

HEMATOLOGY OF ASIAN REDTAIL CATFISH (*Hemibagrus nemurus*) REARED IN MEDIA WITH REGULAR ADDITION OF PROBIOTICS

Rona Lestari^{1*}, Henni Syawal¹, Iesje Lukistyowati¹

¹Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau
Kampus Bina Widya KM. 12,5, Simpang Baru, Kec. Bina Widya, Pekanbaru, Riau 28293

*ronalestari8@gmail.com

ABSTRACT

Multi-cell probiotic containing bacteria *Nitrosomonas* sp., *Nitrobacter* sp., and *Bacillus* sp. This research was conducted from March to May 2021 at the Fish Disease and Parasite Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Riau. This study aimed to analyze the hematology of Asian redbtail catfish reared in media that was added with probiotics periodically and to obtain the best time interval for giving probiotics to the rearing medium. The method used in this study is an experimental method by applying a Completely Randomized Design (CRD) with five treatment levels and three replications. The treatments used were the administration of probiotics at a dose of 0,01 mL/L of water with different time intervals, P0 (without treatment), P1 (5 days once), P2 (10 days once), P3: (15 days once), and P4 (20 days once). Fish were reared for 50 days. The results show that the Asian redbtail catfish reared with the addition of probiotics in the rearing medium had a significant effect ($P < 0.05$) with the best time interval being once every 5 days in the P1 treatment, seen from the total erythrocytes 2.43×10^6 cells/mm³, hematocrit level 28.67%, hemoglobin level 7.40 g/dL, total leukocytes 9.92×10^4 cells/mm³, leukocrit level 1.83%, and glucose 49.67 g /dL. Water quality during the study was still in the normal range that could support the growth of Asian redbtail Catfish, namely temperature 26-27.1 C, pH 5.8-6.5, DO 6.0-7.4 mg/L, and ammonia 0.0011-6.5 0.0047 mg/L.

Keywords: *Hemibagrus nemurus*, Hematology, Probiotics, Water quality

I. INTRODUCTION

Efforts to meet market needs for Asian redbtail catfish (*Hemibagrus nemurus*) mostly still rely on catches from nature. If the fishing effort continues, it is feared that its existence in nature will be threatened with extinction. The production of Asian redbtail catfish originating from public waters in the Riau area is 20.40 tons/year¹. To fulfil market demand without disturbing fish populations in nature, it is necessary to make efforts to increase the intensive cultivation of Asian redbtail catfish.

The enlargement of the Asian redbtail catfish is still experiencing several problems, namely slow growth, and high cannibalism in the larval and seed stages.

The cultivation of Asian redbtail catfish in ponds requires quite a long time, which is around 4-5 months to reach a consumption size with an average weight of 200-250 g/fish². In addition, the problem in aquaculture is that there is often a decrease in water quality caused by organic waste, such as leftover feed and feces. Poor water quality can cause fish stress so that their appetite decreases and they are susceptible to disease.

The use of probiotics in water is one of the factors that can support the improvement of water quality for fish living media and provide a beneficial effect on the host. The bacteria present in multi-cell booster probiotics are (*Nitrosomonas* sp., *Nitrobacter* sp., and

Bacillus sp.). The advantages of these bacteria are that they can improve and maintain water quality, oxidise organic compounds derived from leftover feed, feces, and dead organisms, can reduce toxic metabolites, can reduce the growth of harmful bacteria, provide natural food, and grow several types of beneficial bacteria³.

Fish health status can be seen from blood parameters, namely total erythrocytes, hematocrit values, hemoglobin values, total leukocytes, leukocrit levels, and blood glucose. According to Sarkiah et al.⁴ total erythrocytes, hematocrit levels, and hemoglobin levels are the main indicators to determine changes in fish activity. Several studies related to the addition of probiotics to Asian redtail catfish rearing media⁵, adding probiotics to rearing media can improve the health of red tilapia⁶. Research on the use of probiotics in aquaculture is usually carried out to improve water quality and fish growth, while the use of multi-cell probiotics in rearing media to improve the health of Asian redtail catfish has not been carried out. Therefore, the authors are interested in researching the hematology of Asian redtail catfish raised in media-added probiotics periodically.

The purpose of this study was to analyze the hematology of Asian redtail catfish reared in media supplemented with probiotics regularly and to obtain the best time interval for giving probiotics to the rearing medium.

2. RESEARCH METHOD

Time and place

This research was carried out from March to May 2021 at the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine, Universitas Riau.

Materials

There were 300 Asian redtail catfish as the test fish with an average length of 5-7 cm and an average weight of 3.22-4.06 g/fish. Then the fish feed is in the form of

commercial feed PF1000. The next ingredients are molasses, probiotics, clove oil, Hayem's solution, Turk's solution, EDTA, and 0.1 N HCL.

Meanwhile, the tools used are maintenance containers, drains, strings, microtubes, haemocytometers, objects and cover glasses, binocular microscopes, microhematocrit reader, microhematocrit centrifuge, haemometer, glucose, capillary hematocrit, cryoceleal, DO meter, thermometer, pH meter, and spectrophotometer.

Methods

The method used in this study was an experimental method by applying a completely randomized design (CRD) with 5 treatment levels and 3 repetitions. The treatment applied at this research stage was the addition of probiotics at different time intervals with the same dose. The addition of probiotic doses refers to research by Putra et al.⁷, namely 0.01 mL/L of water. The treatment applied is:

- P0 : Not added probiotics (Control)
- P1 : Every 5 days given probiotics as much as 0.01 mL/L
- P2 : Every 10 days given probiotics as much as 0.01 mL/L
- P3 : Every 15 days given probiotics as much as 0.01 mL/L
- P4 : Every 20 days given probiotics as much as 0.01 mL/L

Procedure

Preparation of Containers and Water Media Maintenance

The container used in the study was a container with a capacity of 100 L. Before the container is used, it must first be cleaned and filled with water until it is full then given 25 ppm of KMnO₄ for 24 hours so that the container is free from pathogenic microorganisms, then the container is rinsed with water until clean then dried for 24 hours. Then the container is filled with 60 L of water and given aeration, the water used comes from the

well water that has been precipitated beforehand.

Provision of Carbon Sources and Probiotics

Giving a carbon source (molasses) into the rearing container refers to Putra⁵ the best dose is 0.02 g/L water. Molasses was added to the maintenance medium with as much as 0.01 mL/L of water. The purpose of giving molasses is to a source of nutrition for probiotic bacteria, then give aeration to each research container. Flock formation was indicated by the change in the colour of the water to brownish turbid on the second day after giving molasses. On the third day, 0.01 mL/L of water was added with a multi-cell commercial booster. The probiotics given contain the bacteria *Nitrosomonas* sp., *Nitrobacter* sp., and *Bacillus* sp.

Maintenance of Test Fish

The Asian redbtail catfish seeds measuring 5-7 cm in length and an average weight of 3.22-4.06 g/head came from fish farmers in Lipat Kain Village, with the criteria of the seeds being active, not deformed, and having a relatively uniform size. Stocking of seeds was carried out in the afternoon with a stocking density of 1 fish/3 L of water. Fish were fed 3 times/day at the rate of 5%/bb/day. Feeding was carried out at 08.00, 13.00, and 18.00 WIB. The feed used is commercial feed PF1000 with a protein content of 39-41%, 5% fat, 6% fiber, 16% ash, and 10% moisture content. This research was conducted for 50 days.

Blood Sampling

Blood observations were carried out three times, namely early before treatment, the second day 25th, and the third day 50th. Blood samples were taken in the morning. Before the blood was taken, syringes and microtubes were prepared beforehand and rinsed with 10% EDTA. Then, the fish were anesthetised with clove oil at a dose of 0.1 mL/L of water. Blood

was taken from the caudal vein with an angle of 45° using a 1 mL syringe. The blood taken was then put into a microtube and stored in a cool box filled with ice cubes to prevent the blood from clotting and immediately observed at the Parasites and Fish Diseases Laboratory.

Parameters measured

Total Erythrocytes

Total erythrocytes were calculated following the Klontz procedure (1994)⁸:

$$\sum \text{Erythrocytes} = \sum n \times 10^6 \text{ cells/mm}^3$$

Information:

n = The number of erythrocytes in the 5 small boxes in the viewing room

10^6 = Dilution factor

Hematocrit Levels

The percentage of hematocrit is calculated following the procedures⁸. i.e. the blood sample in the microtube tube is inserted into the capillary tube. After the blood reaches 4/5 of the tube, the end of the tube (marked red) is plugged with Crystoseal. The capillary tube containing blood was centrifuged for 3-5 minutes at 11000 rpm. The length of the erythrocyte deposits in the capillary tube was measured using a Microhematocrit reader and expressed in percent as % blood volume.

Hemoglobin levels

The calculation of hemoglobin uses the Sahli method with the Sahlinometer⁸, namely first 0.1 N HCL is put into the salinometer tube to a scale of 10 (bottom line on the *Salinometer*), then the tube is placed between the two standard colors. Fish blood samples were taken with a Sahli pipette of as much as 0.02 ml and left for 3 minutes. Subsequent dilutions were carried out by adding distilled water little by little using a pipette until the color was the same as the standard color and the results were expressed in g/dL.

Total Leukocytes

The procedure for calculating total leukocytes refers to Klontz (1994)⁹.

$$\sum \text{Leukocytes} = \sum n \times 50 \text{ cells/mm}^3$$

Information:

$\sum n$ = Total number of leukocytes in the 4 large squares

50 = Dilution factor

Leukocrit Levels

Leukocrit measurements refer to Kurniawan⁸. The blood sample is placed in a leukocrit capillary tube up to approximately 4/5 of the tube, the end of the capillary is plugged with cryoseal, then centrifuged for 3-5 minutes at a speed of 11,000 rpm with the position of the tube with the same volume facing each other so that the centrifugal rotation is balanced. Then the percentage of leukocrit values is read using Microhematocritreader and expressed in percent as % blood volume.

Glucose Levels

Four microliters of blood used are dripped onto a strip that has been attached to the device. The tool will immediately

read the fish's blood glucose level and display it on the screen, then record it.

Water quality

The water quality parameters measured were temperature, pH, DO, and NH₃. The tools used are a thermometer, pH meter, DO meter and spectrophotometer. Water quality measurements were carried out twice, namely at the beginning before treatment, and at the end of the 50th day of the study.

Data analysis

Fish hematologic data obtained during the study is presented in tabular form. Then the homogeneity test. Water quality parameter data is entered into the table and then analyzed descriptively.

3. RESULT AND DISCUSSION**Total Erythrocytes**

Observation of changes in red blood cells can be used as an indicator of stress in Asian redtail catfish caused by environmental factors. The average total erythrocyte results of the Asian redtail catfish during the study can be seen in Table 1.

Table 1. Total erythrocytes of Asian redtail catfish (*Hemibagrus nemurus*)

Treatment	Total Erythrocytes (10 ⁶ cells/mm ³)		
	Day 0	25th day	50th day
P0	1.76±0.04	1.82±1.82a	1.91± 1.91a
P1	1.75±0.02	2.22±2.22c	2.43± 2.43c
P2	1.74±0.02	1.97± 1.97b	2.22± 2.22b
P3	1.74±0.02	1.89± 1.89b	2.18± 2.18b
P4	1.76±0.04	1.94± 1.94b	2.15± 2.15b

Note: P₀: Not added probiotics (Control), P₁: Added probiotics every 5 days as much as 0.01 mL/L, P₂: Added probiotics every 10 days as much as 0.01 mL/L, P₃: Added probiotics every 15 days as much as 0.01 mL/L, P₄: Added probiotics every 20 days as much as 0.01 mL/L.

Based on Table 1, the total erythrocytes of Asian redtail catfish during the study were still within the normal range, following the opinion of Lukistyowati & Syawal⁹, that the number of erythrocytes in normal Asian redtail catfish ranges from 1.522 – 2.912 million cells/mm³. The results of the analysis of variance (ANOVA) based on Table 1,

showed that the periodic administration of multi-cell probiotics in the rearing medium had a significant effect on total Asian redtail catfish erythrocytes (P<0.05). The results of the Newman-keuls test showed that the P₁ treatment was significantly different from the P₂, P₃, and P₄ treatments, and very significantly different from P₀.

Treatment P₁ experienced a higher increase in total erythrocytes than P₂, P₃, and P₄. This can happen because the more often probiotics are added to the rearing media, the more probiotic bacteria in the waters will always be available and will help improve water conditions, so that stress levels decrease, fish appetite increases, and oxygen content in the blood increases. Then, the bacteria will enter the fish's body and help control the microbial balance in the digestive tract so that the body's immunity and fish growth increase. This is in line with Putra⁶ that the addition of multi-cell probiotics to the rearing medium can increase the total erythrocytes of red tilapia (*Oreochromis* sp) seen from the initial total erythrocyte value of 88.0×10^4 cells/mm³ and at the end of the study 220.6×10^4 cells/mm³.

The increase in total erythrocytes indicates that the fish has adapted to its environment. This is in line with the opinion of Syawal & Siregar¹⁰ which states

that increased levels of erythrocytes in the blood indicate increased oxygen content in the blood and fish begin to adapt to the environment. Total erythrocytes are also influenced by several factors including age, size, physical activity, species, sex and feed nutrition¹¹.

The increase in total erythrocytes indicated that the Asian redtail catfish were in good health so they experienced optimal growth, especially in the P₁ treatment. According to Feliatra¹², the provision of probiotics in fish-rearing media aims to maintain microbial balance and control pathogens in the digestive tract to prevent pathogens from taking the nutrients needed for fish to live.

Hematocrit Levels

Hematocrit observation aims to determine the health status of fish, one of which is anemia. Observations of the hematocrit of Asian redtail catfish during the study can be seen in Table 2.

Table 2. Hematocrit levels of Asian redtail catfish (*Hemibagrus nemurus*)

Treatment	Hematocrit Level (%)		
	Day 0	25th day	50th day
P0	20,67±0.58	21.33± 2.31a	23.00± 2.00 a
P1	20,67±0.58	27,33± 1.53 b	28,67± 0.58c
P2	20.00± 1.00	24,67± 0.58 ab	26,33± 0.58 b
P3	19.67±0.58	22.00± 1.00 a	25,67±1.16ab
P4	19.67±1.16	21.67± 2.52 a	23,67±1.16ab

Based on the data in Table 2, the hematocrit value of the Asian redtail catfish during the study was still within the normal range, because normal hematocrit values in the Asian redtail catfish ranged from 17.84-29.72%¹³. The results of the analysis of variance (ANOVA) showed that the periodic administration of multi-cell probiotics in the biofloc rearing medium had a significant effect on the hematocrit levels of the Asian redtail catfish ($p < 0.05$). The results of the Newman-keuls test showed that the hematocrit levels in treatment P₁ were significantly different from treatments P₂,

P₃, and P₄, very significantly different from treatment P₀.

The Asian redtail catfish reared in media with the addition of multi-cell probiotics showed that the fish were in good health, especially in the P₁ treatment. The hematocrit level of P₁ experienced the highest increase in hematocrit compared to P₂, P₃, and P₄, but was still within the normal range. According to Putra⁶, the addition of multi-cell probiotics to the rearing medium for red tilapia reared for 40 days can increase the hematocrit level seen from the initial hematocrit value of 5.33% and 21.33% at the end.

The provision of multi-cell probiotics in rearing media has the aim of improving water quality. Good water quality can reduce fish stress so that fish appetite increases. Bacteria that grow in water media can intensively convert organic waste into a collection of microorganisms, which can then be used by fish as a food source⁶. Increased hematocrit levels are associated with an increase in total erythrocytes. A high number of

erythrocytes will also be followed by an increase in the percentage of hematocrit. According to Fitria et al.¹⁴ the percentage of hematocrit levels is related to total erythrocytes.

Hemoglobin levels

The results of observations on the hemoglobin levels of the Asian redbtail catfish during the study can be seen in Table 3.

Table 3. Hemoglobin levels of Asian redbtail catfish (*Hemibagrus nemurus*)

Treatment	Hemoglobin Level (g/dL)		
	Day 0	25th day	50th day
P0	4.67±1.16	5.00± 1.00 a	5,20± 0.20 a
P1	4.67±1.16	6,33± 0.42 b	7,40± 0.20 d
P2	5,33±1.16	6.00±0.20ab	6,53± 0.11c
P3	4,33±1.53	6.00±0.56ab	6,20± 0.20c
P4	4,33±1.53	5,20± 0.20 a	5,67± 0.31 b

Based on Table 3, the hemoglobin level of the Asian redbtail catfish during the study was within the normal range. Salasia et al.¹⁵, stated that the normal hemoglobin level of freshwater fish ranged from 5.05-8.33 g/dL. The results of the analysis of variance (ANOVA) showed that the regular administration of multi-cell probiotics in the rearing medium had a significant effect on the hemoglobin level of the Asian redbtail catfish ($p<0.05$). The results of the Newman-keuls test showed that the hemoglobin levels in the P₁ treatment were significantly different from the P₂ and P₃ treatments, and very significantly different from the P₀ and P₄ treatments.

Hemoglobin (Hb) levels are affected by the number of erythrocytes and hematocrit. The correlation between hemoglobin and erythrocytes is that erythrocytes contain hemoglobin which functions to bind oxygen which is used for catabolic processes so that energy is produced¹⁶. The lower the number of red blood cells, the lower the hemoglobin level in the blood¹⁷.

Asian redbtail catfish cultured on a rearing medium added with multi-cell

probiotics had higher hemoglobin levels, especially in P₁ which was given multi-cell probiotics every 5 days. The difference in hemoglobin levels is related to the condition of the water quality of the fish-rearing medium. This is because the addition of multi-cell probiotics to the rearing medium functions to decompose toxic gases in the rearing medium so that ammonia levels will decrease. Fish reared in media water with lower water quality conditions have lower blood hemoglobin levels, this is because fish experience stress¹⁸.

Total Leukocytes

Leukocytes are also one of the factors that are examined to determine fish health through observation of total leukocytes. The results of observations of the total leukocytes of Asian redbtail catfish during the study can be seen in Table 4.

Based on the data in Table 4, it can be seen that the total leukocytes of the Asian redbtail catfish during the study were still within the normal range, as reported by Hartika et al.¹⁹ the number of white blood cells (leukocytes) in normal fish generally ranges from 2.00×10^4 cells/mm³

to 15.00×10^4 cells/mm³. The results of the analysis of variance (ANOVA) showed that the regular administration of probiotics in the rearing medium had a significant effect on the total leukocytes of the Asian redtail catfish ($p < 0.05$). The results of the

Newman-keuls test showed that total erythrocytes in the P₁ treatment were significantly different from the P₀ and P₄ treatments, but not significantly different from the P₂ and P₃ treatments

Table 4. Total Leukocytes of Asian redtail catfish (*Hemibagrus nemurus*)

Treatment	Total Leukocytes (10 ⁴ cells/mm ³)		
	Day 0	25th day	50th day
P0	6,77±0.27	7,76±0.39 ^a	8,48± 0.27 ^a
P1	6,61±0.27	8.81± 0.15 ^b	9,92± 0.03 ^c
P2	6,63±0.25	8.53± 0.03 ^b	9.69±0.34 ^{bc}
P3	6,61±0.27	8,44± 0.41 ^b	9.40±0.03 ^{bc}
P4	6,77±0.27	8.51±0.07 ^b	9.30± 0.29 ^b

Giving multi-cell probiotics to the media can increase the total leukocytes which is an indicator of the immune response in fish. The increase in total leukocytes was higher in P₁ compared to P₂, P₃, and P₄. This is because the more often the addition of probiotics is carried out, the more probiotic bacteria in the waters will always be available, then they will enter the fish's body and increase the immunostimulant. This is in line with the research of Septiarini et al.²⁰, that giving probiotics to carp can increase the non-specific immune response seen from the total leukocyte value of $4.09 - 3.49 \times 10^4$ cells/mm³, the percentage of neutrophils is 8.25- 18.75%, monocytes 3-6.25%, and lymphocytes 75-88.75%.

According to Wulandari et al.²¹ the way leukocytes work, both monocytes and neutrophils in the blood tend to increase oxygen consumption through the oxidation process of NADPH and form oxygen radicals in the form of superoxide anions (O₂⁻), hydroxyl radicals (OH⁻), hydrogen peroxide (H₂O₂), and lipid peroxide (ROOH). These compounds are potential antibacterial that can protect fish bodies from pathogenic infections. The main function of neutrophil cells is the destruction of foreign material through phagocytosis, neutrophils will increase in number as a form of immune response to

the presence of an antigen or foreign protein¹⁶.

The content of multi-cell probiotics, namely *Nitrobacter* sp., *Bacillus* sp., and *Nitrosomonas* sp. Multi-cell probiotics given to fish-rearing media will utilize nitrogen and carbon waste as a source of energy, these bacteria develop and form flocks and will then be utilized by fish. Probiotic bacteria will be eaten by fish and utilized for growth and improving fish health. Probiotics contain lipopolysaccharide which can stimulate the immune system by increasing phagocytic activity by neutrophils and monocytes in the blood²⁰.

According to Sukendar et al²² who stated that probiotics can stimulate non-specific immune responses. The use of probiotics is beneficial in increasing the population of bioremediation agent bacteria because probiotic bacteria can prevent pathogenic bacteria from multiplying in the live media of cultivated animals by fighting the emergence of other bacterial colonies so that the bacteria that grow are expected to be bioremediation agent bacteria²³.

Leukocrit Levels

The results of measuring leukocrit levels of Asian redtail catfish during the study can be seen in Table 5.

Table 5. Leukocrit Levels of Asian redtail catfish (*Hemibagrus nemurus*)

Treatment	Leukocrit Level (%)		
	Day 0	25th day	50th day
P0	1.33±0.58	1.17±0.29	1.17±0.29
P1	1.00±0.00	1.83±0.29	1.83±0.29
P2	1.50±0.50	1.50±0.50	1.67±0.29
P3	1.00±0.00	1.33±0.29	1.50±0.50
P4	1.50±0.50	1.50±0.29	1.33±0.29

Based on the data in Table 5, it can be seen that the leukocrit levels of the Asian redtail catfish during the study ranged from 1.00-1.83%. Leukocrit levels at the beginning of the study ranged from 1.00- 1.50%, on the 25th day it ranged from 1.17 - 1.83%, and on the 50th day it ranged from 1.17 - 1.83%, where P1 has the highest value of 1.83%. Leukocrit levels are still within the normal fish range. According to Titrawani et al.²⁴ the normal range of fish leukocytes ranges from 1-2%. According to Syaieba et al.²⁵, low leukocrit levels, the possibility of chronic infection, low nutritional quality, lack of vitamins, and the presence of contaminants, when leukocrit levels are high, is caused by the initial stages of infection and stress.

Leukocrit levels are affected by total leukocytes, in treatment with high total

leukocytes it will cause leukocrit levels to increase. The leukocrit condition of fish also depends on the condition of the fish at the time of sampling, the length of time between sampling and blood measurement, and the measurement procedure used. Water temperature also affects leukocrit conditions. Relatively high water temperatures allow pathogenic microorganisms to develop and attack fish, so fish defend themselves by forming relatively large numbers of white blood cells. According to Titrawani et al.²⁴ high leukocrit levels are an adaptation of fish.

Fish Blood Glucose

The results of measuring blood glucose in Asian redtail catfish during the study can be seen in Table 6.

Table 6. Glucose levels of Asian redtail catfish (*Hemibagrus nemurus*)

Treatment	Glucose Level (g/dL)		
	Day 0	25th day	50th day
P0	27,33±1.15	42,33± 2.52 ^b	78,67±1.52 ^d
P1	27,67±0.58	43,33± 1.53 ^b	49,67±3.06 ^a
P2	26,67±0.58	36.00±2.00 ^a	55.00± 3.51 ^b
P3	27,67±0.58	37,33±2.52 ^a	56,33± 3.61 ^b
P4	26,67±0.58	46,33± 1.53 ^b	61,67±1.53 ^c

Based on the data in Table 6, it can be seen that the glucose levels of the Asian redtail catfish during the study were still within the normal range. According to Rahardjo et al.²⁶, normal fish blood glucose levels contain 40-90 mg/dL. The smallest blood glucose level of the Asian redtail catfish was achieved in fish reared in maintenance media with the addition of probiotics once every 5 days P₁, namely 49.67 mg/dL. The energy requirement

from glucose to deal with stress can be fulfilled if glucose in the blood can immediately enter the cells⁶.

Giving probiotics to the rearing medium can improve water quality and probiotic bacteria that are in the rearing water will enter the intestines of the fish so that it has a beneficial impact on the fish. Giving probiotics can improve growth performance and immune response to fish reared²⁷. The results of the analysis of

variance (ANOVA) showed that the periodic administration of probiotics in the biofloc-rearing medium had a significant effect on the glucose levels of the Asian redbtail catfish ($p < 0.05$). The results of the Newman-keuls test showed that glucose levels on the 50th day of treatment P_1 were significantly different from treatments P_2 ,

and P_3 , and very significantly different from P_4 and P_0 .

Water quality

The results of measuring water quality parameters during the study showed that all parameters measured were still within the normal range for the life of the Asian redbtail catfish (Table 7).

Table 7. Water quality during research

Parameter	P0	P1	P2	P3	P4	Baku Mutu (PP No.22 Tahun 2021)
Temperature ($^{\circ}\text{C}$)	26-27,1	26-27	26-27	26-27	26,1-27,1	25 – 30
pH	6,1-6,5	6,1-6,3	5,8-6,4	6,1-6,3	6.0-6.1	6 – 9
DO (mg/L)	6,3-7,3	6,2-7,3	6,1-7,4	6.0-7.1	6.0-6.1	>4
Ammonia (mg/L)	0.0025-0.0047	0.0011-0.0017	0.0019-0.0030	0.0020-0.0031	0.0022-0.0030	< 0.2

Based on Table 7 above, it can be concluded that the range of water quality in each treatment is still within the tolerance standard for Asian redbtail catfish seeds where the water quality is still in good condition in the maintenance of Asian redbtail catfish seeds. The temperature during the study ranged from 26 – 27.1 $^{\circ}\text{C}$. The pH value during the study ranged from 6.4-7.1. DO values during the study ranged from 6.0-7.4. Ammonia during the study ranged from 0.0011-0.0015. The value of water quality during the research was still following the quality standards²⁸.

Administration of multi-cell probiotics containing *Bacillus* sp., *Nitrosomonas* sp., and *Nitrobacter* sp. intended to accelerate the decomposition of organic matter, to maintain the concentration of ammonia. Whereas the P_0 treatment showed a higher ammonia value from week to week, this was due to the absence of probiotic bacteria that were able to break down the remaining feces and leftover feed. This is in line with the research¹⁹, giving probiotics to tilapia-

rearing media can maintain water quality with ammonia levels at the end of the study, namely 0.11 mg/L.

4. CONCLUSIONS

Giving probiotics once every 5 days gave the best results for total erythrocytes 2.43×10^6 cells/mm³, hematocrit level 28.67%, hemoglobin level 7.40 g/dL, total leukocytes 9.92×10^4 cells/mm³, leukocrit level 1.83%, and glucose 49.67 g/dL. The water quality during the study was still within the normal range which could support the growth of the Asian redbtail catfish, namely temperature 26-27.1 $^{\circ}\text{C}$, pH 5.8-6.5, DO 6.0-7.4 mg/L, and ammonia 0.0011 -0.0047mg/L.

Based on the research results, it can be suggested to cultivators provide additional probiotics every 5 days at a dose of 0.01 mL/L of water because it can improve the health and weight growth of cultivated fish. In future studies, it is recommended to conduct research by administering probiotics more frequently and observing leukocyte differentiation.

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