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Immunomodulator effect of fish herb solution on bonylip barb (*Osteochillus vitattus*) Infected by *Aeromonas hydrophila* bacteria

Rosidah, Muhammad Fathi Dhiaulhaq, Kiki Haetami and Roffi Grandiosa

Abstract
The research aims to determine the addition of herbal solutions on feed which could improve the immune system of Bonylip barb infected by *Aeromonas hydrophila* that indicated from the increased number of leukocyte and clinical signs. The method used in this research was Completely Randomized Design (CRD) with five treatments and three replications. The treatments are addition of herbal solutions on feed with the dose of 0 mL/kg (A), 100 mL/kg (B), 200 mL/kg (C), 300 mL/kg (D), 400 mL/kg (E). The treatments was maintainance for 21 days. At the end of maintenance, bonylip barb was infected by *Aeromonas hydrophila* with a density of 10^8 cfu/mL. Observations number of total leukocytes counted before treatment, after treatment and after infected by *Aeromonas hydrophila*. The observations of clinical signs were damage surface of the body of fish test, shocks response and feeding response. The Data of total leukocytes count after treatment and after challenged were analyzed by ANOVA and followed by Duncan's test, while data of the clinical signs were analyzed descriptively. The results showed that the addition of herbal solutions concentrate as much as 200 mL/kg was the most effective to improve immune response of bonylip barb. During 21 days of treatments, total leukocytes count increased 36,92±2,34% from 44867 cell/mm^3 to 71067 sel/mm^3. Clinical symptoms were seen in the experiment from fish fed with addtional herb solution, however no significant damage was shown while recovery after experimental infections was faster compared with other treatments.

Keywords: *Aeromonas hydrophila*, Bonylip barb (*Osteochilus vittatus*), Herbs solution, Leukocyte

1. Introduction
Aquaculture production in Indonesia has continued to increase over the last few years. Based on data from the Indonesian Ministry of Maritime Affairs and Fisheries, total production in 2015 reached 15.63 million tons and the value continued to increase until total production in 2017 reached 16.11 million tons (Statistik.kkp.go.id). Efforts to increase the production of fish farming are carried out with an intensification system in ponds and floating net cages by increasing the density of the fish being cultivated. These conditions can cause a decrease in water quality and cause fish to experience stress [7].

Fish under stress conditions can be easily attacked by disease-causing pathogens. One of the opportunistic bacteria that easily attack fish under stress is *Aeromonas hydrophila* [10]. These bacteria can cause mortality rates in fish reaching 95% with a density of 10^8 cfu/mL [15]. The disease caused by the bacterium *A. hydrophila* is called Motile Aeromonas Septicemia (MAS), with clinical symptoms of inflammation, bleeding to ulcers on the surface of the fish's body, bulging stomach, enlarged eyes, and ending in death [2].

Disease control in fish can be done by giving chemicals that act as an antibacterial, namely antibiotics. However, the continuous use of antibiotics will have an unfavorable impact, including resistance of pathogens to these antibiotics, polluting the environment, and causing residues on the fish body so that it is dangerous for humans who consume them [17].

The most appropriate action so that fish do not get sick is to take preventive measures, by increasing the fish's natural body resistance. Giving immunostimulants is one way to increase the fish body's resistance to disease. Immunostimulant materials that are safer, cheaper, easily available, and environmentally friendly are derived from plants (herbs). Several studies have proven that herbal ingredients can increase the fish's body resistance, which is indicated by increasing the number of white blood cells. According to Harikrishnan *et al.* (2011) the use of several types of herbal ingredients as fish herbs is known to have a positive impact on the

fish's immune system, based on the active substances contained in these plants, including flavonoids, saponins, and triterpenoids. The composition of herbal ingredients as a constituent of fish herbs, including turmeric, ginger, Javanese ginger, greter galingale, noni, tropical almond leaves, betel leaves and stems, molasses, and yeast.

This study aimed to determine the addition of the concentration of fish herbs into the feed that could increase the body's resistance of nilem fish to attack by *A. hydrophila*, seen from the increase in the number of white blood cells and the clinical symptoms that arise.

2. Materials and Methods

2.1 Material

The materials used included *Osteochilus vitattus* as test fish as many as 400 5-7 cm in size from the Ciparanje Cultivation Laboratory, FPIK Unpad, isolates of *A. hydrophila* bacteria from the Research Institute for Freshwater Aquaculture and Fisheries Extension Bogor, herbal medicine for fish. with the composition of turmeric, ginger, javanese ginger, greter galingale, noni, tropical almond leaves, betel leaves and stems, molasses, yeast and commercial feed PF 500, agar medium NA (Nutrient Agar), NB (Nutrient Brooth), distilled water and turk's solution.

2.2 Method

The research method used was Completely Randomized Design (CRD) with five treatments and three replications. The treatment used was the addition of fish herbs into the feed with a concentration of:

A: without the addition of fish herbs (control)
B: 100 mL/kg
C: 200mL/kg
D: 300 mL/kg
E: 400mL/kg

The fish were given herbal medicine for 21 days, then challenged with *Aeromonas hydrophila* bacteria with a density of 10^8 CFU/ml by intramuscular injection. Observations of clinical symptoms were carried out for 14 days after being challenged. Preparation of the test feed was started by weighing 3% of the weight of the test fish, then mixed with fish herbs using a disposable syringe according to the treatment, allowed to stand for 12 hours. Feeding was carried out twice a day, at 08.00 and 16.00 WIB.

2.3 Culture of *Aeromonas hydrophila* isolate

Bacterial isolates were inoculated on Nutrient Agar (NA) media using ose needle with scratches on the culture media, then incubated at 30ºC for 24 hours. The bacteria that grew were taken using a 1 ose needle, then dissolved in Nutrient Broth (NB) liquid medium and incubated using a shaker incubator at 37ºC at 150 rpm for 24 hours. The results of culture using NB media were taken 2 mL and put into a cuvette and then the absorbance value was seen on the spectrophotometer until it showed the number 0.235 or equivalent to a density of 10^8 cfu/mL.

2.4 Challenge Test with *Aeromonas hydrophila*

The test fish (*Osteochilus vittatus*) that had been treated with herbal medicine were then challenged with *A. hydrophila* bacteria at a density of 10^8 cfu/mL through intramuscular injection. The bacteria injected were 0.1 mL/individual. Observation of clinical symptoms was carried out for 14 days after being challenged.

2.5 Observation of White Blood Cells

Observations of blood samples were carried out three times, namely before being given treatment, after being treated for 21 days and after 14 days of challenge test with *Aeromonas hydrophila*. The procedure for observing white blood cells follows the procedure of Blaxall and Daisley 1973.

2.6 Data Analysis

The average percentage increase in the white blood cell count of *Osteochillus vitattus* was analyzed by the F test, if there was a significant difference, it was continued with Duncan's test with a 95% confidence level to see the difference between treatments. Data on clinical symptoms including body surface damage, response to feed and shock were analyzed descriptively.

3. Results and Discussion

3.1 White Blood Cell Count after Treatment

The test fish after being treated with fish herbs for 21 days experienced an increase in the number of white blood cells (leukocytes) in all treatments. The increase in the number of white blood cells for each treatment was different (Figure 1).

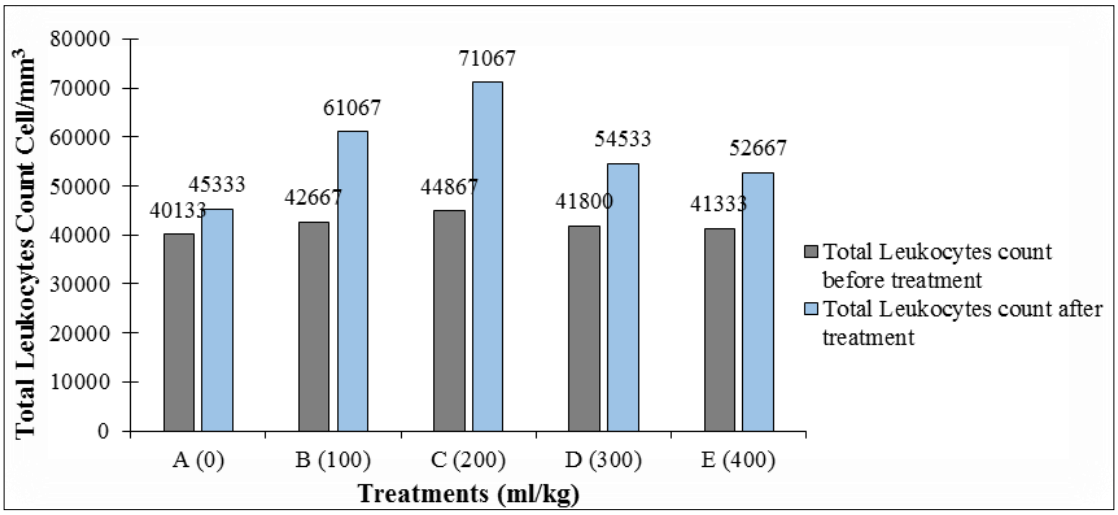

Fig 1: White Blood Cell Count Before and After Giving Fish Herb

In Figure 1, it can be seen that the average range of the white blood cell count of the tested fish before being treated was 4.01 x 10^4 - 4.48 x 10^4 cells/mm, after being given the treatment, it increased to 4.53 x 10^4 - 7.10 x 10^4 cells/mm^3 cells/mm. According to Mulyana and Mumpuni (2016) the number of white blood cells was around 7.05 x 10^4 cells/mm^3 which was still within normal limits. The highest number of white blood cells occurred in fish that were given fish herbs with a concentration of 200 mL/kg, at a concentration of more than 200 mL/kg there was a decrease in the number of white blood cells, but the number was still higher than the control (treatment A). The percentage increase in the white blood cell count for each treatment was different for each treatment (Table 1).

Table 1: Average Increase in White Blood Cell Count of *Osteochillus vitattus* After Giving Fish Herbs

Treatment	Average White Blood Cell Count (cells/mm^3)		
	Before giving herbal medicine fish	**After giving herbal medicine fish**	**Percentage increase in white blood cell count (%)**
A (Control)	40133	45333	11,41±1,42^{a}
B (100 mL/kg)	42667	61067	30,02±3,49^{cd}
C (200 mL/kg)	44867	71067	36,92±2,34^{d}
D (300 mL/kg)	41800	54533	23.30±7,44^{bc}
E (400 mL/kg)	41333	52667	21,37±2,33^{b}

Based on Table 1, the test fish that were not given herbal medicine (control) had the lowest percentage increase in the number of white blood cells compared to other treatments that were given herbal medicine. The results of the analysis of variance showed that the administration of fish herbal medicine had a significant effect on increasing the white blood cell count of nilem fish. The Duncan test results at a 5% confidence level in treatment A were significantly different from the other treatments and produced the lowest percentage increase in white blood cells. This shows that the fish herbal medicine has an immunomodulatory effect that can increase the body's resistance of *Osteochillus vitattus*, which is indicated by an increase in the number of white blood cells. As according to Yonar *et al.* (2019), white blood cells can be used as an indicator of the body's defense against disease. Curcumin contained in turmeric and temulawak can increase the immune response in fish seen from the number of white blood cells. Research conducted by Yonar *et al*. (2019), showed that giving curcumin to feed by 2% increased the white blood cell count of rainbow trout by 29.04 x 10^3 cells/mm^3 and was significantly different from the control treatment, which was 24.16 x 10^3 cells/mm^3. Nya and Austin (2009), in their research showed that the addition of 0.5 g of ginger to 100 grams of feed had the highest leukocyte value compared to other treatments, which amounted to 243.7 x 10^3 cells/mm^3 in rainbow trout. The results of research by Abdel-Tawwab and Abbas (2017), showed that supplementation of turmeric flour at an optimal dose of 2.0 g/kg of feed could increase feed utilization for carp (*Cyprinus carprio*). This is because the curcumin in turmeric flour inhibits the growth of pathogens in the digestive organs, thereby increasing the absorption of nutrients in feed digestion. Treatments B1 (100 mL/kg) and C (200 mL/kg) did not show a significant difference, but the highest percentage increase was found in treatment C, which was 36.92±2.34%. This is because the active ingredients contained at a concentration of 100 mL/kg are still insufficient to induce white blood cells. Treatment D (300 mL/kg) and E (400 mL/kg) did not show a significant difference and experienced a decrease in the percentage increase in white blood cells compared to treatments B and C. It is possible that at these concentrations there was a change in the taste (palatability) of the feed, because of the herbal ingredients it contains such as ginger, japanese ginger, greater galingale, noni, tropical almond leaves, betel leaves are too high, so that feed cannot be consumed optimally, it can be seen from the remaining feed in the aquarium. The description above shows that the concentration of fish herbal medicine at 200 mL/kg (treatment C) is the best concentration to increase fish resistance.

3.2 Increased White Blood Cell Count after Challenge Testing with *A. hydrophila*

After the *Osteochillus vitattus* were treated with fish herbal medicine for 21 days, then a challenge test was carried out with *A. hydrophila* bacteria. Based on observations after being challenged with *A. hydrophila*, the white blood cell count showed higher results than the white blood cell count before being challenged. The graph of the increase in the number of white blood cells after being challenged can be seen in Figure 2.

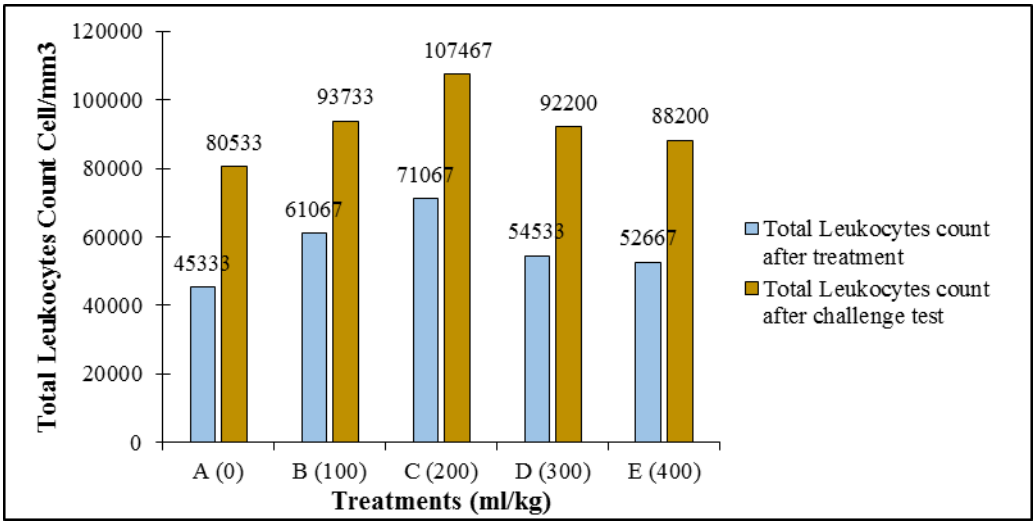

Fig 2: White Blood Cell Count after Challenge Testing with *A. hydrophila*

The lowest white blood cell count occurred in treatment A (control) which was 8.05 x 104 cells/mm^3 and the highest in treatment C (200 mL/kg) which was 10.7 x 10^4 cells/mm^3. The percentage increase in white blood cell count in treatment A (control) was 43.71±0.06%, while the percentage increase in white blood cell count in fish treated with herbal medicine (treatments B, C, D, and E) ranged from 33.85± 0.01% - 40.78±0.03%. Based on the results of the analysis of the variety of herbal medicine, there was a significant effect on the increase in the number of white blood cells after being challenged. The results of Duncan's test at a 5% confidence level of treatments A, D and E did not show a significant difference but were significantly different from treatment C. Treatment B was not significantly different from treatment C (Table 2).

An increase in the number of white blood cells in *Osteochillus vitattus* after being challenged showed an infection from *A. hydrophila* that attacked the fish's body. According to Nugroho *et al.* (2017), that the *Betta* sp. which was challenged with *A. hydrophila* experienced an increase in white blood cells in the control treatment with the highest value of 12.36 ± 0.19 x 10^3 cells/mm^3.

Table 2: Average Increase in White Blood Cell Count of *Osteochillus vitattus* After Infected with *A.hydrophila*

Treatment	Average White Blood Cell Count (cells /mm^3)		
	Before challenge test	After challenge test	Percentage increase in white blood cell count (%)
A (Control)	45333	80533	43,71±0,06c
B (100 mL/kg)	61067	93733	34,76±0,05ab
C (200 mL/kg)	71067	107467	33,85±0,01a
D (300 mL/kg)	54533	92200	40,78±0,03bc
E (400 mL/kg)	52667	88200	40,39±0,03bc

Based on Table 3, the percentage value of the increase in white blood cells was highest in treatment A (control), although it was not significantly different from treatments D and E. This showed that the fish were still in a sick condition due to the attack of *A. hydrophila*. In addition, it may be caused by the high content of saponins in the fish herbs in treatments D (300 mL/kg) and E (400 mL/kg) causing the immunostimulants to not work optimally. According to Hashemi and Davoodi (2012) [9], saponins in amounts that exceed normal limits will act as immunosuppressors, namely substances that can reduce the immune system.

Treatment C had the lowest increase in white blood cells, although it was not significantly different from treatment B. This showed the lowest increase in white blood cells because fish in this treatment could use white blood cells better to fight the attack of *A. hydrophila* bacteria. Yakubu *et al.* (2020), stated that fish given tropical almond leaf extract at a dose of 62.5 g/kg had high phagocytic activity in hybrid red tilapia after four weeks of rearing and challenged with *S. agalactiae* bacteria. This indicates that the administration of fish herbal medicine containing tropical almond leaves functions as an immunostimulant seen from a good immune response.

3.3 Clinical Symptoms of Damage to the Body Surface of Infected *Osteochillus vitattus A. hydrophila*

The results of observations of body surface damage that occurred in test fish infected with *A. hydrophila* bacteria were seen 24 hours after infection. Damage to the body surface was observed in the form of peeling scales (Figure 3c), dropsy (Figure 3b), ulcers (Figure 4), hemorrhagic (Figure 3d), and inflammation. Based on the results of observations of body surface damage that appears different for each treatment (Table 3).

Table 3: Observations of Body Damage on *Osteochillus vitattu*

Days to	Treatment														
	A			B			C			D			E		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	ac	ab	ab	ab	ab	ac	a	a	b	bc	ac	abc	ab	ab	ac
2	abc	abcd	abc	ab	ab	ac	ab	ac	b	bc	ac	abc	ab	ab	ac

3	abcd	abcd	abcd	abc	ab	abc	ab	ac	bc	abc	acd	ab	abc	ab	abc
4	abcd	acd	acd	acd	abc	ab	ab	abc	abc	abcd	acd	abd	abc	abc	abc
5	abd	acd	acd	acd	abc	abd	abc	ab	ac	acd	acd	abd	bcd	acd	abcd
6	abd	acd	acd	ad	ad	ad	bc	ab	ac	acd	abd	abd	bcd	acd	acd
7	M	M	acd	ad	ad	ad	c	a	a	M	bd	ad	bd	cd	ad
8	M	M	M	a	ad	d	c	a	a	M	bd	ad	bd	cd	ad
9	M	M	M	a	a	d	c	a	a	M	bd	d	d	d	ad
10	M	M	M	a	a	d	c	a	a	M	d	d	M	M	d
11	M	M	M	-	a	-	-	-	-	M	d	d	M	M	d
12	M	M	M	-	-	-	-	-	-	M	d	d	M	M	d
13	M	M	M	-	-	-	-	-	-	M	d	d	M	M	d
14	M	M	M	-	-	-	-	-	-	M	d	d	M	M	d

Information: (a) Scales peeling, (b) dropsy, (c) Hemorragic, (d) ulcers, (-) no clinical symptoms

Fig a: Healthy Fish

Fig b: Dropsy

Fig c: Exfoliating Scales

Fig d: Hemorrhagic/Hyperemia

Ulcers appeared for the first time in treatment A on the 2nd day (Figure 4), while in other treatments the ulcers occurred on the 4th day for treatment D and day 5 for treatment E. Treatments that did not show ulcers were Treatment C. This is in accordance with the statement of Yardimci and Aydin (2011), skin redness or hyperemia is one of the early clinical symptoms of *A. hydrophila* infection, the mechanism of *A. hydrophila* is to enter receptor cells and break down host cells by producing extracellular enzymes. such as hemolysins, proteases, and elastases that cause inflammation and eventually develop into ulcers (Figure 4).

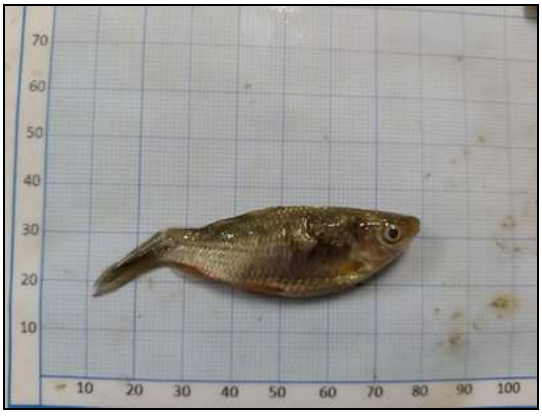

Fig 4: Ulcer

All treatments at the beginning of infection with *A. hydrophila* bacteria died. Starting from day 7, the test fish in treatment A experienced total death, while the other treatments began to experience total death on day 7 for treatment D and day 10 for treatment E. Test fish in treatment A experienced more severe body damage, this shows that the test fish whose body resistance is not induced by immunostimulants has low body resistance to attack by *A. hydrophila*, as seen from the lowest white blood cell count (Figure 1, Table 1). The herbal content found in fish herbs (flavonoids, alkaloids, curcumin, volatile, saponins and terpenoids) can activate the body's defense system in fish by increasing the number of white blood cells. Increasing the number of white blood cells (leukocytes) will reduce the activity of *A. hydrophila* bacteria by phagocytosing these bacterial cells so that the infection power is lower and can reduce the number of infected fish [6].

3.4 Clinical Symptoms Response to Shock

Based on the results of observations of fish responses to shock varied for each treatment (Table 4). Based on Table 4, on the first day of observation, treatments A (control) and D (300 mL/kg) did not show a response to shock, while treatments B (100 mL/kg), C (200 mL/kg) and E (400 mL/kg) on showing a response even though the response is still low. A decrease in response to each treatment after being tested against *A.*

hydrophila was also marked by the behavior of fish that were seen swimming in a tilted position, jumping to the surface of the aquarium, and swimming in groups around the aeration [8].

Table 4: Results of Observation of Responses to Surprises

Days to	Treatment														
	A			B			C			D			E		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	+	-	-	+	+	-	-	-	-	+	-
2	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+ +	+	+	+	+	+	+
4	+	+	+	+ +	+ +	+	+ +	+ +	+ +	+	+	+	+	+	+
5	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+	+	+	+
6	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+ +	+	+	+
7	M	M	+	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+ +	+	+	+
8	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	+	+	+
9	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	+	+	+ +
10	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	M	M	+ +
11	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	M	M	+ +
12	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	M	M	+ +
13	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	M	M	+ +
14	M	M	M	+ +	+ +	+ +	+ +	+ +	+ +	M	+ +	+ +	M	M	+ +

Information: (-) No response, (+) Low response to shock, (++) Response to normal shock, (M) dead

The normal response began to be seen in treatment C on day 3, while in other treatments it was seen on day 4 for treatment B, on day 6 on treatment D and day 9 on treatment E. Treatment A (control) was still showed a low response to shock on day 6 and total death began on day 7 until the end of the observation. Total mortality also occurred in treatment D on day 8 and treatment E on day 10.

Treatment C showed a good effect from the administration of immunostimulants in the form of herbal fish which was seen from the response to shock starting from normal on the 3rd day until the end of the observation. As Hardi *et al*. (2017), the addition of herbal ingredients (*Boesenbergia panduratu, Solanum ferox, Zingiber zerumbet*) through feed showed that fish swimming returned to normal after being infected with *A. hydrophila* and *Pseudomonas* sp. The opposite was seen in treatments D (300 mL/kg) and E (400 mL/kg), although both treatments were given herbal fish in the feed, it is possible that the high content of saponins in the herbal medicine could cause the fish to be poisoned so that the response to shock was low. According to Ardulanisa (2017), high doses of noni fruit extract can cause saponins which have the potential to be toxic as well as antibacterial in Tawes fish (*Barbonymus gonionotus*).

3.5 Clinical Symptoms of Response to Feed

Observation of the response of *Osteochillus vitattus* to feed was carried out for 14 days after infection with *A. hydrophila* bacteria. These observations were made to determine the metabolic changes that occur due to infection with *A. hydrophila* bacteria in the fish's body through the response to feed. According to Roy *et al*. (2018), the target organ of fish attacked by *A. hydrophila* bacteria is the kidney which can change metabolic processes in the body. Based on the observations, the response of the test fish to the feed varied in each treatment (Table 5).

Table 5: Results of Observation of Responses to Feed

Days to -	Treatment														
	A			B			C			D			E		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+ +	++	+	+	+	+	+	++
3	+	+	+	+	+ +	+ +	+ +	+ +	+ +	+	+	+	+	+	+ +
4	+	+	+	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+	+	+	+ +
5	+	+	+	+ +	+ +	+ +	+ +	+ +	+ + +	+ +	+	+ +	+ +	+ +	+
6	+	+	+	+ +	+ +	+ +	+ +	+ +	+ + +	+ +	+	+	+ +	+ +	+
7	M	M	+	+ +	+ +	+ +	+ + +	+ +	+ + +	+	+ +	+	+ +	+ +	+
8	M	M	M	+ +	+ +	+ + +	+ + +	+ + +	+ + +	M	+ +	+ +	+	+	+
9	M	M	M	+ +	+ +	+ + +	+ + +	+ + +	+ + +	M	+ +	+ +	+	+	+ +
10	M	M	M	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	M	+ +	+ +	M	M	+ +
11	M	M	M	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	M	+ + +	+ +	M	M	+ +
12	M	M	M	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	M	+ + +	+ + +	M	M	+ + +
13	M	M	M	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	M	+ + +	+ + +	M	M	+ + +
14	M	M	M	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	M	+ + +	+ + +	M	M	+ + +

Information: (+) Low response, (++) Medium response, (+++) Normal response to feed, (M) Dead

Based on Table 5, on the first day of observation after being challenged with *A. hydrophila* it showed a decrease in response to feed. Seen in treatment C (200 mL/kg) already showed a moderate response to feed, while in other treatments showed a low response to feed. Normal response to feeding began to be seen on day 5 for treatment C, on treatment B (100 mL/kg) on day 7, on day 11 for treatment D (300 mL/kg), and on day 12 for treatment E (400 mL/kg). Meanwhile, treatment A (control) from the beginning of observation until day 6 showed a low response to feed, and

then on day 7, there was total death, while in treatment D and E the response showed fluctuations until death occurred on day 8 for treatment D. and on day 10 for treatment E.

Treatment A experienced the worst response to feed, this showed that the test fish that were not induced by immunostimulants in the form of herbal fish had low body resistance to attack by *A. hydrophila* bacteria. Giving fish herbs with high concentrations showed a poor response to feed. This is the effect of the high saponin content in the fish herbs seen in treatments D (300 mL/kg) and E (400 mL/kg). According to Hashemi and Davoodi (2012) [9], saponins in amounts that exceed normal limits will act as immunosuppressors, namely substances that can reduce the immune system.

Administration of immunostimulants at the right concentration showed the best results seen in treatment C (200 mL/kg). Starting on day 5 showed the fastest normal response compared to other treatments. According to Chen *et al*. (2018), it is stated that herbal plants can induce appetite in several species of fish, besides that the anti-microbial content found in fish herbs such as flavonoids plays a role in eliminating negative microbes in the digestive tract of fish. Lestariningsih's research (2015) showed the results of adding meniran flour containing flavonoids to feed were able to increase the number of LAB (Lactic Acid Bacteria) and reduce the number of *Escheria coli* bacteria in the digestive tract.

4. Conclusion

Based on the research that has been done, it can be concluded that:

1. Fish herbal medicine given through feed on *Osteochillus vitattus* has an effect as an immunomodulator, indicated by an increase in the percentage of white blood cell count by 21.37±2.33% to 36.92±2.34%.
2. Concentration of 200 mL/kg resulted in the highest increase in white blood cell count (36.92±2.34%), showing clinical symptoms in the form of body surface damage, response to shock and response to the lightest feed.

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