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Hematological Response Studies of Climbing Perch (*Anabas testudineus*) Fed with Fermented Feed on the Fermentation of Kelakai Leaves (*Stenochlaena palustris*)

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© 2023 The Authors. This open access article is distributed under a (CC-BY License) Abstract: The purpose of this study was to analyze the nutritional value of fermented feed on kelakai leaves that are feasible as climbing perch feed and the hematological response of climbing perch raised by fermentation-based feeding of kelakai leaves. The study was carried out using an experimental method on the maintenance of climbing perch which was fed based on fermented leaves with 5 treatments and 3 tests. The parameters observed are the nutritional content of feed based on fermented leaves and hematological responses which include erythrocyte, leukocyte, hemoglobin, and hematocrit values. The results of the research on the nutritional content of feed showed that fermented feed on kelakai leaves has a protein content of 27%-31%, crude fiber content of 4%-7%, water content of 12%-14%, ash content of 11%-13%, fat content of 4%-6%. It was concluded that there was a decrease in fiber content and fat content and an increase in protein content, water content, and ash content. It happens gradually in line with the increase in probiotic dose. The results of the study on the hematological response of climbing perch are erythrocytes range from 1.65-3.91, leukocytes range from 223.23-287.43, hematocrit ranges from 15.30-35.07 and hemoglobin ranges from 5.37-10.33. The results of the proximate analysis of feed based on the fermentation of kelakai leaves showed that the values of water content, ash content, crude fiber, protein, and fat were in the recommended range. The hematological response of climbing perch shows good results and is in the normal range.

Keywords: Artificial feed; Hematological; Kelakai

Introduction

Climbing perch is a type of fish that is easy to adapt, breed, and receive the artificial feed, disease resistance, and tolerance to environmental changes (Slamat et al., 2019). A problem that often becomes an obstacle is that the provision of feed requires costs reaching 60–70% of the components of production costs (Emma, 2006). An alternative can be done by making self-made feed by utilizing sources of raw materials that are easy to obtain, sufficient in quantity, relatively cheap, and nutritionally qualified (Akbar, 2012). The results of research by Ansyari et al. (2020), stated that the shoots of kelakai young leaves contain higher protein (27.13%) than old kelakai leaves (26.79%). According to Wijaya et al. (2017) in Petricka et al. (2018), kelakai contains high Fe (41.55 ppm), CU (4.52 ppm), vitamin C (15.41 mg/100g), beta carotene (66.99 ppm), and folic acid 11.30 ppm). The use of kelakai flour has been carried out for climbing perch feed (Ansyari et al., 2020), where the composition of kelakai flour up to 30% can replace soybean flour in feed formulations.

The obstacle to the use of kelakai for fish feed is the high crude fiber content of 10.45% for young leaves and 15.62% for old leaves (Fatmawati et al., 2016), while the

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need for fish for crude fiber is no more than 8% (Mujiman, 2000). This improvement in nutritional value can be improved through the silage of Fauzana et al. (2021) or the fermentation of feed ingredients or feed using microbes (addition of probiotics) (Arief et al., 2014). The results of Fauzana et al. (2021) the nutritional content of fresh kelakai leaves and silage of kelakai leaves with a storage period of 1-6 days contain nutrients that are very suitable for use as a feed with a moisture content of 9.49-11.55%, ash content of 0.72–0.76%, crude fiber content of 2.01-2.23%, fat content of 0.22-0.26% and protein of 26.41-31.44% (Handayani et al., 2017).

Research related to hematological and feed responses has been conducted by Prasetio et al. (2017) on barley fish fed with aloe powder and Eviani et al. (2019) on tilapia fed with gamal leaf extract, which can improve hematological responses in the body and have a good influence on fish health.

Based on several studies on the use of feed for climbing perch farming, studies on the use of kelakai leaves that go through a fermentation process in feed formulations to determine their effect on the hematological response of climbing perch have not obtained adequate information. Kelakai will be included in the inventory of wetland-specific plants that have the potential to be developed as a source of fish feed ingredients. The objectives of this study are as follows: 1) Analyze the nutritional value of fermented feed based on kelakai leaves that are feasible as climbing perch feed; and 2) Analyze the hematological response of climbing perch raised by fermentation-based feeding of kelakai leaves.

Method

Research Time

The research was conducted on March 8 – May 14, 2022, at the Faculty of Fisheries and Marine Affairs, Banjarbaru, South Kalimantan Province. The manufacture of test feed was carried out at the Nutrition and Animal Feed Laboratory, proximate analysis testing at the Venterinir Banjarbaru Hall, and the maintenance of test fish were carried out in the Wet Laboratory.

Research Tools and Materials

Tools used: aluminum baking sheet, small basin, oven, storage machine, knife, pot, pellet mold, measuring cup, erlenmeyer, large basin, hapa, rubber rope, bottle, digital scale, digital ruler, blower, hose and stone aerator, faucet aerator, scoop net, sponge, hose, DO meter, pH meter, spectrophotometer, jar, cool box, plastic baking sheet, glass, rag, syringe and EDTA microtube. Ingredients used: climbing perch, kelakai, fish meal, bran flour, tapioca flour, vitamin and mineral mix, fish oil, EM4 probiotics, molasses, aquades, plastic bags, plastic clips, EDTA 10%, alcohol 70%, ice cubes, tissues, label paper, and tissues.

Research Procedure

Preparation of kelakai floury oung leaves are separated between the leaves and the stem and then dried. The dried kelakai leaves are then mashed and filtered to produce fine kelakai flour. Fermentation of kelakai flour the flour is weighed and mixed with water until moist and steamed for 15 minutes. EM4 probiotics are activated first by adding 3% molasses (Widayati et al., 1996) which has been diluted with aquades, then added to kelakai leaf flour according to the treatment dose (Setyono et al., 2004). The homogeneous flour is then tightly closed and allowed to stand for 3 - 7 days at room temperature. Making fermentation-based feed flour the ingredients of the feed maker are mixed with the composition following (Fatmawati et al., 2016). All feed ingredients that have been mixed ever are only then melded into pellets and dried. Preparation of maintenance containers maintenance container is first disinfected by soaking using water mixed with disinfectant liquid. Water quality conditions are maintained by replacing water with a 25% calling technique once a day (Akbar, 2018). Preparation of test fish climbing perch that are densely kept stocking fish in each basin amount to 20 heads with a water level of 25 cm. The test fish were kept in a basin for 60 days (Mujiman, 2000). Fish sampling Blood sampling of fish is carried out at the end of the study used for the observation of leukocytes, erythrocytes, hematocrit, and hemoglobin.

The experimental design in this study used a Complete Randomized Design with 5 treatments and 3 tests, namely:

Treatment A : Kelakai flour + 0% probiotics Treatment B : Kelakai flour + 5% probiotics Treatment C : Kelakai flour + 10% probiotics Treatment D : Kelakai flour + 15% probiotics

Treatment E : Kelakai flour + 20% probiotics

Observation Parameters

Water quality measurements are carried out at the beginning (H + 1) and every 2 weeks, namely on H + 14, H + 28 H + 45, and H + 60. Temperature, degree of acidity (pH), dissolved oxygen (DO), and ammonia are the observed parameters of water quality. Hematological Response observation of hematological responses was carried out at the end of the study (H + 60) by taking blood samples of climbing perch on each treatment. The blood sample was then analyzed at the Banjarbaru Veterinary Center.

Data Analysis

The data obtained are analyzed by normality test and homogeneity test. Test data that has been in the assumptions accordance with the assumptions is then carried out by a one-way ANOVA. Test results that show real/significant differences, followed by Middle-Value Difference testing.

Result and Discussion

Nutritional Content of Feed

The results of the nutritional content of the feed (Table 1) show that there is an increase in water and ash protein and a decrease in crude fiber and crude fat. The largest increase occurred in the protein content of the E treatment at 3.07% compared to the treatment. The increase in protein levels of the E treatment is to the

research of Lumbanbatu et al. (2018), namely there is an increase in protein levels in each gradual treatment from 2-5% with the addition of EM4 probiotics to the feed. This is by Rachmawati et al. (2006) said that the addition of EM4 in artificial feed can increase the protein content of the feed.

The protein content in treatment A, treatment B, and treatment C is still within the protein range needed by climbing perch according to Kordi (2013), which is protein between 24-28%. The protein content in treatment D and treatment E according to the protein needs of fish in general ranges from 20-60%. Research on pellet feeding with different protein content (28%, 30%, and 32%) found that climbing perch had a length growth of 1.90 cm and a weight growth of 1.02 grams with the best results in feeding 32% protein pellets (Akbar, 2018).

Table 1. Nutritional Content of Feed Based on Fermented Kelakai Leaves (Nutrition and Animal Feed Laboratory, 2022)

Treatment	Crude Protein (%)	Crude Fiber (%)	Water (%)	Ash (%)	Crude Fat (%)
A	27.83	7.96	12.84	11.74	5.11
В	28.29	7.96	12.65	11.75	4.32
С	28.54	4.88	13.28	12.12	4.98
D	30.44	5.28	13.29	12.31	4.96
E	30.90	5.42	13.22	12.26	4.74

The result the decrease occurred in the protein content of treatment C by 3.08% compared to treatment A and treatment B. Decrease in crude fiber occurred by the research of Lumbanbatu et al. (2018) there was a decrease in crude fiber levels in each treatment gradually from 2-4% with the addition of EM4 probiotic 5-20 ml/kg in feed. The higher the dose of probiotics used, the lower the crude fiber content contained in the feed.

The crude fiber content in the study was still around the crude fiber content needed by climbing perch. The crude fiber content in fish feed should be below 8% (Mudjiman, 2000). The results of the crude fiber analysis of the test fish range from 2-8% which means that the test feed has a crude fiber content that suits the needs of the fish. According to Murtidjo (2011), a good percentage of coarse fiber in the content of feed for freshwater fish in the frying period is 5%, while for the enlargement period it is 6%.

The results of the feed moisture content analysis have changed in each treatment. The water content of feed in this study ranged from 12.65-13.29%, which is still higher than SNI (2006) where the quality requirements for water content that must be met are a maximum of 12%. High water content in the sample can also result in damage to the sample such as decay by microbes and chemical reactions such as fat oxidation which is influenced by the amount of water in the sample (Kemendikbud RI, 2013).

The results of the analysis of feed ash content increased in each treatment with the highest results in the E treatment with an ash content of 12.26% compared to other treatments. The ash content in the feed represents the mineral content of the feed, the corresponding content is 3-7 % (Winarno, 1997). The ash content in the test feed ranges from 11-13%. This shows a very high ash content, not by the needs of fish because it has an excess mineral content.

The results of the analysis of feed fat content decreased in each treatment. Good feed generally contains 4-18% fat, while according to Suyanto (1994), the optimal fat content in supporting fish growth is 2.57%. The fat content in this study ranged from 4-6% which is still within the optimal range of fish feed based on literature and does not exceed SNI (2006) where the quality requirements for fat content that must be met are a maximum of 12%.

The results obtained in the research on the fermentation of kelakai leaves using EM4 probiotics can be seen that giving 10% probiotics can reduce crude fiber content and increase protein content in kelakai leaves. Giving 6-8% probiotics containing cellulolytic bacteria Bacillus cereus and proteolytic Trichoderma sp and Penicillium on lamtoro leaves fermented for 7 days can reduce crude fiber content and increase protein content

(Putri et al., 2012). The application of EM4 probiotics to PF-800 commercial pellet feed for a 24-hour fermentation period at a dose of 20 ml/kg of feed experienced an increase in the protein of 5.77% and a decrease in the crude fiber of 3.16% compared to unfermented feed (Hanafie et al., 2021).

The high increase in crude protein in the E treatment is due to the large number of levels of inoculum given (Hermawan et al., 2014). The difference between each EM4 level means that there are differences in the number of microorganisms that work to remodel waste, the more microorganisms that play a role in the overhaul process, the more substrate decomposes because of which bacteria contribute a high protein compared to other treatments. The low decrease in treatments A and B compared to other treatments is because the level of EM4 use given is still small, that is so microbial activity in degrading cellulose is not optimal. This indicates that the higher the use of inoculum levels is expected to lower crude fiber, as more and more microbes can overhaul or degrade fermented materials. This is the opinion of Ratnakomala et al. (2007) in Sijabat (2016) which states that the addition of inoculum will further accelerate the fermentation process and the more the substrate is degraded.

Hematological Response

The Fingerprint Analysis Test (ANOVA) produced F count < F table 5% and 1% then the results showed that fermentation-based feeding of kelakai leaves had no real effect on climbing perch erythrocytes (Table 2). The average erythrocytes of climbing perch fed fermented with kelakai flour fermentation (Figure 1) ranged from 1.65-3.91. According to Irianto (2005), the number of erythrocytes in teleostei fish ranges from (1.05-3.0) x 106/ μ L.

The erythrocytes of climbing perch are higher than the normal limit, this is suspected to be stress in the fish. This condition was corroborated by Hardi et al. (2011), who stated that handling when taking blood samples (injections) or due to pathogenic infections can cause stress in fish so which can increase erythrocytes. According to Fitriana (2019) affects the number of erythrocytes in fish, the condition of hypoxia at the time of blood draw is very likely to occur an increase in erythrocytes. In this study sample, the average number of erythrocytes can still be expressed within the normal range and fish can be declared healthy. The eating response according to Amlacher (1970) can also have an influence on blood composition including the number of erythrocytes which also affects hematocrit.

Table 2. Average erythrocytes of Climbing perch (x 106 $/\mu$ L)

Demotition		Treatment						
Repetition	А	В	С	D	E			
1	2.34	3.54	3.97	1.36	1.54			
2	2.30	4.01	2.49	1.20	3.72			
3	2.83	2.81	5.28	2.40	3.57			
Total	7.47	10.36	11.74	4.96	8.83			
Average	2.49±0.30 ^a	3.45±0.60 ^a	3.91±1.40 ^a	1.65±0.65 ^a	2.94±1.22ª			

Note: the same letter indicates no real difference

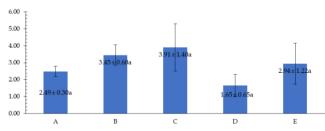


Figure 1. Average graph of climbing perch erythrocytes

The Fingerprint Analysis Test (ANOVA) produced F count > F table 5% and 1% then the results showed that fermentation-based feeding of leaves had a very noticeable effect on climbing perch leukocytes (Table 3). The result of the coefficient of diversity (0.49). The results of the BNJ test found that treatment A was very different markedly from treatment B, treatment C, and treatment E. Treatment A was not significantly different

from Treatment D. Treatment B was significantly different from Treatment C.

This leukocyte value provides clues about the health of fish and helps to determine the onset of abnormalities (Fitriana, 2019). The average leukocytes of climbing perch were fed fermented with kelakai flour (Figure 2). It ranges from 223.23-287.43. Leukocyte values of freshwater fish are in the standard value range of 200-300 x 10 3/µL (Bastiawan et al., 1995). The number of leukocytes will increase when the fish is exposed to infection because it is an active unit in the body's defense system, and leukocytes play a role in fighting infectious diseases (Kimball, 1988). Then the number of leukocytes can also decrease if the body condition is stressed. Factors affecting the normal number of such leukocytes should be controlled during the maintenance of fish so that the fish are always in good health (Fatmawati et al., 2018).

Domotition			Treatment		
Repetition	А	В	С	D	E
1	216.70	271.90	287.40	241.40	281.30
2	221.10	262.20	290.10	231.20	278.70
3	231.90	252.20	284.80	236.00	283.50
Total	669.70	786.30	862.30	708.60	843.50
Average	223.23±7.82ª	262.10±9.85b	287.43±2.65b	236.20±5.10 ^a	281.17±2.40 ^b

Table 3. Average Climbing Perch Leukocytes (x $103/\mu$ L)

Note: the same letter indicates no real difference

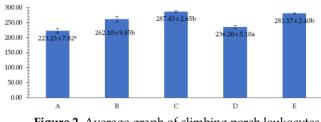


Figure 2. Average graph of climbing perch leukocytes

The Fingerprint Analysis Test (ANOVA) produced F count < F table 5% and 1% then the results showed that feeding based on fermented leaves kelakai had no real effect on climbing perch hematocrit (Table 4).

The average hematocrit of climbing perch fed fermented with kelakai flour ranges (Figure 3) between 15.30-35.07. Fange (1992), states that hematocrit in several teslostei fish ranges from 20-40%. According to Salasia et al. (2001), the hematocrit value is directly

Table 4. Average Climbing perch Hematocrit (%	Climbing perch Hematocrit (%)
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related to the number of fish erythrocytes, meaning that the hematocrit value will increase if the number of erythrocytes increases. The results obtained in this study also showed that the higher the hematocrit value, the higher the number of erythrocytes (Nabib et al., 1989).

According to Bastiawan et al. (2017), when fish are exposed to disease infections or appetite decreases, the hematocrit value becomes low. The increase in hematocrit value means improving the health status of fish, this is by the opinion of Wedemeyer et al. (1977), according to which hematocrit levels can be used as a clue to find out the low protein content, vitamin deficiency, or fish getting infections (Kurniasih et al., 2021). Numbers et al. (1990) state that fish hematocrit varies depending on nutritional factors and the age of the fish. The results of the research of Lestari et al. (2019) show qualitatively that the higher the hematocrit value, the higher the number of erythrocytes.

Demetition			Treatment		
Repetition –	А	В	С	D	E
1	17.80	28.30	31.60	12.10	15.70
2	19.10	32.00	24.40	11.90	31.20
3	23.60	23.10	49.20	21.90	29.20
Total	60.50	83.40	105.20	45.90	76.10
Average	20.17 ± 3.04^{a}	27.80±4.47 ^a	35.07±12.76 ^a	15.30±5.72 ^a	25.37±8.43 ^a

Note: the same letter indicates no real difference

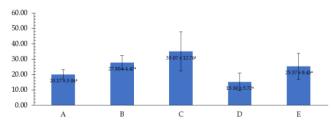


Figure 3. Average graph of climbing perch hematocrit

The Fingerprint Analysis Test (ANOVA) produced F count < F table 5% and 1% then the results showed that fermentation-based feeding of kelakai leaves had no real effect on climbing perch hemoglobin (Table 5). The average hemoglobin of climbing perch fed fermented with kelakai flour (Figure 4) ranges from 5.37-10.33. According to Salasia et al. (2001) the hemoglobin

concentration of freshwater fish ranges from 5.05-8.33 gr/dl. According to Hastuti et al. (2011), the large amount of hemoglobin in the blood indicates the ability to transport oxygen from the gills to the tissues by the blood. Most teleostei fish (hard-boned fish) have hemoglobin in the same erythrocytes as in other vertebrates. Stressful states can affect the physiological activity and hemoglobin levels in fish. The physiological state of fish blood varies greatly, depending on environmental conditions such as humidity, temperature, and pH (Adelbert, 2008).

Hemoglobin levels are in harmony with the number of erythrocytes, the higher the hemoglobin level the higher the number of erythrocytes. Hemoglobin levels are related to the number of erythrocytes but are not necessarily correlated with the number of erythrocytes because hemoglobin is the pigment content of red blood cells. Hemoglobin levels do not undergo significant changes, although the number of erythrocytes rises (Lagler et al., 1977).

According to Lagler et al. (1977) in Kurniaji (2015) and (Purwanti et al., 2014) states, that hemoglobin (Hb) levels in fish blood correlate with erythrocyte counts. The level of hemoglobin (HB) in the blood of fish is closely related to the number of erythrocytes.

Table 5. Average Climbing perch Hemoglobin (g/dl)

Hemoglobin transports oxygen along with Fe (iron) in the blood, low levels of HB are thought to cause anemia in fish. Thus, the results of the average amount of hemoglobin in this study sample still showed a normal range, and the fish were declared healthy. This condition is suspected that it is closely related to the type of climbing perch that can take oxygen with an additional breathing device in the form of a Labyrinth (Asyari, 2017).

Denstition			Treatment		
Repetition -	А	В	С	D	E
1	6.60	10.10	10.90	4.10	4.50
2	6.40	11.00	5.50	3.70	10.40
3	8.10	8.30	14.60	8.30	10.10
Total	21.10	29.40	31.00	16.10	25.00
Average	7.03±0.93ª	9.80±1.37 ^a	10.33 ± 4.58^{a}	5.37±2.55 ^a	8.33±3.32 ^a

Note: the same letter indicates no real difference

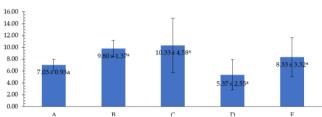


Figure 4. Average graph of climbing perch hemoglobin

A study on the effects of giving kelakai extract (Stechnolaena palustris) for 1 week in white rats showed that hemoglobin levels improved twice as high as the control treatment (Negara et al., 2017). According to Petricka et al. (2018), based on the results of the research provides evidence that kelakai (Stechnolaena palustris) is effective in increasing hemoglobin levels. Significant increase in hemoglobin levels (3.24 g/dl) after taking kelakai (Stenochlaena palustris) for a week ($p \le 0.05$). It is proved that kelakai (Stechnolaena palustris) is an alternative option to increase hemoglobin levels naturally.

Hemoglobin from remodeled red blood cells will be released and carried into the liver to be used as iron derived from the released hemoglobin earlier (Suwarno, 2014). According to Negara et al. (2017), it turns out that kelakai extract can increase hemoglobin levels that have decreased. Hemoglobin levels increase because Hemoglobin (Hb) is a protein that contains iron (Fe) with a high content of iron and protein contained in the kelakai which can trigger the formation of hemoglobin levels so that hemoglobin levels over the not too long period have increased very rapidly (Aminah et al., 2021).

Water Quality

Measurements of water quality during the maintenance of climbing perch (Table 6) such as pH, temperature, dissolved oxygen, and ammonia should be at an optimal range by the provisions of water quality for fish farming. Drastic changes in water quality are very dangerous for fish because they can trigger stress in fish so that the metabolic rate increases (Effendie, 2002).

The results of measuring water temperature in the maintenance media of climbing perch fry during the study obtained temperatures in each treatment, which ranged from 26.12-26.82ºC. This is to the statement of Kordi (2013) which states that the optimal temperature for the growth of climbing perch ranges from 25-32°C and the Ministerial Decree (2014) which states that climbing perch for enlargement grows well at temperatures between 15-30°C.

	Table 6. Average	Water Qua	lity (Source	: Primary	Data,	2022)
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		Parameter		
Treatment	Temperature (°C)	pН	DO (mg/L)	Ammonia (mg/L)
А	26.82	7.1	6.68	1.002
В	26.62	6.82	6.36	1.176
С	26.62	6.84	6.84	1.05
D	26.4	6.98	6.74	1.548
Е	26.12	7.04	6.06	1.518
References*	25-32	6-8.5	3-7	< 0.016

Kordi, 2013

According to Irianto (2005), aquatic organisms have a degree of tolerance to temperature with a certain range that plays a very important role in growth, incubation of eggs, feed conversion, and resistance to diseases. Kohno et al. (1986) in Pramono et al. (2006), reported that water temperatures that are too low can cause metabolic processes to be slow, which can affect the growth rate of fish larvae will be slow to grow. High temperatures can cause an increase in the metabolic processes of fish which increases the intensity of sewage so that the oxygen content decreases.

The results of measuring the degree of acidity (pH) in this study ranged from 6.82-7.1 in each treatment. The pH value in each such treatment is quite good for the life of the fish. The optimal pH value according to Kordi (2013), ranges from 6-8.5. Types of fish that live originally in swamps have the resistance to survive in a very low or high pH range, which is between 4-11, especially climbing perch. pH that is less than 6 and more than 9.5 for a long time affects the growth and reproduction of fish.

Dissolved oxygen in this study ranged from 6.06-6.84 mg/l. The oxygen level suitable for the growth of climbing perch according to Kordi (2013) is 3-7 mg/l and the opinion of Khairuman et al. (2008) that the dissolved oxygen needed in fish farming is at least 3 mg/L.

The results of ammonia measurements during the study ranged from 1,002-1,176 mg/l. The optimal ammonia level for climbing perch farming is <0.016 mg/L (Kordi, 2013) while Haryanti (1995), stated that the growth of climbing perch fry is still good where ammonia levels in the water are still below the appropriate tolerance limit of 0.50 mg/L, so as not to cause disturbances in test fish. Ammonia levels above 1.5 mg/L are toxic to fish commercially (Chen et al., 2006).

Ammonia poisoning in fish will result in increased oxygen consumption, damage to the gills, and reduce the ability of the blood to transfer oxygen (Boyd, 1988). This condition during maintenance does not occur because ammonia levels during maintenance can still be tolerated by climbing perch. As the value of ammonia increases the need for oxygen will also increase. The accumulation of the above factors is one of the causes of the growth of climbing perch.

Ammonia management during the study was minimized by changing water with a 25% sampling technique to reduce ammonia concentrations and the aeration system can increase the supply of dissolved oxygen in the maintenance container so that death can be suppressed during the study. This can be seen from the results of dissolved oxygen which is still in the optimal range and survival is still high (Norhayati et al., 2020).

Conclusion

The content of fermentation-based feed leaves has increased protein and decreased crude fiber content, occurring gradually in line with the increase in the dose of probiotics used. Fermentation-based feeding of flour shows the hematological response of climbing perch with erythrocyte, leukocyte, hematocrit, and hemoglobin values to be in the optimal range in each treatment.

Author Contributions

This authors in this research are divided into executor and advisor.

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Conflicts of Interest

The author declares no conflict of interest this research.

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