

# THE EFFECT OF ADDITION OF PROBIOTICS ON THE GROWTH AND HEALTH PERFORMANCE OF SNAKEHEAD FISH (*CHANNA STRIATA*) SEEDS IN THE BUCKET FISH CULTIVATION METHOD

Pengaruh Penambahan Probiotik Terhadap Performa Pertumbuhan dan Kesehatan Benih Ikan Gabus (*Channa striata*) di Sistem Budikdamber

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### ABSTRACT

This study aims to analyze the effect of the addition and optimal dose of probiotics on the growth performance and health of snakehead fish (channa striata) seeds in the budikdamber system. The budikdamber system is an innovation in fisheries cultivation in limited land that is easy to apply at home by the community at an affordable cost. The study was conducted using a completely randomized design (CRD) consisting of 4 treatments of probiotic doses and a control without probiotics, with each treatment repeated three times. The treatments used were (A) without the addition of probiotics (control), (B) the addition of probiotics as much as 0.6 ml/L of water, (C) the addition of probiotics as much as 0.8 ml/L of water, (D) the addition of probiotics as much as 1.0 ml/L of water, and (E) the addition of probiotics as much as 1.2 ml/L of water which were maintained for 40 days. The test fish used were 5-6 cm in size and weighed 1-2 grams. The container used was a plastic bucket with a volume of 80 liters with a water volume of 70 liters where the snakehead fish were placed with a stocking density according to the treatment. The amount of feed given was 5% of the fish biomass. The results obtained during the study showed that treatment C with the addition of probiotics as much as 0.8 ml/L of water produced the highest absolute length growth of 2.71±0.05 cm, the highest absolute weight growth of 2.87±0.27 grams, a specific growth rate of 1.69±0.02%, survival of 89±0.03%, the number of erythrocytes 1,356,800±0.02 and the number of leukocytes 120,617±0.07

Keywords: Probiotics, Snakehead Fish, Budikdamber, Growth, Health

### ABSTRAK

Penelitian ini bertujuan untuk menganalisis pengaruh penambahan dan dosis optimal probiotik terhadap performa pertumbuhan dan kesehatan benih ikan gabus (*channa striata*) di sistem budikdamber. Sistem budikdamber merupakan inovasi budidaya perikanan di lahan terbatas yang mudah diterapkan di rumah oleh masyarakat dengan biaya yang terjangkau. Penelitian

dilakukan dengan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 4 perlakuan dosis probiotik dan kontrol tanpa probiotik, dengan masing-masing perlakuan diulang sebanyak tiga kali. Perlakuan yang digunakan adalah (A) tanpa penambahan probiotik (kontrol), (B) penambahan probiotik sebanyak 0,6 ml/L air, (C) penambahan probiotik sebanyak 0,8 ml/L air, (D) penambahan probiotik sebanyak 1,0 ml/L air, dan (E) penambahan probiotik sebanyak 1,2 ml/L air yang dipelihara selama 40 hari. Ikan uji yang digunakan berukuran 5-6 cm dengan bobot 1-2 gram. Wadah yang digunakan merupakan ember plastik dengan volume 80 liter dengan volume air sebesar 70 liter yang dimana ikan gabus ditempatkan dengan padat tebar sesuai dengan perlakuan. Jumah pakan yang diberikan sebanyak 5% dari biomassa ikan. Hasil yang didapatkan selama penelitian menunjukan bahwa perlakuan C dengan penambahan probiotik sebanyak 0,8 ml/L air menghasilkan pertumbuhan panjang mutlak tertinggi sebesar 2,71±0,05 cm, pertumbuhan bobot mutlak tertinggi 2,87±0,27 gram, laju pertumbuhan spesifik 1,69±0,02 %, kelangsungan hidup  $89\pm0,03\%$ , jumlah eritrosit 1.356.800±0,02 dan jumlah leukosit 120.617±0,07.

Kata Kunci: Probiotik, Ikan Gabus, Budikdamber, Pertumbuhan, Kesehatan

#### **INTRODUCTION**

Snakehead fish (Channa striata) is a type of freshwater fish that is popular for cultivation because of its ability to survive in less than optimal water quality conditions. This is due to the presence of additional respiratory organs (diverticula) that allow this fish to take oxygen directly from the air (Djokosetiyanto *et al.*, 2017). Snakehead fish (Channa striata) has high economic value and beneficial nutritional content, especially its carcass albumin. This albumin content is known to play an important role in accelerating the wound healing process, making snakehead fish a potential commodity with strategic value (Mustafa *et al.*, 2012).

Based on data from the Ministry of Maritime Affairs and Fisheries of the Republic of Indonesia, snakehead fish production in 2015 was recorded at 6,490 tons, which then increased significantly to 21,987 tons in 2019 (KKP 2020). The increasing market demand for snakehead fish has encouraged various efforts to increase the production of this fish. However, one of the main challenges in cultivating snakehead fish (Channa striata) is the high mortality rate caused by the limited tolerance of fish to extreme changes in water quality, especially related to pH and temperature parameters. In addition, snakehead fish cultivation often uses concrete/earthen ponds which require very high costs. One step to optimize snakehead fish production is to apply the budikdamber method (fish farming in buckets) (Apriliani *et al.*, 2021).

Budikdamber is a potential solution for fish farming in limited areas, with higher water use efficiency. This method is easy to apply by people at home with relatively affordable capital, so it can help meet the nutritional needs of the family (Andhikawati *et al.*, 2021). In this system, fish farming is the main activity, while vegetable production is an additional business that has economic value (Saparinto and Susiana 2024). However, one of the technical obstacles often faced in budikdamber is maintaining water quality. High levels of ammonia in budikdamber can be toxic to fish. Poor water conditions can cause various growth and health disorders in fish. Therefore, water quality must be maintained to remain clean and free from harmful contaminants. Sudden changes in water quality can trigger stress in fish, resulting in decreased growth and health performance (Wahyuningsih 2020).

Problems found such as growth, survival, water quality and health can be overcome by using appropriate probiotics in aquaculture activities. Various studies have proven that the application of probiotics in fish farming can increase growth, survival rates, feed utilization efficiency, and fish immune responses (Huerta Rabago *et al.*, 2019). However, most of the studies conducted to date have focused more on the use of probiotics as additives in feed. In contrast, the application of probiotics directly to water or in maintenance media is still

relatively under-explored, although this method has the potential to have a positive impact on fish growth and health. (Jahangiri & Esteban, 2018). The use of probiotics directly in water is one solution to increase growth and maintain the health of snakehead fish, because probiotics can increase the fish's resistance to stress and disease (Martinez Cruz *et al.* 2012).

#### **RESEARCH METHODS**

## **Place and Time**

The research was conducted for 40 days in January 2025 – February 2025. The location of the research was in Building 4 of the Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor.

#### **Tools and Materials**

The tools used in this study were 15 buckets with a size of 80 liters, a set of aeration tools, namely aerators, aeration taps, hoses, and aeration stones, 7 in 1 water test tools, DO meters, digital scales, drinking glasses, hydroton, millimeter blocks, cellphone cameras, stationery, and ladles. The materials used were 1050 snakehead fish with a length of 5-6 cm and a weight of 1-2 grams obtained from Farm Ajis Bibit Parung, Bogor, West Java, commercial feed PF1000, Probiotic BIOM-S, Rajawali Water Spinach Seeds.

#### **Experimental Design**

This study was conducted using an experimental method, namely using a Completely Randomized Design (CRD). The treatment used in this study was the addition of probiotics as much as 0.6 - 1.2 ml / L of water with a density of 1 tail / liter (Dzulhadi *et al.*, 2025; Fadillah *et al.*, 2025)

### Procedure

The implementation stage of the research includes preparation of water spinach plants, preparation of test fish, preparation of containers, management of water quality, data collection by measuring the length and weight of fish, observation of the number of fish per bucket, observation of the feed eaten, measurement of the length and weight of dead fish, measurement of water quality and observation of fish health including red blood cells and white blood cells. Fish that have been acclimatized in fiber tubs will be sorted and spread into buckets as much as 1 fish/liter which are given a feed dose of 5% of fish biomass with a feeding frequency of 3 times a day. The research was conducted for 40 days with water changes every 5 days, data collection of length and weight as much as 30% and collection of water quality data is carried out every 10 days including temperature, pH, DO, ammonia, nitrite and nitrate and blood counts are calculated at the end of the study. The parameters measured include: (1) Absolute length growth (Lucas *et al.*, 2015), (2) Absolute weight growth (Everhart *et al.*, 1975 in Effendie 1997), (3) Specific Growth Rate (Effendie, 1997), (4) Feed Conversion Ratio (Effendie, 1997) (5) Survival rate (Effendie, 1997) (6) Red Blood Cell Count (Blaxhall, 1972)

(1)  $\Delta L = Lt - Lo$ 

Note :

- $\Delta W$  : Absolute Length Increase (g)
- Wt : Average length of final study (g)
- Wo : Average initial length of research (g)

(2)  $\Delta W = Wt - Wo$ 

Note :

ΔW : Absolute Weight Gain (g)
 Wt : Final average weight of the study (g)
 We : Initial average weight of the study (x)

Wo : Initial average weight of the study (g)

$$(3) SGR = \frac{\ln Wt - \ln Wo}{t} \times 100\%$$

Note:

SGR : Specific growth rate Wt : Flat weight - flat at time t (g) Wo : Initial average weight (g) t : Time (day)

(4) 
$$FCR = \frac{F}{(Wt+D)-Wo}$$

Note :

FCR : Feed Conversion Ratio

 $Wt \qquad : Average \ weight \ of \ fish \ at \ the \ end \ of \ the \ study \ (g)$ 

Wo : Average weight of fish at the beginning of the study (g)

D : Weight of fish that died during the study

F : Total feed given (g)

$$(5) SR = \frac{Nt}{No} \times 100\%$$

Note :

SR : Survival rate (%)

Nt : Number of fish alive at the end of the study (tails)

No : Number of fish alive at the start of the study (tails)

(6) Eritrosit = 
$$(\frac{A}{N}) \times (\frac{1}{V}) \times Fp$$

Note :

A : The number of erythrocytes is counted
N : The number of boxes counted from the haemacytometer
V : Volume kotak haemacytometer

Fp : Dilution factor

(7) Leukosit = 
$$\frac{N}{0,4}$$
 × Multiplier factor

Note :

Ν	: Leukocyte count
0,4	: Blood volume in the slide object glass of the haemacytometer
Multiplier factor	: With multiple dilutions (20 times)

### **Data Analysis**

The data obtained from the measurement results of absolute length increase, absolute weight increase, specific growth rate, feed conversion ratio, survival rate, number of red blood cells and number of white blood cells will be analyzed using the F Test or ANOVA (Analysis Of Variance) with a confidence level of 95%. If the analysis results show a significant difference between treatments, it will be continued with the Duncan test to find out which treatment gives the best results, while water quality data will be analyzed descriptively.

### RESULT

### **Absolute Length Growth**

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 1. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the absolute length growth parameters of snakehead fish.



Figure 1. Absolute Length Growth Graph of Snakehead Fish Seeds

### Absolute Weight Gain

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 2. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the absolute weight growth parameters of snakehead fish.



Figure 2. Absolute Weight Growth Graph of Snakehead Fish Seeds

## **Specific Growth Rate**

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 3. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the specific growth rate parameters of snakehead fish.



Figure 3. Specific Growth Rate Graph of Snakehead Fish Seeds

## **Feed Conversion Ratio**

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 4. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L did not show significant differences (P>0.05) in the feed conversion ratio parameters, but there was a tendency for an increase in the feed conversion ratio from treatment 0.6 to 1.0.



Figure 4. Graph of Feed Conversion Ratio of Snakehead Fish Seed

## **Survival Rate**

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 5. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the survival parameters of snakehead fish.



Figure 5. Survival Graph of Snakehead Fish Seeds

### Water Quality

The range of water quality values for the maintenance of snakehead fish seeds with the addition of different probiotics in the Budikdamber system for 40 days of research can be seen in Table 1 and Table 2. Based on Table 1 and Table 2, it can be seen that the water quality during the maintenance of snakehead fish seeds in the study has been in accordance with the water quality standards for snakehead fish cultivation based on the quality standard data listed in Table 1 and Table 2.

Turaturant	Physical Parameters			
reatment	Temperature (°C)	DO (mg.L <sup>-1</sup> )	pН	
А	25,6 - 27,5	4,2 - 7,1	6,2 - 7,4	
В	26,3 - 28	4,2 - 7,2	6,1 - 7,8	
С	25,6 - 27,8	4,5 - 7,2	6,2 - 7,4	
D	25,7 - 27,8	4,2 - 6,7	6,3 - 7,4	
E	26,2 - 27,9	4,2 - 7,2	6,1 - 7,6	
	23 - 27	0,2 - 8,6	4 - 7	
Optimum Range	(Muflikha <i>et al.</i> 2008)	(BPBAT Mandiangin 2014)	(BPBAT Mandiangin 2014)	

Table 1. Water Quanty Physical Parameters	Table 1	. Water	Ouality	Physical	Parameters
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Tursturset	Chemical Parameters			
Ireatment	Ammonia (mg.L <sup>-1</sup> )	Nitrite (mg.L <sup>-1</sup> )	Nitrate (mg.L <sup>-1</sup> )	
А	0 - 0,25	0 - 0,1	0	
В	0 - 0,25	0 - 0,1	0	
С	0 - 0,25	0 - 0,1	0	
D	0 - 0,25	0 - 0,1	0	
Е	0 - 0,25	0 - 0,1	0	
	< 0,02	< 0,06	< 20	
Optimum Range	(BPBAT Mandiangin 2014)	(SNI 2014)	(SNI 2014)	

### Table 2. Water Quality Chemical Parameters

### **Red Blood Cell Count**

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 6. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the parameters of the number of red blood cells in snakehead fish.



Figure 6. Number of Red Blood Cells in Snakehead Fish Seed

### White Blood Cell Count

The results of the study on the addition of probiotics to the growth and health of snakehead fish seeds in the Budikdamber system are presented in Figure 7. The study, which lasted for 40 days, showed that the addition of different probiotics, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, had a significant effect (P<0.05) on the parameters of the number of red blood cells in snakehead fish.



Figure 7. Number of White Blood Cells in Snakehead Fish Seeds

### DISCUSSION

### **Absolute Length Growth**

The addition of probiotics tested, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, showed a significant effect (P<0.05) on the absolute length increase of snakehead fish. Treatment A (without the addition of probiotics, control) produced the lowest length increase, namely  $1.77 \pm 0.21$  cm during the maintenance period. In contrast, the highest absolute length increase of  $2.71 \pm 0.05$  cm was achieved in treatment C, with the addition of probiotics of 0.8 ml/L of water.

Research by Dzulhadi *et al.*, (2025) showed that a dose of 0.8 mL/L resulted in an absolute length increase of tilapia of 4.37 cm, the highest value among other treatments. The effectiveness of this dose is due to the population of probiotic bacteria that is sufficient to improve water quality and support digestion. The ability of Bacillus Sp. to improve growth performance depends on the dose (Elsabagh *et al.*, 2018). Kuebutornye *et al.*, (2019) emphasized that adding probiotics, for example, Bacillus sp., to the maintenance water will increase beneficial bacteria and multiply them in the fish pond. Xu *et al.*, (2013) stated that Bacillus sp. can produce extracellular enzymes and antimicrobial peptides that control pathogenic bacteria and improve water quality. The quality of the aquatic environment directly affects the performance and welfare of the cultivated organisms (Hura *et al.* 2018).

### **Absolute Weight Gain**

The addition of probiotics tested, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, showed a significant effect (P<0.05) on the absolute weight gain of snakehead fish. Treatment A (without the addition of probiotics, control) produced the lowest weight gain, namely  $2.87 \pm 0.27$  grams during the maintenance period. In contrast, the highest absolute weight gain of  $4.20 \pm 0.14$  grams was achieved in treatment C, with the addition of probiotics as much as 0.8 ml/L of water.

The results of the study by Dzulhadi *et al.*, (2025) showed the best dose of probiotic bioms on the growth of tilapia (Oreochromis niloticus) of 0.8 mL/L which resulted in the highest absolute weight gain of 12.40 grams compared to other treatments. This dose allows the population of probiotic bacteria such as Bacillus sp. develop indirectly in the digestive tract, so that they are able to secrete major digestive enzymes such as protease and amylase which can accelerate the breakdown of feed into efficiently absorbed nutrients (Elsabagh *et al.*, 2018; Setiawati *et al.*, 2013). The positive effects of water-added probiotics can be attributed to the biological performance of Lactobacillus sp., Bacillus sp., and Saccharomyces cerevisiae, which are the main probiotic components in BIOMS. These probiotic species play several important roles in improving water quality parameters in aquaculture systems, including reducing organic matter (Hlordzi *et al.*, 2020), reducing ammonia (Li *et al.*, 2022), stabilizing pH and nutrient cycling (Huang *et al.* 2022; Said *et al.* 2022).

## **Specific Growth Rate**

The addition of probiotics tested, namely 0.6 ml/L of water, 0.8 ml/L of water, 1.0 ml/L of water and 1.2 ml/L of water, showed a significant effect (P<0.05) on the specific growth rate of snakehead fish. Treatment A (without the addition of probiotics, control) produced the lowest specific growth rate, namely  $1.41 \pm 0.06\%$  during the maintenance period. In contrast, the highest specific growth rate of  $1.69 \pm 0.02\%$  was achieved in treatment C, with the addition of probiotics as much as 0.8 ml/L of water.

The results of the study by Dzulhadi *et al.*, (2025) showed that at this dose, fish achieved an SGR of  $1.98 \pm 0.12$ , much higher than other doses. This effectiveness is closely related to the sufficient abundance of probiotic bacteria, especially Bacillus sp., which are indirectly able to actively secrete the main digestive enzymes such as protease, amylase, and lipase in the digestive tract. These enzymatic activities accelerate nutrient hydrolysis and increase absorption efficiency, which has a direct impact on growth acceleration (Omar *et al.*, 2024; Zorriehzahra *et al.*, 2016). Bacillus sp. bacteria ferment indigestible nutrients, including dietary fiber, in the large intestine and thus produce oligosaccharides, oligopeptides, and simpler molecules, including short-chain fatty acids, which increase their absorption, improve the immune system, and thus increase growth and reduce mortality (Standen *et al.*, 2016).

## **Feed Conversion Ratio**

Feed Conversion Ratio (FCR) of snakehead fish seeds ranged from  $0.91 \pm 0.04\%$  to  $1.03 \pm 0.12\%$ . The highest FCR value, which was  $1.03 \pm 0.12$ , was obtained in treatment A (control), while the lowest FCR value, which was  $0.91 \pm 0.04\%$ , was found in treatment B with the addition of probiotics of 0.6 mL/L.

Low FCR generally indicates good feed efficiency, because the amount of feed needed to produce body weight gain is relatively small (DKPD 2010). However, the insignificant difference between treatments indicates that the addition of probiotics through water has not provided a strong enough biological effect in increasing the efficiency of feed utilization. This can be caused by several factors.

The method of applying probiotics directly to the water medium is likely less effective in ensuring that probiotic bacteria reach and colonize the fish's digestive tract optimally. According to Newaj-Fyzul *et al.*, (2014) the success of probiotic colonization depends on the media of administration. Application through water tends to cause most bacteria to be suspended in the environment, not entering the fish's body. The use of the same type and quality of feed and controlled cultivation environmental conditions can cause relatively stable feed efficiency across treatments. Thus, even though there is probiotic treatment, its main role in increasing feed efficiency has not been clearly seen.

#### Survival rate

The survival rate of snakehead fish seeds ranges from  $80 \pm 0.04\%$  to  $89 \pm 0.03\%$ . The lowest survival rate was recorded in treatment A (control) at  $80 \pm 0.04\%$ , while the highest survival rate was achieved in treatment C, namely with the addition of 0.8 mL/L of probiotics, which reached  $89 \pm 0.03\%$ . These results indicate that variations in probiotic doses have a significant effect on the survival of snakehead fish seeds in the bucket cultivation system (budikdamber).

Research by Dzulhadi *et al.*, (2025) showed that at this dose, fish were able to achieve a survival rate of 82%, higher than other doses. This success is due to the sufficient number of probiotic bacteria to work effectively in the digestive system and culture media. The combination of bacteria such as Bacillus sp., Lactobacillus sp., and Saccharomyces cerevisiae plays an important role in suppressing pathogens, stabilizing intestinal microflora, and strengthening the immune response of fish (Iribarren *et al.*, 2012; Putri *et al.*, 2012). Probiotics, especially Saccharomyces cerevisiae, are known to be able to increase phagocytosis and non-specific immune responses, thereby strengthening the body's resistance to internal and external infections (Apriyan *et al.* 2021).

### Water Quality

Water quality can affect the growth and survival of farmed fish. According to Muflikha et al. (2008) that the temperature range of 23 - 27 oC is safe for snakehead fish to be cultivated and reproduce well. According to BPBAT Mandiangin (2014) the water quality standards for snakehead fish cultivation are pH 4-7, DO 0.2-8.6 mg/L, and ammonia <0.02. Kordi (2011) added that snakehead fish can live in waters with low dissolved oxygen content of up to 2 mg/l because they have additional respiratory organs. The good pH range for snakehead fish growth is 6.5-8.5 (Kordi and Ghufran 2013). The results of measuring ammonia levels in the maintenance media during the study ranged from 0 - 0.25 mg.L-1. Ammonia levels of 0.25 mg.L-1 indicate that they have passed the optimum limit. Based on BPBAT Mandiangin (2014), the ammonia level for snakehead fish is <0.02 mg.L-1. However, snakehead fish can tolerate this ammonia level. According to Bijaksana (2011), the ammonia range of 0.30 - 0.70 mg.L-1 can still support the growth of snakehead fish. This high ammonia is caused by the accumulation of metabolic waste and leftover feed in a limited container, without adequate water circulation and biofiltration support. The probiotics used contain Bacillus, Lactobacillus, and Saccharomyces, not specifically tasked with converting ammonia into nitrite or nitrate. If this process is not followed by the activity of nitrifying bacteria such as Nitrosomonas, then the accumulation of ammonia has the potential to increase and can have a negative impact on water quality and fish health (Martínez-Córdova et al., 2015)

The results of measurements of nitrite and nitrate levels respectively in the maintenance media during the study ranged from 0 - 0.1 mg / L and 0. Nitrite levels are considered high for snakehead fish, because the optimal tolerance limit according to BPBAT Mandiangin (2014) reaches 0.06 mg / L. The increase in nitrite levels indicates that the first stage of the nitrification process, namely the oxidation of ammonia to nitrite by Nitrosomonas bacteria, has occurred. This is because there are naturally formed Nitrosomonas bacteria originating from the maintenance media, so that ammonia can be partially converted into nitrite, but has not been optimally continued to the next stage, namely the conversion of nitrite to nitrate by Nitrobacter bacteria. This is thought to be due to the low population of Nitrobacter bacteria, or environmental conditions such as temperature, pH, and dissolved oxygen levels that do not support their activity. According to Park *et al.*, (2010), the nitrification process is highly dependent on the balance of microbial populations and the stability of water quality parameters. If there is an imbalance, nitrite can accumulate and be toxic to fish and nitrate is not detected.

### **Red Blood Cell Count**

Based on observations, the red blood cells of snakehead fish fry ranged from 757,883  $\pm$  0.08 - 1,356,800  $\pm$  0.02. The lowest red blood cells of 757,883  $\pm$  0.08 were obtained in snakehead fish fry given treatment A (control). The highest number of red blood cells of 1,356,800  $\pm$  0.02 was obtained in treatment C (Addition of Probiotics 0.8 mL/L of water). According to Robert's research (2012), the number of erythrocytes in Teleostei fish ranged from (1.05-3.0) x 10^6 cells/mm^3. The increase in erythrocytes at a dose of 0.8 mL/L is thought to be due to the sufficient number of probiotic bacteria to increase digestive efficiency, improve water quality, and support the immune system. Sharma *et al.*, (2022) explained that Saccharomyces cerevisiae is able to stimulate the immune system, including phagocyte production and hematopoietic activity, thereby increasing the number of blood cells. Baisakhi *et al.*, (2024) also stated that probiotics in the right dose can increase hematological parameters such as erythrocytes and leukocytes.

In contrast, in treatment A (control/without probiotics), the lowest number of erythrocytes was recorded at  $757,883 \pm 0.08$  cells/µL. This number indicates a less than optimal physiological condition of the fish, due to the lack of microbial support in increasing metabolism and suppressing pathogens in the digestive tract. An unstable environment and water quality that tends to deteriorate without probiotics can also cause stress, which is known to suppress the immune system and reduce erythrocyte production (Latha *et al.* 2024; Seenivasan *et al.* 2012).

### White Blood Cell Count

Based on observations, the white blood cells of snakehead fish fry ranged from  $80,983 \pm 0.06 - 120,617 \pm 0.07$  blood. The lowest white blood cells of  $80,983 \pm 0.06$  were obtained in snakehead fish fry given treatment A (control). The highest number of red blood cells of  $120,617 \pm 0.07$  was obtained in treatment C (Addition of Probiotics 0.8 mL/L of water). According to Hartika et al. (2014) the number of leukocytes in fish ranges from 20,000 - 150,000 cells per mm3 of blood.

The increase in the number of leukocytes in treatment C indicates that a dose of 0.8 mL/L of probiotics provides optimal stimulation of the snakehead fish immune system. Leukocytes, especially granulocytes and lymphocytes, play an important role in fighting infections and maintaining the stability of the immune system (Harikrishnan et al., 2011). Probiotics including Bacillus sp., Lactobacillus sp., and Saccharomyces cerevisiae produce immunostimulant compounds such as  $\beta$  glucan and peptidoglycan that trigger the non-specific immune system. β Glucan from S. cerevisiae has been shown to increase the number of leukocytes and stimulate phagocytic and respiratory burst activities in various fish species, while peptidoglycan and βglucan from bacterial probiotics stimulate the activity of leukocyte cells such as granulocytes and macrophages, increasing the initial immune response to pathogens (Kazun et al., 2022; Torres et al., 2024). Conversely, the low number of leukocytes in the control treatment (without probiotics) reflects a weak immune response due to the absence of assistance from microorganisms to stimulate the production of white blood cells. This condition makes fish more susceptible to infection and environmental stress (Seenivasan et al., 2012). A decrease in leukocytes can indicate immunosuppression or a condition of the fish's body that is not ready to face disease challenges, both from the environment and internally (Wang et al. 2020).

### CONCLUSION

The addition of probiotics with the treatment of 0.6 ml/L water, 0.8 ml/L water, 1.0 ml/L water and 1.2 ml/L water showed significant differences (P<0.05) in absolute length growth, absolute weight growth, specific growth rate, survival, number of red blood cells and

number of white blood cells. However, there was no significant difference in the feed conversion ratio. Treatment C (0.8 mL/L) in terms of effectiveness and efficiency was superior because it gave a better effect compared to other treatments with absolute length growth and absolute weight growth values of  $2.71 \pm 0.05$  cm and  $2.87 \pm 0.27$  grams, specific growth rate and feed conversion ratio of  $1.69 \pm 0.02\%$  and  $0.91 \pm 0.04\%$ , survival  $89 \pm 0.03\%$ , number of red blood cells and number of white blood cells 1,356,800 and 120,617.

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