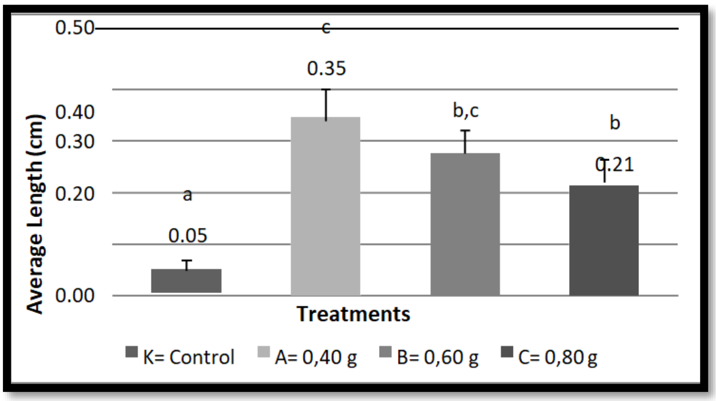

Figure 2: Average Absolute Length of Fish

The results of the analysis of variance (ANOVA) show that the treatment has an effect on the absolute length increase rate with a significant value (0.001<0.05). Furthermore, further tests were carried out in the form of Duncan's test, with the results of the control treatment significantly different from other treatments.

Mahardika *et al*. (2017) explained that the growth rate of fish is influenced by internal factors in the form of the condition of the fish where with good body conditions, they can digest food properly so that it supports growth, while external factors are the quality of the environment where the fish feed. Sainah *et al*. (2016) added that the speed of fish growth is affected by the amount of protein that can be absorbed through feed and utilized by the body as a building block for body protein, where protein is used to maintain metabolic activity and growth.

4.1.4.3. Fish-Specific Growth Rate

The specific growth rate is the percentage of the difference between the final weight and the initial weight, divided by the length of time of maintenance. Specific growth rate (SGR) is the speed of growth over time and is a parameter used to determine the ability of fish to utilize feed nutrients to be stored in the body and converted into energy (Putri, 2014).

The results showed that there were differences between each treatment where treatment A showed a higher increase in weight compared to the other treatments with an average of 0.55%, followed by treatment B with an average of 0.32%, treatment C with an average of 0.15% and treatment K (Control) showed very low results with an average of -0.41%. The observation results can be seen in figure 3.

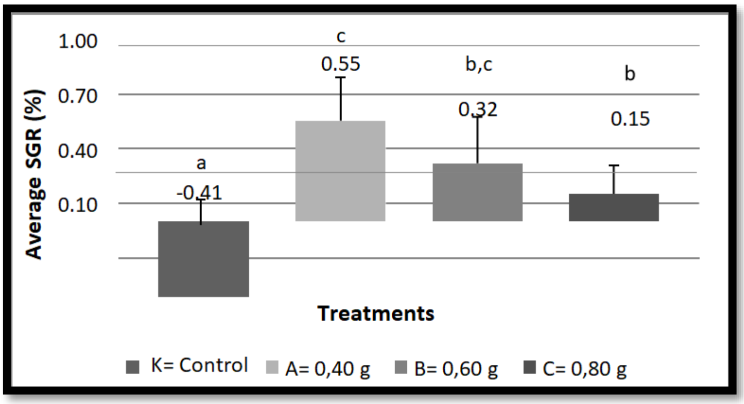

Figure 3: Nilai Rata-Rata Laju Pertumbuhan Spesifik

The results of the analysis of variance (ANOVA) showed that the treatment had an effect on the specific growth rate with a real value (0.002<0.05). Furthermore, further tests were carried out in the form of Duncan's test, with the results of the control treatment significantly different from other treatments.

The increase in body weight of the test fish in this study occurred allegedly due to wound healing in the test fish and an increase in response to the feeding. This is in accordance with the statement of Zhang *et al*. (2015) that the content of essential oils has a fragrant aroma that can stimulate the digestive glands to arouse fish appetite. Meanwhile, the low growth in the control treatment was caused by the loss of appetite of fish and decreased response to eating fish, thus disrupting the process of fish growth. This is in line with the statement of Abdullah (2008), which states that the low response to eating and loss of appetite is caused by organ damage in changes in swelling of the liver, kidneys, and bile.

4.1.4.4. Survival Rate of Fish

The results of observations on survival parameters for 15 days of observation showed that there were changes in the survival rate of fish from several treatment groups on the survival of tilapia. Treatment with betel leaf extract (Piper betle) resulted in a different survival rate than the control treatment.

The treatment with the highest survival of tilapia was treatment A: treatment of 0.40 g of betel leaf extract, where the survival value on the 15th day was 60.00%, followed by treatment B: treatment of 0.60 g of betel leaf extract with the survival value on the 15th day was 46.67% and treatment C: 0.80 g of betel leaf extract with a survival value on the 15th day of 33.33%, while the lowest survival rate was in the control treatment: without betel leaf extract treatment with a survival value on the 15th day of 0%.

The results of Kruskal-Wallis H analysis showed that treatment with betel leaf extract had an effect on the survival of sig. (P<0.05). On the 15th day, the fish survival showed a significantly different value, namely the sig. (0.034<0.05) thus the result is that there is an effect of adding betel leaf extract (Piper betle) to feed for the treatment of tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Then a further test was carried out in the form of a PostHoc test, with the results of the control treatment significantly different from treatment A.

4.2. Effective Concentration of Betel Leaf Extract in Feed for Treatment of Tilapia

This study used the addition of betel leaf extract (Piper betle) to feed with control dose (without betel leaf extract) and betel leaf extract doses of 0.40 g, 0.60 g, and 0.80 g. The results of this study indicate that the effective concentration in the treatment of tilapia infected with *A. hydrophila* bacteria is a dose of 0.40 g, where at this dose, the fish recovery rate is relatively faster both in terms of behavior, morphology, and anatomy, compared to extract doses of 0.60 g and 0.80 g where the recovery rate of the fish was relatively slower. Whereas in the control treatment (without betel leaf extract), the condition of the fish did not show a positive change.

4.3. Water Quality Parameters

4.3.1. Temperature of Water

Based on the water quality observations that have been made, the results show that the average water temperature for each treatment ranges from 27.1°C–28.3°C. This shows that the quality of the temperature in the aquarium is still in the range of good conditions for the life of tilapia.

- In treatment A, the average water temperature in the morning was 27.1°C, and in the evening, it was 28.3°C;
- In treatment B, the average water temperature in the morning was 27.1°C and 28.3°C in the evening,
- In treatment C, the average temperature water in the morning was 27.2°C and 28.2°C in the afternoon, and
- In the control treatment, the average water temperature in the morning was 27.2°C and 28.3°C in the evening. Changes in temperature that occur in each treatment are quite important things that can affect fish life.

4.3.2. The Level of Acidity (pH) of Water

In observing the water quality parameters in the form of pH (acidity), it is known that the pH value of the water in each treatment is different. pH measurements were carried out twice a day, i.e., in the morning and evening, for 15 days. In general, the ideal pH number ranges from 4-9, but for optimal growth for tilapia, the ideal pH ranges from 6-8; this indicates that in each treatment, the pH value is still in normal conditions.

4.3.3. Dissolved Oxygen of Water

In observing the water quality parameters in the form of dissolved oxygen, it was found that the value of dissolved oxygen in each treatment was different. In treatment A, the average value of dissolved oxygen in the morning is 5.4 ppm, and in the afternoon is 6.0 ppm. In treatment B, the average value of dissolved oxygen in the morning is 5.6 ppm, and in the afternoon is 6.0 ppm. In treatment C, the average value of dissolved oxygen in the morning is 5.5 ppm, and in the afternoon is 5.8 ppm. In the control treatment, the average value of dissolved oxygen in the morning was 5.4 ppm, and in the afternoon was 5.8 ppm. This shows that the dissolved oxygen value in the aquarium is in the range of good conditions for the life of tilapia, where the optimal dissolved oxygen level is more than 5 ppm. Meanwhile, dissolved oxygen values of less than 3 ppm and 4 ppm can disrupt fish life.

4.3.4. Ammonia (NH_3-N)

In observing the water quality parameter in the form of ammonia (NH_3-N), the result was that the value of ammonia in each treatment was the same. Ammonia measurements were carried out twice, namely on day 1 and day 15, where the average value of ammonia during the observation was 0.25 ppm. This shows that the ammonia value in the aquarium is in the range of good conditions for the life of tilapia, where the ideal range of ammonia concentration in water for fish life is not to be more than 1 ppm.

5. Conclusion

Giving betel leaf extract (Piper betle) to feed has an effect on treating tilapia infected with Aeromonas hydrophila bacteria, where the percentage of behavior, morphology, anatomy, growth rate, and survival of fish is better than without betel leaf extract. The dose of betel leaf extract (Piper betle) 0.40 g/100 g of feed is an effective dose compared to other treatments, with the fastest healing process of clinical symptoms (behavior and anatomy), has a positive effect on the internal organs of fish, producing the highest absolute weight with an average of 0.72 ± 0.30 g, an average absolute length of 0.35 ± 0.07 cm, an average specific weight of 0.55 ± 0.24 % and survival rate with an average of 60 ± 1.00 %.

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