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EFFECT OF BETEL LEAF EXTRACT (*Piper betle L.) ON Aeromonas hydrophila* DISEASES IN TILAPIA (*Oreochromis niloticus*) SEED

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ABSTRACT

Seed is essential in aquaculture activities, but tilapia hatcheries often suffer from disease attacks caused by Aeromonas hydrophila bacteria, *causing* losses *for hatchery* businesses. One of the natural ingredients for treating *Aeromonas hydrophila* is betel leaf extract. This study aims to analyze the effect of betel leaf extract (*Piper betle L.) on Aeromonas* disease *hydrophila* on fish fry tilapia (*Piper betle L.*). This study used a completely randomized design (CRD) with 5 treatments and 3 replications. The treatment used was a dose of betel leaf extract (P1) 0.4 ml, (P2) 0.6 ml, (P3) 0.8 ml, (P4) positive control, (P5) negative control. The parameters observed were survival rate, specific growth rate, feed conversion ratio, absolute length growth, erythrocytes, hemoglobin, hematocrit, leukocytes, differential leukocytes, total bacteria count, and water quality. The results of this study showed that before and after the challenge test, the best average values were P3 leukocytes (1.06 and 1.4 x 10 5), erythrocytes (0.90 and 0.93 x 10 5), hemoglobin (10.9 and 9.7), hematocrit (10.7 and 10.3), total bacteria (7 x 10 1). Then the worst value on P1 leukocytes (0.74 and 0.87 x 10 5), erythrocytes (0.86 and 0.75 x 10 5), hemoglobin (10.7 and 7.7), hematocrit (10.3 and 9.3), total bacteria (74 x 10 2). The study concluded that the best treatment was found in treatment 3 with a dose of 0.8 ml of betel leaf extract, which significantly increased the immune system in tilapia seeds.

INTRODUCTION

Tilapia is a biota with a high economy. Robisalmi *et al.,* (2020), stated that the amount of tilapia production in the world in 2015 reached 5,576,800 tons, and Indonesia is the second largest tilapia producer after China, with a value of 1,100,000 tons. The need for tilapia continues to increase yearly because this fish is quite popular and also called Aquatic chicken. Even now, it is known as an aquatic turkey.

In tilapia, production must, of course, be directly proportional to the availability of seeds on the market so that the needs of cultivators can be *met*. This bacterium is antiseptic,

infects quickly if the stocking density increases and is capable of causing seed mortality to reach 90%.

To deal with bacterial infections, it is necessary to have a drug solution that is safer and easier to obtain to control diseases caused by the bacterium *Aeromonas hydrophila. That* drug is betel leaf extract because it can potentially treat *Aeromonas hydrophila bacterial illness. As* we know, betel leaf is a traditional plant whose existence is rare. They need help to come by. Fish (Sumarya, 2020) showed that the methanol content of betel leaves could be immunomodulatory. Betel leaf extract has the chemical element Hydroxicavikol (HC), which also has a role as an immunomodulator.

4-*allylprocatechol* is a secondary metabolite that can act like other secondary metabolites in various plants. The hydroxychavicol in betel leaves is the main phenolic compound (Sumarya, 2020). The phenol content in betel leaves can help the wound-healing process and increase the number of macrophages migrating to the wound area, thereby increasing the production of cytokines which will activate fibroblasts in the wound tissue (Deru *et al.,* 2019).

METHODOLOGY

This research activity was carried out for 60 days with a tilapia seed size of 7-10 cm. This research was carried out using an experimental method using a Completely Randomized Design (CRD) with 5 treatments with 3 replications each. The treatment arrangement used was a dose of betel leaf extract (P1) 0.4 ml, (P2) 0.6 ml, (P3) 0.8 ml, (P4) positive control, (P5) negative control, and continued with the challenge test using *Aeromonas hydrophila* bacteria, which were injected on the back of the fish as much as 4 x 10 4 CFU/ml per head. The parameters observed included SR, SGR, FCR, total length, erythrocytes, hemoglobin, hematocrit, leukocytes, differential leukocytes, total bacteria, and water quality.

RESULT

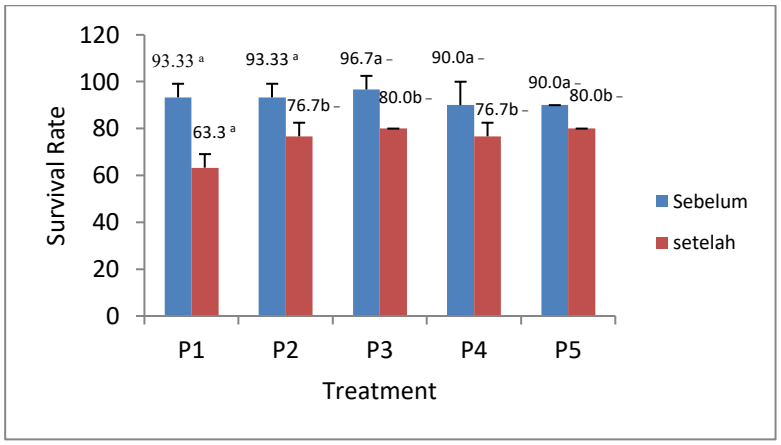

Figure 1. Survival Rate (SR)

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given, betel leaf extract gave significantly different results (P< 0.05).

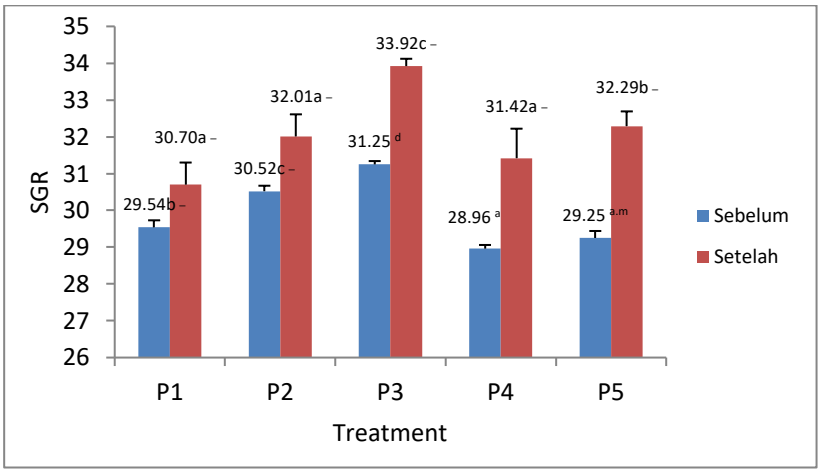

Figure 2. Specific Growth Rate (SGR)

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

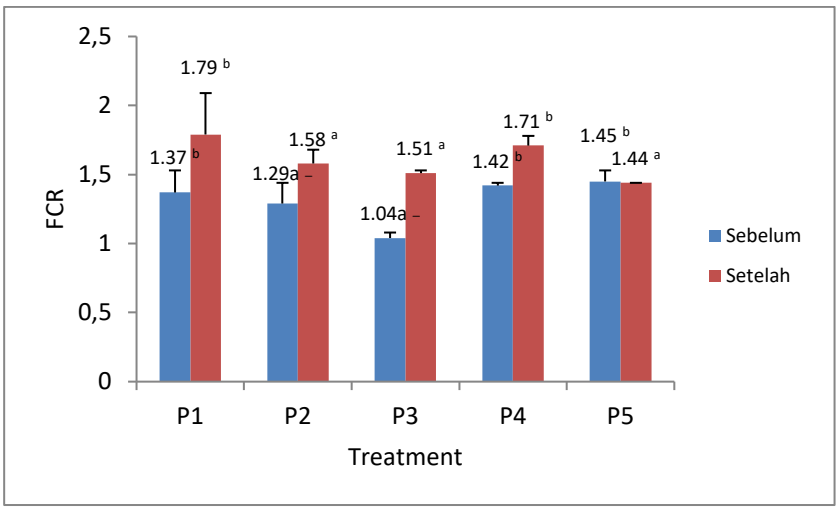

Figure 3. Feed Conversion Ratio (FCR)

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

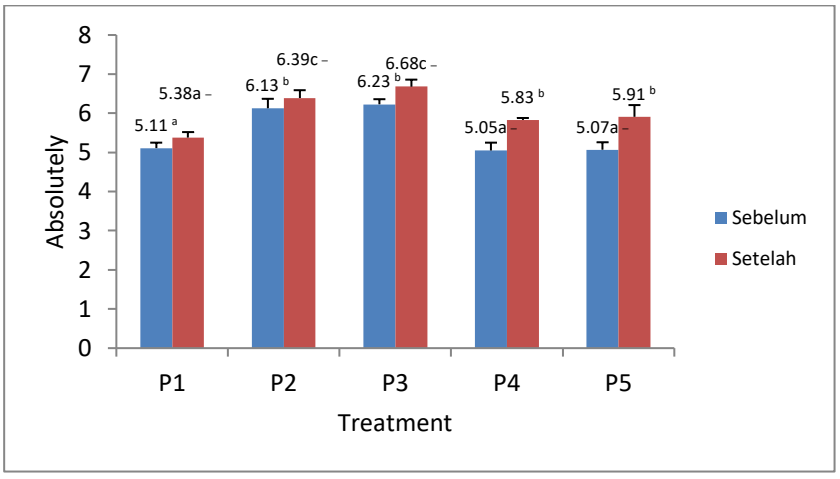

Figure 4. Absolute Length Growth

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

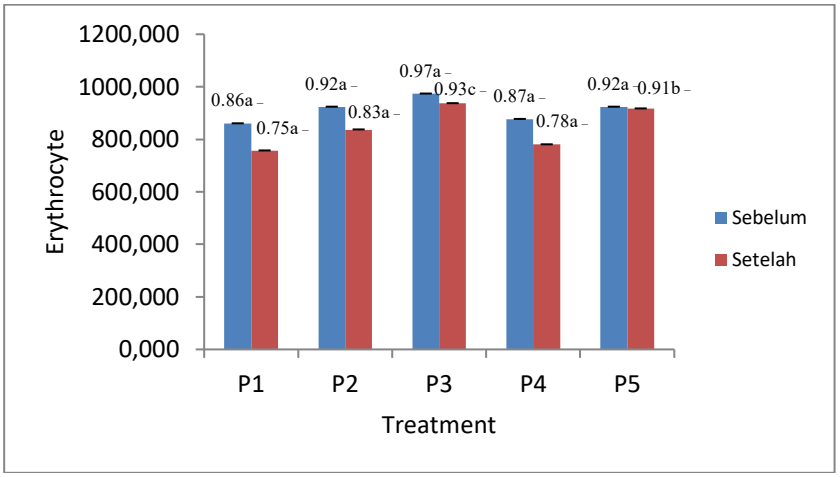

Figure 5. Erythrocytes

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

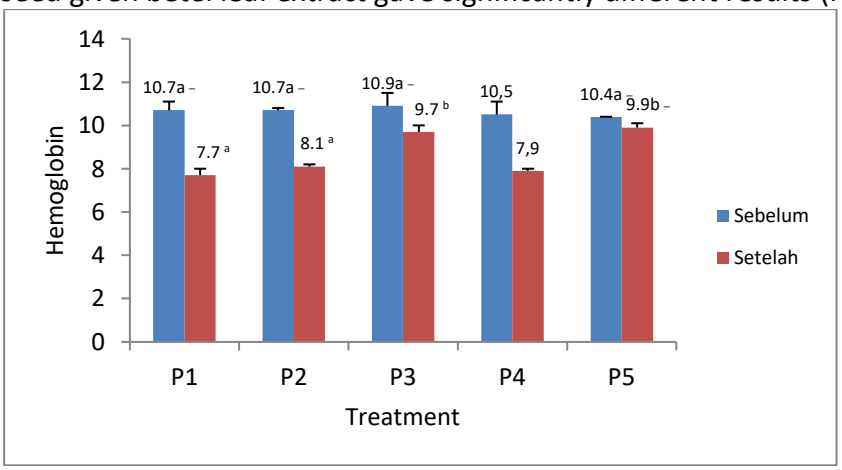

Figure 6. Hemoglobin

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

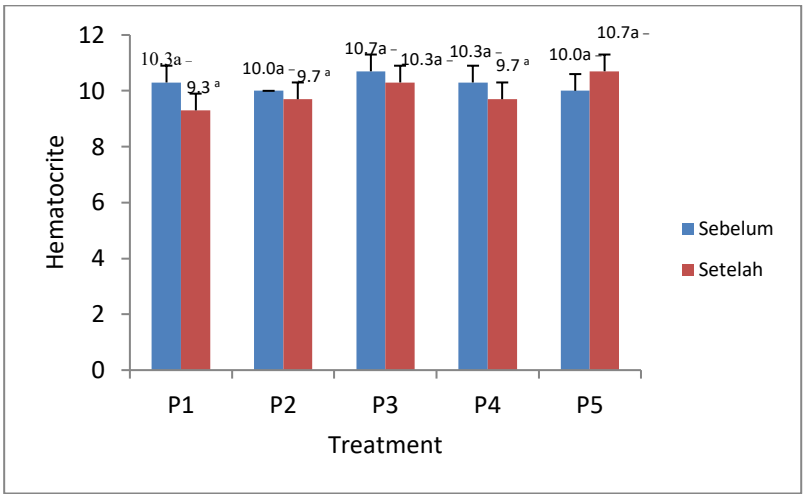

Figure 7. Hematocrit

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract had no significant effect.

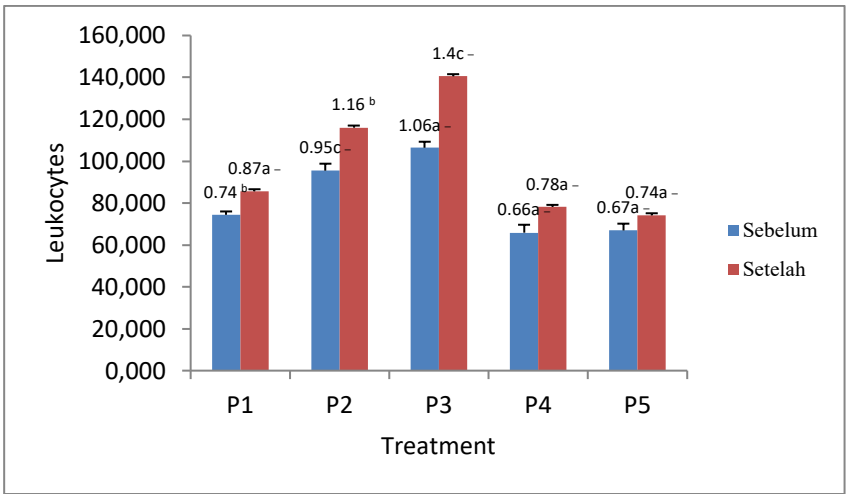

Figure 9. Leukocytes

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

(Table 1) Total leukocyte differential values before the challenge test

Treatment	Basophils	Eosinophils	Neutrophils	Lymphocytes	Monocytes	Platelets
P1	0%	2% [a]	7% [a]	66% [a]	1% [a]	24% [a]
P2	0%	1% [a]	6% [a]	70% [b]	1% [a]	22% [a]
P3	0%	1% [a]	6% [a]	70% [b]	1% [a]	21% [a]
P4	0%	3% [a]	7% [a]	63% [a]	1% [a]	26% [a]
P5	0%	2% [a]	8% [a]	64% [a]	1% [a]	26% [a]

(Table 2) The number of leukocyte differential values after the challenge test

Treatment	Basophils	Eosinophils	Neutrophils	Lymphocytes	Monocytes	Platelets
P1	1% [b]	3% [b]	9% [b]	61% [a]	5% [c]	20% [a]

P2	1% [b]	4% [c]	10% [b]	60% [a]	3% [a]	21% [a]
P3	1% [b]	3% [b]	7% [a]	65% [b]	4% [a]	20% [a]
P4	1% [b]	2% [a]	10% [b]	61% [a]	5% [b]	23% [a]
P5	0% [a]	1% [a]	4% [a]	64% [b]	3% [a]	28% [b]

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

Total Bacteria	P1	P2	P3	P4
Initial Challenge Test	4×10^4	4×10^4	4×10^4	4×10^4
End of Challenge Test	74×10^2	16×10^2	7×10^1	52×10^2

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract gave significantly different results (P< 0.05).

NO	Parameter	P1	P2	P3	P4	P5	P6	Optimum Value
1	Temperature	26,1-27	27.2-27.8	27.2-27.9	27.9-28.7	27.2-28.7	27.9-28.6	25-32 ^{0}C (BSNI, 2009)
2	pH	7,2-7,9	7,4-7,9	7,3-7,7	7,4-7,7	7,4-7,7	7.5-7.8	6.5-8.5 (BSNI, 2009)
3	DO	5,6	7-7,4	5-6,5	5.1-5.5	5-5,8	5,8-5,9	$\geq$3 mg/L (BSNI, 2009)

The results of the *analysis of variance* (ANOVA) showed that before and after the challenge test on tilapia seed given betel leaf extract had no significant effect.

DISCUSSION

The results of this study indicated that the administration of betel leaf extract on rearing tilapia seeds had a significant effect (< 0.05) on the survival of tilapia seeds. The best value of the survival rate before the challenge test is found in P3 at 96.7 %, and the lowest is in P4 and P5 at 90 %. Then the highest SR value after the challenge test was found in P3 at 80% and the lowest in P1 at 63.3%.

Betel leaf extract with the chemical element hydroxychavicol also functions as an immunomodulator. The difference influences the increasing SR of tilapia seed in the level of extract doses given in each treatment to fish affected by bacteria, and this is caused by the more substantial Hydroxicavicol content so that it can increase the immune system in fish so that the defense against disease attacks is getting better. This is the opinion (Sumarya, 2020).

Judging from the data, the best value before the challenge test was at P3 of 31.25%, and the lowest was at P4 of 28.96%. This value was still in good condition because the fish seeds were still not infected with bacteria, but after the challenge test, their growth began to decline slightly. It was seen from the data that the highest value at P3 was 33.92%, and the lowest was at P1 at 30.70. This was because the dose level of betel leaf extract was higher at P3 than P1, so it could affect the body's defense system to fight bacterial infections, which could directly affect the appetite of tilapia seeds that were reared so that growth slows down. However, this value is still within the normal range in the opinion (Suryanto & Suprianto, 2021) that several studies on determining the amount of protein in feed for economically important

fish for aquaculture activities have been carried out and obtained protein content values that varied between 30% -55% depending on species, age and cultivation method applied.

Based on the opinion of (Aisiah et al., 2016), the higher the dose of betel leaf extract, the less the number of bacteria that can survive. This indicates that increasing the dose can increase the active ingredient, which acts as an antibacterial, so its efficacy in preventing bacterial growth is more significant.

The lowest (best) FCR value during the study period before the challenge test was found in P3 with a dose of 0.8 ml of betel leaf extract with a value of 1.04 g, while the highest value was in P5 without giving betel leaf extract with a value of 1.45 g this is quite good because the response of fish to good feed is different when after the challenge test the response of fish to low feed can be seen from the data the lowest value is found at P5 of 1.44 g and the highest at P1 of 1.79 g this is caused by the influence *Aeromonas hydrophila* infection which causes a decrease in appetite. This is the opinion of (Ihsanudin et al., 2014) that the feed conversion value is quite good. The range is between 0.8-1.6, where 1 kg of tilapia consumption is obtained from 0.8-1.6 kg of feed. (Mentari & Khairil, 2016) The use of betel leaf extract in tilapia can inhibit bacterial populations due to the phenol content in betel leaf extract, which is as much as 30%, where phenol compounds denature bacterial cell proteins resulting in the cessation of bacterial cell metabolic activities due to all metabolic activities. Enzymes, namely proteins, catalyze bacterial cells.

During maintenance before the challenge test, the highest value was found at P3 with a value of 6.23 cm, while the lowest value was found at P4 with a value of 5.05 cm. This value was considered reasonable because the fish were not infected with bacteria, so the fish were still in good health. Then, P3, with a value of 6.68 cm and the lowest at P1 of 5.38 cm, is different after the challenge test. The growth slows down when seen from the data due to bacterial infection resulting in decreased appetite and affecting the growth of tilapia fry. This is the opinion of Aziz & Barades (2021) that external and internal conditions influence fish growth. External factors consist of feed and water chemistry physics, then internal factors consist of genetics and physiology, for example, fish health.

Red blood cells, but this value is still in average condition. From the results of observations, the number of erythrocytes before the challenge test was highest at P3 with a value of 0.97 x 10 5, and the lowest was at P1 with a value of 0.86 x 10 5 cells / mm 3. Then after a challenge test, the highest score was in P3 with a value of 0.93 cells/mm 3, and the lowest was in P1 with a value of 0.75 x 10 5 cells/mm 3. High and low numbers of erythrocytes are affected by the ability of betel leaf extract to fight infectious diseases because red blood cells are essential in transporting oxygen in the blood. If *Aeromonas hydrophila bacteria* succeed in infecting tilapia seeds, they can attack the respiratory system, reducing the number of cell pieces. This is the opinion of Hartika *et al.* (2014) that the average value of the number of fish erythrocytes, in general, is in the range of 20,000-3,000,000 cells/mm 3.

Based on hemoglobin observations that have been carried out where the highest value is found at P3, which is equal to 10.9 (g%) with a dose of 0.8 ml of betel leaf extract, and the lowest is found at P5 of 10.4 (g%) this difference in value is due to the influence of betel leaf extract which functions to boost the immune system so that it can fight attacks by *Aeromonas hydrophila bacteria*. Meanwhile, after examining the symptoms caused by these bacteria, such as bruises on the gills, which made it difficult for the fish to breathe, this directly affected the oxygen in the blood. After the challenge test, the highest value was at P5 of 9.9 (g%), and the lowest was at P1. 7.7 this value is below normal levels due to the influence of infection with *Aeromonas hydrophila,* which indicates that the respiratory system of tilapia fry has

begun to be disrupted. Referring to the opinion of Fauzan et al. (2017), the average amount of hemoglobin content in the body of tilapia is in the range of 10-11.1 (g%)

Based on these data, it can be seen that between the treatment with the administration of betel leaf extract and the control treatment, the value was not significantly different because the extract administration did not have a significant effect on the total hematocrit value belonging to red blood cells, betel leaf extract was used to increase fish immunity and which functioned for the body's defense are white blood cells. However, this value is still in the normal range. This is the opinion of (Hartika et al., 2014) that the average value of the hematocrit content in the fish body is in the range of 5-60%.

From the results of observations, the highest leukocyte count before the challenge test was found in P3 with a value of 1.06 x 10 5 cells/mm 3, and the lowest was in P4 with 0.66 x 10 5 cells/mm 3. Then after being challenged, the highest score was found at P3 with a value of 1.4 x 10 5 cells/mm 3, and the lowest was found at P5 with a value of 0.74 x 10 5 cells/mm 3. The high number of leukocytes was due to the addition of 0.8 ml of betel leaf extract at P3, and the low number of values did not include the addition of betel leaf extract at P4 and P5. However, these values are within the normal range. This is the opinion of (Fauzan et al., 2017) that when the value of white blood cells in the fish's body is low, the fish quickly becomes infected with bacteria, and the value of tilapia leukocytes usually is in the range of 20,000-150,000 cells/mm 3.

Based on the opinion of Zainuddin *et al.* (2018), betel leaf extract contains ingredients that act as immunostimulants that can increase the non-specific defense of tilapia, the main chemical ingredients of betel leaf are attic oil, vitamins, organic acids, amino acids, sugars, tannins, fats, starch, and carbohydrates act as antibacterial, antiseptic and immunostimulant.

From these data, it can be seen that before the fish were infected with bacteria, the differential leukocyte value was in an excellent value range because at P1, P2, and P3, the fish were given betel leaf extract where betel leaf can function to boost the immune system of fish so that it causes the value of the types of leukocytes increased because white blood cells play an essential role in defense of the fish body. Then the differential leukocyte value after infection with *Aeromonas hydrophila bacteria* decreased in the type of blood cell whose function was for immunity. However, the value increased in the type of blood cell whose function was to respond and fight against bacterial attacks. According to (Hartika et al., 2014), some fish bodies' efforts to defend themselves from disease infection is to eradicate these pathogens through phagocytic processes.

Fish reared were injected with *Aeromonas hydrophila bacteria* at a density of 10 4 at P1, P2, P3, and P4. This was done to test the ability of betel leaf extract at different doses to fight disease by increasing the immunity of fish and the effects caused by these bacteria. Then the number of bacteria after the challenge test was calculated using the plate count method to determine the ability of each dose of betel leaf extract to fight disease. Growth was carried out by taking fish liver from each treatment, and after growing, the best value was at P3 with the highest total bacteria. few, namely 7 x 10 1 cfu/g and P1 with the highest value with a total of 74 x 10 2 cfu/g. According to BSN (2009) *in* (Kamal et al., 2016), the maximum limit for the number of microorganisms or microbes that can be accepted in the fish body is 1 x 10 2 cfu/g.

All treatments given indirectly affected the life and growth of tilapia fry during the rearing period, including water quality. Judging from the data on water quality values such as temperature, DO, and pH, they were still in normal conditions.

CONCLUSION

The best treatment was found in treatment 3 with a dose of 0.8 ml of betel leaf extract, which naturally increased the immune system in tilapia seeds. This was due to the higher the betel leaf extract content, the more active ingredients for antibacterial, and the resistance to pathogens became good so that it can overcome infections from *Aeromonas hydrophila bacteria* which can affect the survival rate and growth of tilapia fry.

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