Asian Journal of Aquatic Sciences, December 2025. Vol 8, Issue (3) 327-336 e-issn: 2716-4608
p-issn: 2655-366X

PHYSIOLOGICAL AND BLOOD BIOCHEMICAL RESPONSES OF *Pangasianodon hypophthalmus* ENRICHED PELLETS *Citrus jambhiri* L.

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ABSTRACT

Jungga lime (*Citrus jambhiri* L.)/Sundai acid/Limau kuit is a natural ingredient that contains alkaloids, saponins, steroids, tannins, flavonoids, ascorbic acid, essential oils, and limonene, which are antibacterial. This research was conducted from August to December 2024 at the Laboratory of Fish Diseases and Parasites, Faculty of Fisheries and Marine, Universitas Riau. This research aimed to analyze the physiological and biochemical responses of the blood of striped catfish. The method used was experimental with a completely randomized design (CRD) of 1 factor, five treatments, and three replicates. The treatments applied were Kn (without the addition of jungga lime and without being infected with *A. hydrophila*), Kp (without jungga lime and infected with *A. hydrophila),* and jungga lime-enriched pellets at P1 (6.0 mL/kg), P2 (6.5 mL/kg), and P3 (7.0 mL/kg), and infected with *A. hydrophila*. Striped catfish (*P. hypophthalmus*) with a size of 8-12 cm and a weight of 6-7 g were kept in a 54x37x29 cm container, with a stocking density of 1 fish/3 L, and fed 3 times a day and sanitation. On day 32, fish were intramuscularly challenged with *A. hydrophila* (10^8 CFU/mL) at 0.1 mL. The results showed that the best dose was P3 (7.0 mL/kg) with a blood glucose of 79.33 mg/dL, Mg 3.07 mg/dL, Ca 9.87 mg/dL, P 4.03 mg/dl, Lisozyme activity of 259 units, mL, weight growth reached 19.11 g, and length growth reached 5.39 cm. Adding jungga lime to fish feed can improve post-challenge fish blood's physiological and biochemical responses.

Keywords: Jungga lime, MAS disease, *Pangasionodon hypophthalmus*, Blood Biochemical

1. INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) is one of the leading commodities in freshwater fish farming in Indonesia. This fish has the advantage of thick meat and a taste that is favored by consumers, thus driving high market demand. To meet this demand, intensive cultivation with high stocking density is one of the strategies that many farmers apply. However, intensive cultivation systems also increase the risk of stress in fish, which results in decreased immune system and increased susceptibility to pathogen infections[1].

One of the diseases that often attacks striped catfish under stressful conditions is *Motile Aeromonas Septicemia* (MAS), which is caused by *Aeromonas hydrophila* bacteria. This bacterial infection is characterized by changes in behavior, decreased feeding response, body wounds, and bleeding that can cause high mortality of up to 50-100%[2]. Disease control due to bacterial infections is generally carried out by administering antibiotics such as Novobiocin and Oxytetracycline. However, the continuous use of antibiotics can trigger bacterial resistance, which is not only detrimental to the aquaculture sector but also a risk to human health and the environment[3].

In response to these problems, alternative approaches through the utilization of natural materials with

Received: 18 October 2025
Accepted: 12 December 2025

antimicrobial activity began to be developed. One plant that has potential as an antimicrobial agent is jungga acid (*Citrus jambhiri* L.), also known as sundai orange or limau kuit. This plant contains essential oils and limonene compounds that can inhibit the growth of pathogenic microorganisms. According to Sembiring[4], jungga tamarind leaves contain 0.8% essential oil by dry weight and have 10 chemical compounds with moderate antioxidant and antibacterial activity against *Bacillus cereus* and *Escherichia coli*.

In fish farming activities, the effectiveness of using jungga acid as a disease prevention agent needs to be evaluated through physiological and biochemical parameters of fish blood, such as glucose levels, absolute weight growth, and survival rate. These parameters reflect the health status and stress level of fish, which are greatly influenced by environmental quality and pathogen exposure. Blood glucose level, for example, is a commonly used indicator of stress, with the normal range for striped catfish fry being in the range of 36-88 mg/dL[Error! Reference source not found.]. This study aims to obtain the physiological and biochemical responses of the blood of striped catfish.

2. RESEARCH METHOD

Time and Place

This research was conducted from August 2024 to December 2024. Observations of physiological responses such as blood glucose, growth, and fish survival were carried out at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau. Observations of blood biochemistry, such as Calcium, Magnesium, and Phosphorus, were carried out at the Balivet Laboratory, Bukittinggi. In contrast, lysozyme activity was carried out at the Health Laboratory, FPIK, Bogor Agricultural University.

Method

The study used an experimental method with a one-factor Completely Randomized Design (CRD), namely the dose of added jungga acid solution in feed, which consisted of five treatment levels. Each treatment was repeated three times to minimize the error rate, resulting in a total of 15 experimental units. The placement of treatments in each experimental unit was randomized. The treatment used refers to the results of the LD_{50} test of kaffir lime juice solution on striped catfish by Sihombing et al.[Error! Reference source not found.], which is at a concentration of 0.791% through the immersion method for 24 hours. The treatments consisted of a negative control (Kn), which is fish fed without jungga lime solution and without *A. hydrophila* challenge test, positive control (Kp), which is fish fed without jungga lime solution but tested for *A. hydrophila* challenge, and three doses of jungga lime solution in feed, namely P1 (6.0 mL/kg), P2 (6.5 mL/kg), and P3 (7.0 mL/kg), each of which was tested with *A. hydrophila*.

Procedures

Preparation of Jungga Acid (*Citrus jambhiri* L.) Solution

The jungga acid used comes from the Jorong Balai Panjang area, Saniang Baka, X Koto Singkarak District, Solok Regency, West Sumatra. The making of jungga acid is done by selecting ripe fruit with greenish-yellow color characteristics and weighing as much as 100 g. The fruit is washed and dried for 15 minutes. Jungga tamarind fruit was washed and dried for 15 minutes. After drying, the fruit is cut into pieces and then squeezed to produce 36.8 mL. Then the juice is filtered using Whatman paper no 42 and produces 20 mL of pure solution.

Aeromonas hydrophila Isolate Preparation

Aeromonas hydrophila used in this study came from the collection of Parasites and Fish Diseases Laboratory, Faculty of Fisheries and Marine, Universitas Riau. Bacterial isolates were then re-cultured on TSA media and incubated in an incubator for 18-24 hours at 28-30^{o}C. Bacterial colonies

that grew on TSA media were cultured on GSP media and incubated in an incubator for 18-24 hours. After 18-24 hours, the red GSP media turned yellow, indicating that the bacteria growing on the media were bacteria of the genus Aeromonas. Then the bacteria on GSP media were re-cultured on TSA media. Furthermore, making an inoculum. Inoculum that can be used is 18-24 hours old for testing[5].

Aeromonas hydrophila challenge test

Striped catfish were challenged on day 32 with *A. hydrophila* bacteria at a density of 10^8 CFU/mL as much as 0.1 mL/head by infecting intramuscularly using a 1 mL syringe. Before infection, fish were first anesthetized using clove oil as much as 0.1 mL/L water with the aim of reducing stress in fish. After the fish were challenged, they were returned to the aquarium and maintained for 14 days[7].

Profile of Hematology

Blood collection of test fish was carried out 3 times, namely first before treatment, after 30 days of maintenance and 14 days after the challenge test with *A. hydrophila* bacteria. Fish samples were taken from as many as three fish from each container. Before taking blood, fish were first anesthetized with clove oil as much as 0.1 mL / L water for ± 5 minutes in 5 L of water. Fish blood was taken using a 1 mL syringe without 10% EDTA blood was taken from the caudalis vein, then the blood in the syringe was put into a microtube and centrifuged at 3000 rpm for 10 minutes. After that, the serum was taken and transferred to a microtube.

3. RESULT AND DISCUSSION

Clinical Symptoms of Striped Catfish

Clinical symptoms of Siamese jambal fish 14 days post-challenge can be seen in Table 1.

Table 1. Clinical symptoms of striped catfish post-challenge

Treatment	Clinical Symptoms				
	Movement	Appetite	Body Surface	Eye	Fins
Kn	• Active	Normal	• Bright • Normal mucus	Normal	• Fins intact
Kp	• passive	Declining	• Pale • Excess mucus • Ulcers at the infection site • Swelling of the abdomen • Skin flaking	*Exophtalmia*	• Bleeding on the tail and abdomen • Thinning of the caudal and pelvic fins
P1	• passive	Declining	• Pale • Excessive mucus • Ulcers at the infection site • Bleeding around the eye	Normal	• Dorsal fin bleeding • Thinning of the caudal fin
P2	• passive	Declining	• Pale	Normal	• Fins intact

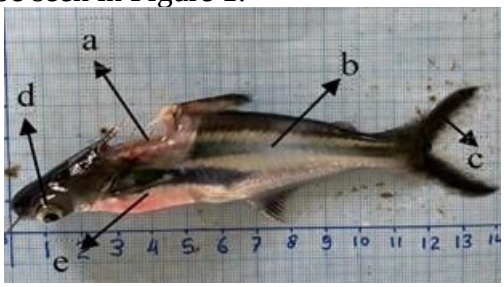
			• Excessive mucus • Ulcer at the site of infection		• Abdominal fin bleeding
P3	• Active	Normal	• Bright • Excess mucus • Infection scars turn into white spots	Normal	• Fins intact

Clinical symptoms after challenge with *A.hydrophila* were different in each treatment. Striped catfish in the negative control (Kn) showed no abnormal symptoms, because no challenge test was conducted. In contrast, fish in the positive control (Kp), which were not given preventive treatment, showed the most severe clinical symptoms in the form of ulcers at the injection site, hemorrhage from the base of the tail to the abdomen, exophthalmia (protruding eyes), and thinning tail fins. In the P1 and P2 treatments, the fish showed similar but slightly milder symptoms, including excessive mucus, ulcers, fin hemorrhage, and fin thinning. In P2, the ulcers on the injection site had started to improve into white spots.

The mildest clinical symptoms were recorded in treatment P3, which was only mild inflammation at the injection site that began to improve after the third day post-challenge. The effectiveness of this treatment is thought to be influenced by the dose of jungga acid solution in the feed, where the dose of 7.0 mL/kg (P3) is more optimal in curing the infection compared to lower doses in P1 and P2. This is in line with the opinion of Pattanayak et al.[8]; Farag et al.[9] which explains that *A. hydrophila* infection triggers symptoms of inflammation, ulcers, skin peeling, exophthalmia, and abnormal swimming behavior due to systemic disorders and tissue damage.

The rapid recovery in P3 is thought to be due to the large amount of vitamin C content from the jungga acid solution, which acts as an antioxidant and immunostimulant, helping increase antibody production and strengthen the fish's immune system against pathogens. According to Mohammed et al.[10], Jungga acid juice contains 70.0 mg/100 g of vitamin C, which functions in neutralizing free radicals and accelerating the physiological recovery of post-infection fish. Thus, the administration of jungga acid solution at the P3 dose is optimal in accelerating healing due to *A. hydrophila* infection in striped catfish. Changes in clinical symptoms of post-challenge fish can be seen in Figure 1.

KP



KN

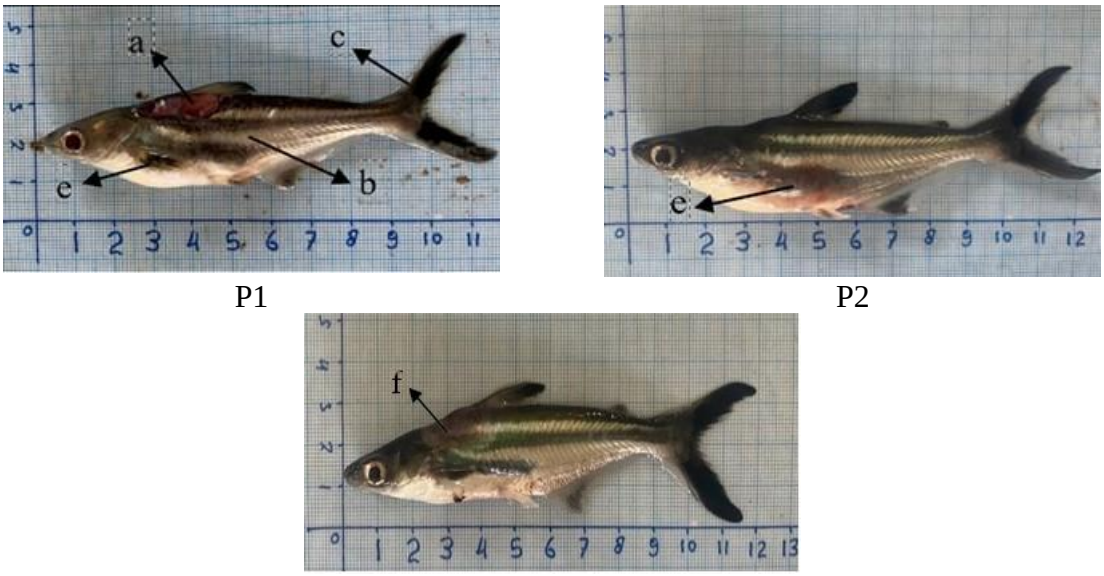


Figure 1. Clinical symptoms of striped catfish after *A.hydrophila* challenge
Description: a: ulcer, b: skin peeling, c: caudal fin thinning, d: exophthalmia, e: abdominal swelling, f: white spots of infection, G = ulcer.

Blood Glucose

The measurement results of blood glucose levels of striped catfish presented in Table 2 show that at the beginning of maintenance (day 0), glucose levels ranged from 43.00-44.67 mg/dL, still within the normal range according to Ariyanti et al.[11] which is 40-90 mg/dL. These levels reflect the initial physiological response of fish in the adaptation phase to the new environment. After 30 days of maintenance, glucose levels increased in all treatments, with a range of 47.00-66.67 mg/dL. This increase was thought to be related to an increase in appetite and metabolic activity. The highest glucose level was recorded in treatment P3 (66.67 mg/dL), followed by P2 (58.67 mg/dL) and P1 (54.33 mg/dL), indicating that the administration of jungga acid solution was able to increase metabolic activity through stimulation of the immune system. This is in line with the flavonoids and vitamin C in jungga acid, which act as immunostimulants.

Table 2. Blood glucose of striped catfish during the study

Treatment	Blood Glucose (mg/dL)		
	Start of maintenance	Day 30th	Day 14th Post-challenge
Kn	44,67	47,00±3,61^{a}	52,00±6,08^{b}
Kp	44,33	47,33±3,51^{a}	24,33±2,52^{a}
P1	43,00	54,33±1,53^{b}	107,67±3,51^{e}
P2	43,00	58,67±2,08^{b}	90,67±6,51^{d}
P3	44,33	66,67±2,52^{c}	79,33±3,51^{c}

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14 days after the challenge test, blood glucose levels of striped catfish ranged from 24.33-107.67 mg/dL. The lowest level was recorded in the positive control (Kp) at 24.33 mg/dL, which was thought to be due to metabolic disorders caused by *A. hydrophila* infection that was not balanced by an adequate immune response. In contrast, the highest level was recorded in treatment P1 (107.67 mg/dL), indicating an acute stress response due to infection and high metabolic activity due to active compounds in jungga acid. According to Addini et al.[12], blood glucose levels are an important indicator of stress in fish, and elevated levels indicate high levels of physiological stress. Elevated glucose can also be caused by the activity of stress hormones such as cortisol, triggered by pathogen infection and immune response.

The ability of P3 to maintain stable blood glucose levels is thought to be due to the active role of bioactive compounds in jungga acid, especially flavonoids and essential oils. Flavonoids such as nobiletin, which are commonly found in citrus-citrus group plants, are known to have antioxidant, anti-inflammatory, and antibacterial properties[13]. In addition, essential oil in jungga tamarind leaves at 0.8% of 450 g dry powder[4] has anti-inflammatory, anti-microbial, and accelerates the recovery of infected tissues[14].

The combination of these compounds strengthens the fish's immune system and helps maintain internal homeostasis, so that glucose levels can be maintained within normal ranges despite the stress of infection. Thus, the administration of jungga acid solution at the optimal dose proved to be effective as a natural immunostimulant and antimicrobial agent in improving the physiological response of striped catfish to bacterial infection.

Blood Biochemistry

The results of blood biochemical observations of striped catfish can be seen in Table 3.

Table 3. Blood biochemistry of striped catfish during the study

Maintenance	Treatment	Magnesium (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Lysozyme Activity (Unit/mL)
Start of maintenance	Kn	2,33	5,80	2,30	218,00
	Kp	2,30	5,63	2,33	218,33
	P1	2,30	5,73	2,30	219,33
	P2	2,30	5,70	2,36	219,66
	P3	2,36	5,76	2,33	219,00
Day 30th	Kn	2,43±0,06^{a}	6,60±0,26^{a}	2,60±0,10^{a}	219,67±0,58^{a}
	Kp	2,37±0,06^{a}	6,67±0,15^{b}	2,57±0,06^{a}	219,00±1,00^{a}
	P1	2,90±0,10^{b}	7,27±0,32^{b}	2,77±0,06^{a}	227,67±2,52^{b}
	P2	3,00±0,10^{b}	8,47±0,15^{c}	3,03±0,58^{b}	232,33±2,52^{c}
	P3	3,33±0,58^{c}	9,33±0,15^{d}	3,53±0,38^{c}	242,67±2,52^{d}
Day 14th Post-challenge	Kn	2,53±0,58^{b}	7,20±0,26^{b}	2,77±0,15^{b}	221,00±1,00^{a}
	Kp	1,97±0,15^{a}	4,97±0,31^{a}	2,00±0,10^{a}	470,00±10,0^{d}
	P1	2,63±0,58^{ab}	8,17±0,15^{c}	3,10±0,10^{c}	246,67±1,53^{b}
	P2	2,80±0,10^{c}	8,97±0,15^{d}	3,40±0,15^{d}	251,00±1,00^{ab}
	P3	3,07±0,39^{c}	9,87±0,15^{e}	4,03±0,15^{e}	259,33±6,03^{bc}

At the beginning of rearing, blood magnesium levels of striped catfish ranged from 2.20-2.36 mg/dL, which is slightly below or close to the lower limit of the normal range according to Bojarski et al.[15], which is 2.51-3.45 mg/dL, and Putra[16], which is 2.4-3.8 mg/dL. After 30 days of maintenance, magnesium levels increased to 2.43-3.33 mg/dL, and post-challenge for 14 days were in the range of 1.97-3.07 mg/dL.

The increase in magnesium levels in P1, P2, and especially P3 after 30 days of maintenance is thought to be due to the stimulating effect of jungga acid solution on the metabolic system and mineral homeostasis. Jungga acid contains active compounds such as flavonoids and saponins that help boost the body's immunity and support the work of organs in maintaining mineral balance. However, after the challenge test, magnesium levels in some treatments decreased, especially in Kp (fish not given jungga acid solution), which only reached 1.97 mg/dL, possibly due to *A. hydrophila* bacterial infection that disrupts metabolism and ion balance. This also suggests that an unstimulated immune system is unable to maintain a homeostatic balance.

Calcium levels at the beginning of the study were in the range of 5.63-5.80 mg/dL, still within the normal range according to Sharma et al.[17], which is 5.4-13.5 mg/dL, but slightly below the range according to Syawal et al.[18], which is 8.6-10.30 mg/dL. After 30 days of rearing, calcium levels increased significantly to 6.60-9.33 mg/dL, and after 14 days post-challenge were in the range of 4.97-9.87 mg/dL. This increase was particularly noticeable in P3, which showed the highest calcium value (9.87 mg/dL) post-challenge. This indicates the positive effect of the tamarind solution in supporting mineral and immune metabolism. As described in the literature, the flavonoid nobiletin in jungga acid helps to increase the appetite and immunity of the fish, which has an impact on the absorption of minerals such as calcium.

Fish blood phosphorus levels at the beginning of the study ranged from 2.30-3.03 mg/dL, then increased to 2.57-3.53 mg/dL after 30 days of rearing, and 2.00-4.03 mg/dL post-challenge. This value is still within the normal range according to Syawal et al.18, which is 2.5-4.5 mg/dL, although lower than Putra's[16] study (6.3-13.1 mg/dL in fish at different salinities). Phosphorus is essential for energy metabolism and hard tissue (bone) formation, as well as its role in the immune system. The increase in phosphorus levels in treatments P1-P3, especially P3, indicates an increase in the body's metabolic function and defense against stress or infection. The active compounds in jungle acid, such as tannins and saponins, support the body's immune effectiveness in suppressing the spread of pathogens and supporting recovery.

Lysozyme activity is a key indicator of the nonspecific immune system. At the beginning of rearing, lysozyme activity was in the range of 218-219.66 units/mL, then increased to 219-242.67 units/mL after 30 days, and increased sharply post-challenge to 221-470 units/mL. According to Kubilay & Ulukoy[19], the normal lysozyme activity of non-stressed fish is 140-480 units/mL, while in stressed fish it is 320-980 units/mL. The increase in lysozyme activity after the challenge test, especially in P3 (259.33 units/mL), indicates that the fish's immune system is working optimally in the face of infection. In contrast, the extreme increase in Kp (470 units/mL) indicates high stress due to infection without the help of stimulants such as jungga acid. Lysozyme plays an important role as the first line of defense against infection by hydrolyzing the bacterial cell wall. Bioactive compounds in jungga acid, such as flavonoids, saponins, and tannins, are known to increase lysozyme production as an immune response to infection.

Absolute Weight and Length Growth

Table 4, the absolute weight growth of striped catfish ranged from 8.81-19.11 g and

the absolute length of 2.88-5.39 cm. The highest value was found in the P3 treatment, while the lowest was in Kp. Further tests showed that P3 was significantly different from the other treatments, while Kp and Kn were not significantly different. The absolute weight and length growth of striped catfish during the study can be seen in Table 4.

Table 4. Growth of weight and length of striped catfish during the study

Treatment	Absolute weight (g)	Absolute length (g)
Kn	9,41±0,45^{a}	2,88±0,43^{a}
Kp	8,81±0,03^{a}	3,28±0,19^{ab}
P1	10,75±1,16^{b}	3,49±0,67^{ab}
P2	12,78±0,59^{c}	4,23±0,26^{b}
P3	19,11±0,49^{d}	5,39±0,64^{c}

The increased growth in P3 is thought to be due to the content of active compounds in jungga acid solution, such as flavonoids, saponins, and tannins that can increase appetite, improve digestion, and nutrient absorption. After 14 days post-challenge, fish in the P1-P3 treatment still showed better growth than the positive control, indicating that the jungga acid solution also helped maintain the physiological stability of fish after stress due to infection. The observation results of the average weight of striped catfish are presented in Figure 2.

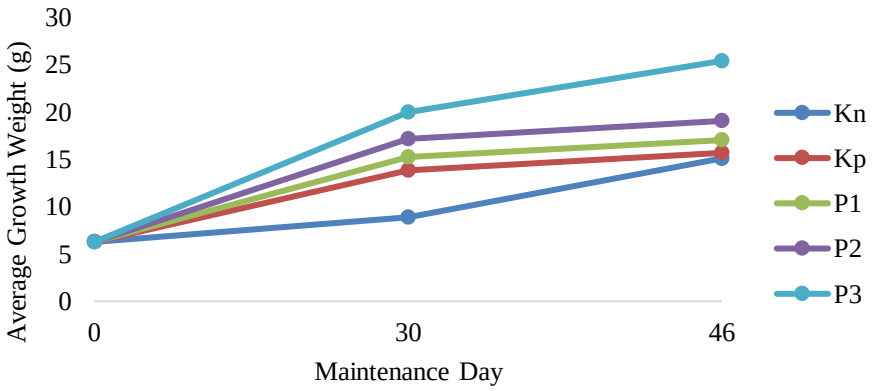

Figure 2. Average weight growth of striped catfish

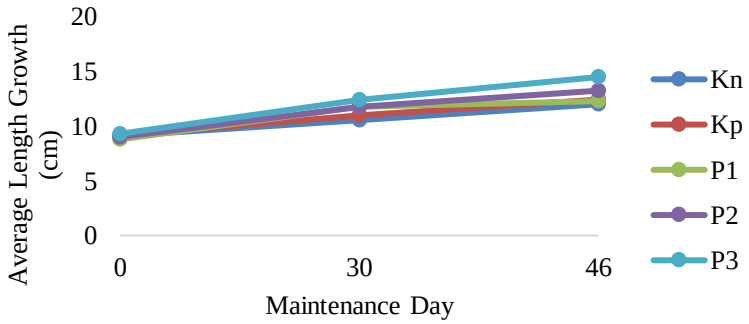

Figure 3. Average length growth of striped catfish

Figure 2 shows that the average weight growth pattern of striped catfish reared for 46 days has increased. Weight growth in maintenance from 0-30 in the P1, P2, and P3 treatments experienced a high increase compared to those without treatment. This is thought to be due to the addition of jungga acid solution to the feed, which is able to

increase fish appetite. After 14 days post-challenge, there was still an increase, but not too high. This is due to the influence of *A. hydrophila* infection that attacks the metabolic system in fish, so that the appetite of fish is not the same as pre-challenge maintenance.

Figure 3 shows that the highest length growth occurred in the P3 treatment (7.00 mL/kg jungga acid solution). This is thought to be due to the content of active compounds in jungga acid, such as vitamin C, which can increase fish appetite, immunity, and metabolism. Increased appetite is also related to the age, size, and eating habits of striped catfish.

The feed used (FF-999) has a protein content of 35%, which is in line with the needs of the fish, while the vitamin C in jungga acid at 70 mg/100 g[10] acts as an antioxidant, accelerates healing, and supports growth. Without jungga acid, growth is not optimal due to low immune stimulation and appetite. This is in line with Fuadi et al.[20] that vitamin C supplementation can increase physical activity and fish appetite. Therefore, P3 is the best treatment because it supports optimal physiological conditions that encourage maximum growth.

4. CONCLUSION

Based on the results of the study it can be concluded that the addition of jungga acid solution to the feed has an effect on the physiological and biochemical responses of blood of striped catfish with the best dose of 7.00 mL/kg feed as seen from the milder clinical symptoms with inflammation in the former injection at 48 hours post-challenge and inflammation slowly began to improve after the third day, glucose levels of 79.33 mg/dL, Calcium 9.87 mg/dL, magnesium 3.07 mg/dL, phosphorus 4.03 mg/dL, lysozyme activity 259.33 units/mL, absolute weight growth of 19.11 g, and absolute length growth of 5.39 cm.

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