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Effectiveness of Minaraya Probiotics on The Growth Rate and Immune Response of Catfish (*Clarias gariepinus*)

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Abstract

The growing demand for catfish in the market is prompting farmers to consistently enhance their production capacity. Several obstacles are encountered during production growth, such as pathogen attacks, suboptimal growth, and low survival rates. This study aimed to determine the effectiveness of Minaraya probiotics with different doses in feed on the growth and immune response of catfish. The method used in this study was an experimental method with a completely randomized design (CRD) consisting of four treatments: control (without probiotics), and treatments P1, P2, and P3 with the addition of Minaraya probiotics at 2, 5, and 10 mL per 2.5 kg of feed, respectively, each with three replications. The parameters observed included survival rate, SGR, DGR, FCR, PER, AF, RB, and leukocyte differentiation. The results showed that the survival rate in all treatments was relatively high, ranging from 95% to 98.89%. The results also showed that the addition of Minaraya probiotics significantly ($p<0.05$) increased growth parameters (SGR and DGR) and feed efficiency (FCR and PER), with the best dose being in treatment 3 (P3) at a dose of 10 mL/2.5 kg of feed. On the other hand, the non-specific immune response also increased in the treatment given Minaraya probiotics, as indicated by increased phagocytic activity, respiratory burst, and proportion of lymphocytes, neutrophils, and monocytes, as well as an increase in the total number of erythrocytes after *Aeromonas hydrophila* infection. The application of Minaraya probiotics in a sustainable cultivation system can improve catfish productivity and health.

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Introduction

Catfish (*Clarias gariepinus*) is a fishery commodity with high economic value in Indonesia and offers significant and promising aquaculture business opportunities (Ardiansyah & Alkemega, 2024). Nationally, catfish production shows a significant upward trend. Based on data from the Ministry of Maritime Affairs and Fisheries (KKP), catfish production volume was recorded at 1.13 million tons in 2023, increasing to 1.17 million tonnes in 2024. Market demand for catfish has led to annual increases in production. Farmers continue to

increase production to meet this market demand. However, in the process of increasing production, several problems often hinder farmers, such as pathogen attacks, suboptimal growth, and low survival rates due to relatively low feed absorption effectiveness (Rarassaria *et al.,* 2021).

One of the most common challenges in catfish production is pathogen attacks, particularly bacterial infections, such as *Motile Aeromonas Septicemia* (MAS), caused by *A. hydrophila*. This disease causes up to 100% mortality in farmed fish (Hamka *et al.,* 2021). Furthermore, suboptimal growth of catfish is also a common issue in catfish farming. Suboptimal growth is generally caused by the fish's low absorption of nutrients in the feed (Setiawan *et al.,* 2022). According to Simanjuntak *et al.* (2022), high-energy feeds with complex structures tend to be difficult to digest, requiring additional energy to break down into simpler forms for efficient absorption by the fish's digestive tract. These various obstacles demand appropriate solutions to support the sustainability of catfish farming. One potential approach is the use of probiotics, which have been shown to boost immunity and support more optimal fish growth.

Probiotics are beneficial microorganisms that can have a positive impact on their hosts, including in fish farming systems. The benefit of probiotics on aquaculture include improved water quality, enhanced fish immunity, increased growth rates, and more effective feed utilization (Rahayu *et al.,* 2024; Sumon *et al.,* 2022). Furthermore, probiotics are known to suppress the population of pathogenic microorganisms, stimulate the immune system, and modulate microbial metabolism through enzymatic activity (Hamka *et al.,* 2021). Various studies have demonstrated the effectiveness of probiotic applications in aquaculture. For example, a study by Putri *et al.* (2022) showed that the use of herbal probiotics can improve the growth and survival of catfish , while Hidayat *et al.* (2024) reported that the addition of the commercial probiotic EM4 to tilapia feed can increase growth, indicating the potential for similar effects in catfish. Based on these findings, it can be concluded that the effectiveness of a probiotic needs to be demonstrated in real-world settings on its host, necessitating in vivo testing for both commercial and indigenous probiotics. One probiotic currently being developed is the Minaraya probiotic, which has the potential to be applied in catfish cultivation.

Minaraya Probiotics is a probiotic product containing a combination of bacteria, fungi, and herbal ingredients that are beneficial for farmed fish. The main bacteria contained in this probiotic product are *Bacillus sp., B. longum,* and *B. bifidum*, which are known to have antibacterial and enzymatic activities and are safe for use in fish farming (Andriani & Pratama, 2022; Latif *et al.,* 2023). Furthermore, S. cerevisiae acts as a probiotic and immunostimulant, improving growth, feed efficiency, disease resistance, intestinal health, and immune responses in various fish species (del Valle *et al.,* 2023). Most of the herbal components in this probiotic are immunostimulants, possessing antibacterial and antioxidant activity, and contribute to strengthening the fish's immune response (Ahmad *et al.,* 2024; Pridayem *et al.,* 2022). With these properties, Minaraya Probiotic has great potential for application in catfish cultivation. Therefore, this study was conducted to evaluate the effectiveness of administering Minaraya Probiotic at different doses in feed on the growth and immune response of catfish.

Materials and methods

Time and Place of Research

This research was conducted from February to April 2025 at the Fisheries Production and Entrepreneurship Development Laboratory, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.

Research Design

This study was conducted using an experimental method to determine the effectiveness of MINARAYA probiotics on the growth and immune response of catfish. The research design used in this study was a Completely Randomized Design (CRD) consisting of four treatments and three replications consisting of K (control); P1 (feed with the addition of probiotics at a dose of 2 mL/2.5 kg of feed), P2 (feed with the addition of probiotics at a dose of 5 mL/2.5 kg of feed) and P3 (feed with the addition of probiotics at a dose of 10 mL/2.5 kg). The

Table 1. Experimental design for catfish rearing period

Treatment	Description
K	Commercial feed without added probiotics (Control)
P1	Artificial feed with added probiotics at a dose of 2 mL/2.5 kg of feed
P2	Artificial feed with added probiotics at a dose of 5 mL/2.5 kg of feed
P3	Artificial feed with added probiotics at a dose of 10 mL/2.5 kg of feed

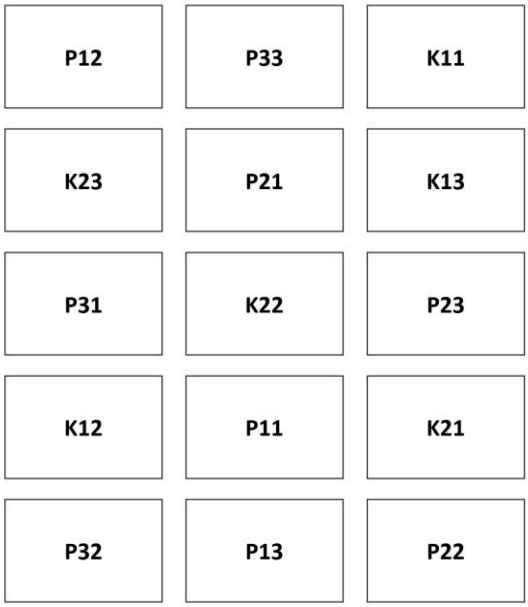

Figure 1. Research Design

detailed research design can be seen in Table 1. This research design was designed systematically to ensure that each treatment can be observed and well-structured. The research design can be seen in Figure 1.

Research Procedures

Preparation of Maintenance (Reraing Period) Containers

The containers used in this study were 15 tank with a diameter of 27 cm and a height of 43 cm. The tank were washed using a sponge and detergent, then rinsed with water until clean and dried. Sterilization of the water medium was carried out by adding 1.5 ppm of chlorine and spreading it evenly in the maintenance tubs and leaving it for 24 hours (Sukoco *et al.,* 2016). The maintenance tank were filled with 60 L of water, or approximately 30 cm from the height of the tank. After that, hoses and aeration stones were installed in each tank. The treatments were placed sequentially and marked according to their location.

Preparation of Test Animals

The test animals used in this study were 3-4 cm catfish seeds weighing 0.6 g. The stocking density for each tank was 1 fish/L, or approximately 60 fish/tank (Pamungkas *et al.,* 2024). The catfish were acclimatized for 30 minutes before use. The acclimatized catfish were then immediately transferred to the rearing tank and given treatment for 30 days. Before treatment, the catfish were subjected to initial sampling to determine their length and weight. Subsequent sampling was conducted every seven days.

Addition of Minaraya Probiotics to Feed

The commercial feed used in this study contains 39% protein. Before use, this commercial feed was added with minaraya probiotics containing *Bacillus* sp. ≥ 1×10^6 CFU/mL, *Bifidobacterium longum* ≥ 1×10^6 CFU/mL, *Bifidobacterium bifidum* ≥ 1×10^6 CFU/mL, *Saccharomyces cerevisiae* ≥ 1×10^6 CFU/mL, ginger (*Zingiber officinale*) 16.67 g/L, Java turmeric (*Curcuma xanthorrhiza*) 16.67 g/L, black turmeric (*Curcuma aeruginosa*) 16.67 g/L, turmeric (*Curcuma domestica*) 16.67 g/L, aromatic ginger (*Kaempferia galanga*) 8.33 g/L, molasses 175 g/L, water 1000 mL. The mixing process begins by weighing the feed according to the 5% biomass. Next, the minaraya probiotics were mixed evenly into the commercial feed. After the feed and probiotics were mixed evenly, the feed was ready to be used or stored in a closed container.

Fish Farming

A total of 900 catfish, with an average initial weight of 0.6 g, were placed in 15 tanks measuring 20 x 43 cm, with a volume of 60 L. The catfish were reared for 30 days. During the rearing period, the fish were fed with different treatments, and 30% water changes were carried out every 3 days. The feed was given at 5% of the fish's body weight, and feeding was carried out twice a day, at 9 a.m. and 4 p.m. Length and weight measurements were taken five times during the rearing period: at the beginning of stocking, and on days 7, 14, 21, and at the end of the rearing period.

Challenge Test

Catfish were challenged with the *A. hydrophila* pathogen after 30 days of rearing period with feed supplemented with the probiotic Minaraya. A total of 10 catfish from each treatment were injected with 0.1 mL of *A. hydrophila* bacterial suspension with a concentration of 10^8 CFU/mL, except for the control (-), which was injected with 0.1 mL of PBS solution. After the injection process, the catfish were kept in a tank. The challenge test lasted for 5 days; after 5 days, the fish were still fed with probiotic Minaraya regularly without any water changes.

Growth and Survival Rate

Survival Rate

Survival rate (SR) during rearing period is calculated using the formula of Effendie (1997):

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

Where:
SR : Survival rate (%)
Nt : Number of fish alive at the end of rearing period (fish)
No : Number of fish at the start of rearing period (fish)

Specific Growth Rate

The specific growth rate (SGR) during rearing period is calculated using the formula of Zonneveld *et al.* (1991):

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

Where:
SGR : Daily growth rate (%)
Wt : Average weight of fish on the final day of rearing period (g)
Wo : Average weight of fish on the first day of rearing period (g)
t : Rearing period time (s)

Feed Conversion *Rate* (FCR)

Feed Conversion Ratio (FCR) during rearing period is calculated using the formula of Zonneveld *et al.* (1991):

$$FCR = \frac{F}{Wt - Wo}$$

Where:
FCR : Feed conversion rate
F : Amount of feed given during the study (g)
Wt : Total fish weight at the end of the study (g)
Wo : Total fish weight at the initial of the study (g)

Protein Efficiency Ratio (PER)

Protein Efficiency Ratio (PER) during rearing period is calculated using the formula of Jafri (1999) :

$$PER = \frac{Wg}{P}$$

Where:
PER : Rasio efisiensi protein
Wg : Total absolute weight (g)
P : Protein intake (g)

Daily Growth Rate (DGR)

Daily Growth Rate (DGR) during rearing period is calculated using the formula of Zonneveld *et al.* (1991):

$$DGR = \frac{\mathrm{LnWt} - \mathrm{LnWo}}{t}\ x\ 100$$

Where:
DGR : Daily Growth Rate (%/day)
Wt : Final average weight (g)
Wo : Initial average weight (g)
t : Rearing period Time (days)

Water Quality

During the rearing period, water quality measurements were routinely conducted by monitoring several important parameters, namely temperature, pH, and dissolved oxygen (DO) levels. These three parameters were used to maintain optimal environmental conditions and promote the growth and health of the catfish.

Immunological Testing

Phagocytic Activity (PA)

Phagocytic activity was measured before and after the challenge test. A total of 50 µL of catfish blood was placed into a microtube, then 50 µL of *Staphylococcus aureus* bacterial suspension dissolved in NaCl solution was added, and then the mixture was homogenized. After that, the mixture was incubated for 20 minutes at 28 ºC. After incubation, 5 µL of the mixture was taken to make a smear preparation, then fixed using 100% methanol for 5 minutes and

dried. The preparation was then immersed in Giemsa solution for 15 minutes. The dried smear preparation was then washed with running water and dried. Observations were made using a microscope with a magnification of 400 times. The calculation of phagocytic activity was carried out using the formula from (Anderson & Siwicki, 1993).

$$PA\ (\%) = \frac{number\ of\ cells\ that\ perform\ phagocytosis}{number\ of\ phagocytic\ cells} \times 100$$

Respiratory Brust (RB) Activity

Respiratory burst activity was measured based on the principle of nitroblue tetrazolium (NBT) reduction, which produces formazan as an indicator of the amount of superoxide anion. A total of 50 µL of blood was added to each well of the titer microplate, then left for 1 hour. After that, the supernatant was discarded and washed twice with 100 µL of PBS solution and then left for another 1 hour. Next, the NBT was discarded, and 100% methanol was added and left for 1 hour. After leaving, it was discarded, and 30% methanol was added and rinsed twice. Finally, the precipitate was dissolved by adding 60 µL of 2N KOH solution and 70 µL of dimethyl sulfoxide (DMSO) to each well of the microplate. The optical density (OD) value of the precipitate was then measured using a microplate reader (Setiyaningsih *et al.,* 2017).

Leukocyte Differential

The differential leukocyte count was performed before and after the challenge test. The procedure for the differential leukocyte count began with a drop of blood on a glass slide, then flattened using another glass slide at a 30° angle and pulled to the end of the slide to create a smear. The smear was then air-dried. The dried blood smear was then fixed using methanol for 5 minutes. Then, the smear was dried again and immersed in a diluted Giemsa solution for 15 minutes. The Giemsa 10% used in this staining process was diluted at a ratio of 1:20. After staining was complete, the smear was rinsed with distilled water and dried again. The prepared smear was observed under a microscope at 400x magnification. The differential leukocyte count was calculated based on cell type, namely lymphocytes, neutrophils, and monocytes, using the following formula:

$$\text{Cell proportions} = \frac{Types\ of\ leukocyte\ cells}{Total\ leukocyte\ cells\ observed} x100\%$$

Number of Erythrocytes

Blood samples mixed with EDTA 20% (v/v) were drawn using a hemocytometer pipette equipped with a special red bead for counting erythrocytes, until they reached the 0.5 mark. Next, Hayem's solution was drawn up to the 101 mark as a diluent. The pipette was shaken for 3 to 5 minutes in a figure-eight motion to ensure homogeneity of the

Table 2. Survival Rate (SR), Specific Growth Rate (SGR), Daily Growth Rate (DGR), Feed Coverion Rate (FCR), Protein Efficiency Rate (PER) values in catfish after being fed with the addition of Minaraya probiotics for 30 days of rearing period.

Growth Parameters	Treatment			
	K	P1	P2	P3
Survival rate (%)	97.78 ± 2,55[a]	95 ± 6,01[a]	97.22 ± 1,92[a]	98.89 ± 1,92[a]
SGR (%)	2.28 ± 0,28[a]	3.49 ± 0,13[b]	3.78 ± 0,28[b]	4.61 ± 0,40[c]
DGR (g)	0.15 ± 0,02[a]	0.29 ± 0,02[b]	0.32 ± 0,01[b]	0.45 ± 0,05[c]
FCR	2.08 ± 0,32[a]	1.30 ± 0,08[b]	1.23 ± 0,08[b]	1.02 ± 0,08[b]
PER (%)	1.57 ± 0,26[a]	2.47 ± 0,14[b]	2.61 ± 0,16[b]	3.14 ± 0,24[c]

blood and solution mixture. The first two drops of the mixture were discarded to remove any air trapped in the pipette, then one drop was placed in the hemocytometer box and covered with a cover glass. The number of erythrocytes was then counted under a microscope in five small hemocytometer boxes at 400x magnification. The results were then calculated using the calculation formula according to Blaxhall & Daisley, (1973).

Number of erythrocytes = E/N x 1/V x FP (cell/mm3)

Where:

E = Number of erythrocytes counted
N = Number of boxes counted
V = Box volume (0.004 mm^3)
FP = Dilution factor

Data Analysis

The research design used was a completely randomized design (CRD). The data obtained were then processed using Microsoft Excel 2021 and SPSS software. Data analysis was performed using one-way ANOVA analysis of variance. If the analysis results showed a significant difference between the treatments, further testing was conducted using the Duncan test.

Result

Growth parameters of catfish after feeding with the addition of Minaraya probiotics

Observations on the growth of catfish after feeding with the addition of Minaraya probiotics at different doses for 30 days showed significant differences between treatments. The results also showed that the addition of Minaraya probiotics significantly increased catfish growth, as seen from the increase in SGR, DGR, FCR, PER, and survival rates. The most effective treatment was Treatment 3 (P3), which significantly improved growth parameters compared to the other treatments. Observations of catfish growth after being fed with Minaraya probiotics at different doses are presented in Table 2 below.

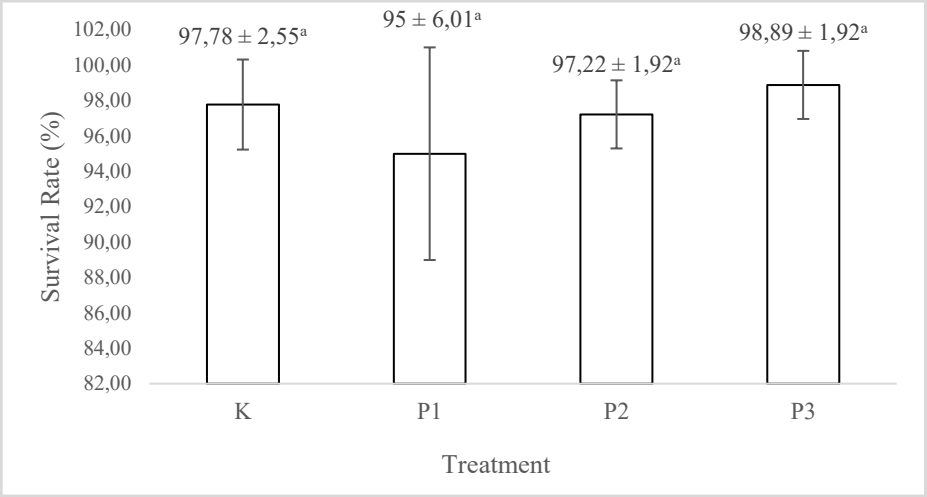

Figure 2. Survival Rate (SR) of catfish after being fed consortium probiotics, with different letters on the bar graph indicating no significant differences (p<0,05) (n=12). The values shown in the graph are the results of the standard deviation and the overall

Survival Rate (SR)

The results showed that the addition of Minaraya probiotics with different doses to the feed, consisting of treatment K (control), P1 (2 mL/2.5 kg feed), P2 (5 mL/2.5 kg feed), and P3 (10 mL/2.5 kg feed), did not provide a statistically significant difference in the survival of catfish ($p > 0.05$). However, the survival rate in all treatments was relatively high, ranging from 95.00% to 98.89%. Treatment P3 produced the highest survival rate of 98.89 ± 1.92%, followed by the control treatment of 97.78 ± 2.55%, P2 of 97.22 ± 1.92%, and the lowest value was in treatment P1 of 95.00 ± 6.01%. These results indicate that although statistically not significantly different, the addition of Minaraya probiotics with the highest dose tends to provide a better survival rate in catfish. The survival rate of catfish fed with different doses of Minaraya probiotics can be seen in Figure 2.

Specific Growth Rate (SGR)

The results showed that addition Minaraya probiotics at different doses significantly affected the specific growth rate (SGR) of catfish ($p < 0.05$). Catfish fed with additional Minaraya probiotics tended to have higher SGR values compared to the control. The highest SGR value was obtained in treatment P3 (10 mL/2.5 kg of feed), which was 4.61 ± 0.40, and was statistically significantly different from all other treatments. Treatments P2 (5 mL/2.5 kg of feed) and P1 (2 mL/2.5 kg of feed) showed SGR values of 3.78 ± 0.28 and 3.49 ± 0.13, respectively; both were significantly different from the control and P3, but did not

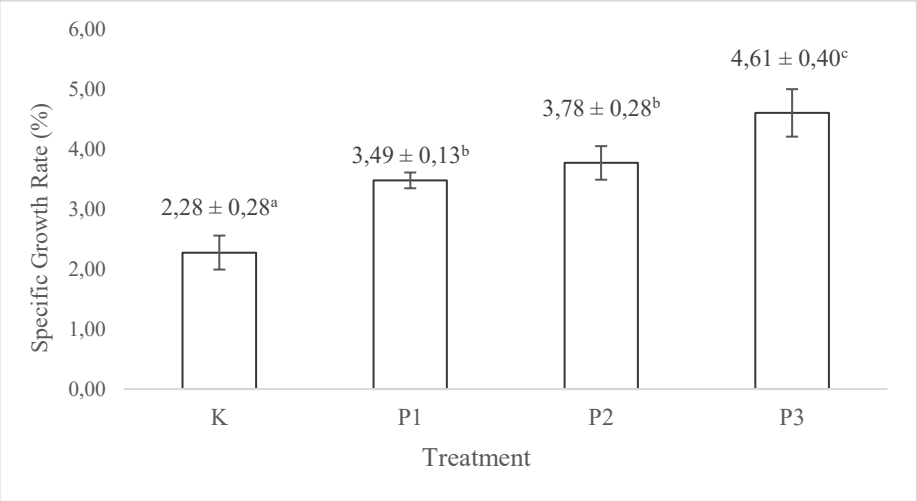

Figure 3. Spesific Growth Rate (SGR) of catfish after being fed consortium probiotics, with different letters on the bar graph indicating no significant differences (p<0,05) (n=12). The values shown in the graph are the results of the standard deviation and the overall

show significant differences from each other. Meanwhile, the control treatment (K) recorded the lowest SGR value of 2.28 ± 0.28, which was statistically significantly different from all treatments with probiotics. Overall, the results of this study indicate that the addition of Minaraya probiotics to feed can increase the specific growth rate of catfish, with a dose of 10 mL/2.5 kg (P3) being the most effective treatment. The SGR values of catfish fed feed supplemented with Minaraya probiotics at different doses can be seen in Figure 3.

Daily Growth Rate (DGR)

The results showed that the addition of Minaraya probiotics at different doses had a significant effect on the daily growth rate (DGR) of catfish ($p < 0.05$). The highest DGR value was obtained in treatment P3 (10 mL/2.5 kg feed), which was 0.45 ± 0.05 g, which was statistically significantly different compared to all other treatments. Furthermore, treatments P2 (5 mL/2.5 kg feed) and P1 (2 mL/2.5 kg feed) showed DGR values of 0.32 ± 0.01 g and 0.29 ± 0.02 g, respectively. These two treatments were significantly different from the control and P3, but did not show significant differences from each other. Meanwhile, the lowest DGR value was found in the control treatment (K), which was 0.15 ± 0.02 g, and was statistically significantly different compared to all treatments given probiotics. Overall, these results indicate that the addition of Minaraya probiotics to feed can increase the daily growth rate of catfish, with P3 being the most optimal dose for supporting growth. The DGR values of catfish fed with different

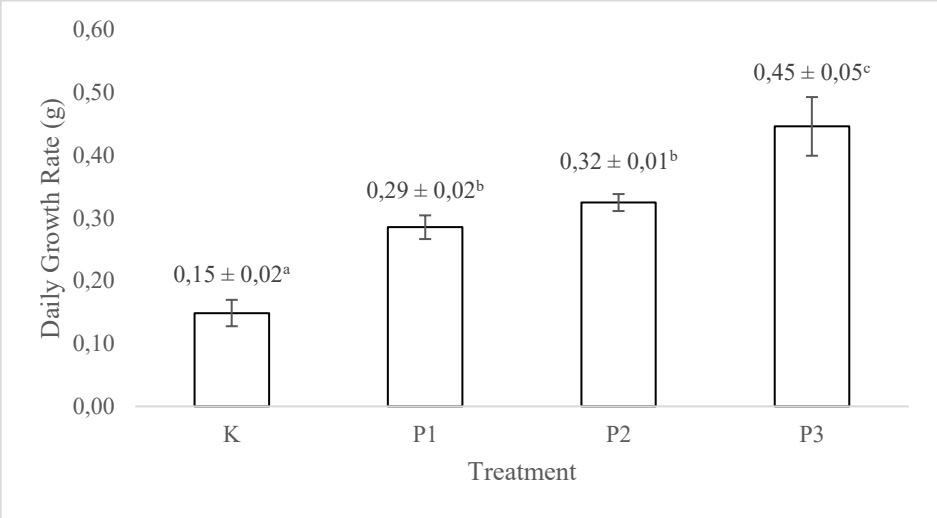

Figure 4. Daily Growth Rate (DGR) of catfish after being fed consortium probiotics, with different letters on the bar graph indicating no significant differences (p<0,05) (n=12). The values shown in the graph are the results of the standard deviation and the overall

doses of Minaraya probiotics are shown in Figure 4.

Feed Conversion Ratio (FCR)

The results showed that the addition of Minaraya probiotics with different doses had a significant effect on the Feed Conversion Ratio (FCR) value of catfish ($p < 0.05$). The best FCR value was obtained in treatment P3 (10 mL/2.5 kg of feed), which was 1,02 ± 0,08 statistically, showing the highest feed efficiency compared to other treatments. Furthermore, treatments P1 (2 mL/2.5 kg of feed) and P2 (5 mL/2.5 kg of feed) had FCR values of 1.30 ± 0.08 and 1.23 ± 0.08, respectively. Meanwhile, the highest (worst) FCR value was found in the control treatment (K) at 2.08 ± 0.32. A lower FCR value indicates better feed efficiency, which means the fish need less feed to produce one kilogram of body weight. Overall, these results indicate that the addition of Minaraya probiotics can improve feed efficiency in catfish, with P3 treatment being the most optimal dose in reducing FCR values and supporting more efficient feed conversion. The FCR values of catfish fed with Minaraya probiotics at different doses can be seen in Figure 5.

Protein Efficiency Ratio (PER)

The results showed that the addition of Minaraya probiotics at different doses had a significant effect on the protein efficiency ratio (PER) in catfish ($p < 0.05$). The PER value increased significantly from the control treatment to the treatment with the addition of probiotics. The highest PER value was obtained in treatment P3 (10 mL/2.5 kg of feed), which was 3.14 ± 0.24, followed by

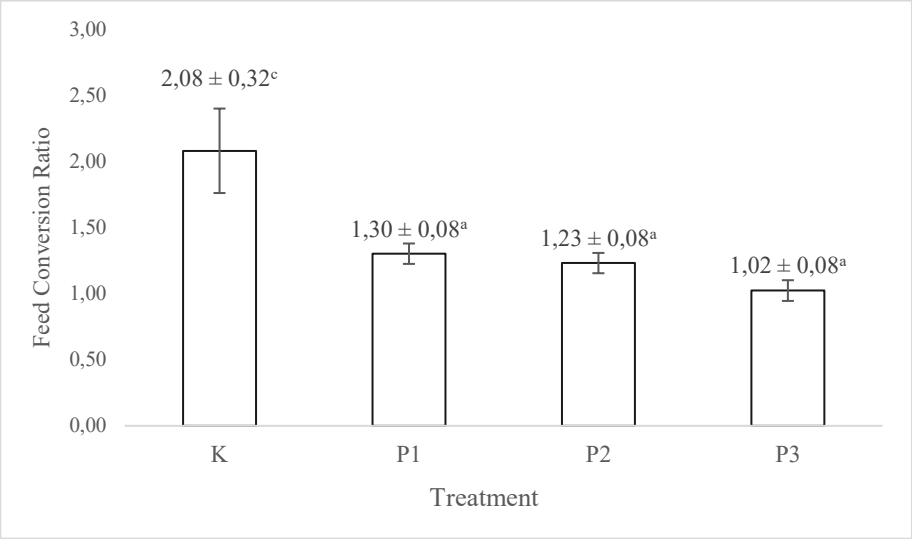

Figure 5. Feed Convertion Rate (FCR) of catfish after being fed consortium probiotics, with different letters on the bar graph indicating no significant differences ($p<0,05$) ($n=12$). The values shown in the graph are the results of the standard deviation and the overall mean.

treatment P2 (5 mL/2.5 kg of feed) at 2.61 ± 0.16, and P1 (2 mL/2.5 kg of feed) at 2.47 ± 0.14. Meanwhile, the lowest PER value was found in the control treatment (K), which was 1.57 ± 0.26. These results indicate that the addition of Minaraya probiotics, especially at the highest dose (P3), was able to optimize protein utilization in feed, which had a positive impact on feed efficiency and catfish growth. The PER value of catfish fed with the addition of Minaraya probiotics at different doses can be seen in Figure 6.

Water Quality

The average values of water quality parameters during the rearing period indicate conditions within the optimal range for catfish cultivation. Measurement results indicate that the water temperature ranged between 26.8 and 27.1°C, pH between 7.6 and 7.7, and dissolved oxygen (DO) levels between 5.3 and 5.7 mg/L. This study indicated that all of these parameters support the growth and survival of catfish, thus concluding that the water quality during the study was appropriate to support successful cultivation. The average values of water quality parameters during rearing can be seen in Table 3.

Immunity parameters of catfish fed with the addition of Minaraya probiotics injected with *A. hydrophylla* bacteria.

The immunity parameters of catfish observed after being challenged with the *A. hydrophylla* pathogen include Phagocytic Activity (PA), Respiratory Burst (RB), Leukocyte Differentiation (Lymphocytes,

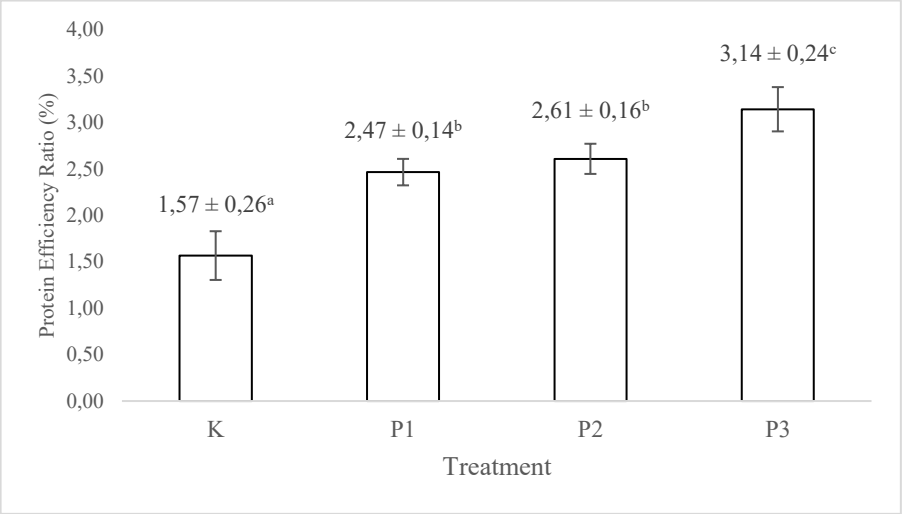

Figure 6. Protein Efficiency Rate (PER) of catfish after being fed consortium probiotics, with different letters on the bar graph indicating no significant differences (p<0,05) (n=12). The values shown in the graph are the results of the standard deviation and the overall mean.

Neutrophils, Monocytes), and Total Erythrocytes. Immunity observations were carried out after *A. hydrophylla* injection, namely on days 1, 3, and 5 post-infection. During the post-injection rearing period, the catfish were still fed with the addition of Minaraya probiotics at different doses. The results of the catfish immunity test are presented in full in Table 4 below.

Phagocytic Activity (PA)

Phagocytic activity was observed on days 1, 3, and 5 after treatment, with each group consisting of a positive control (K+), a negative control (K−), and three treatments, namely P1, P2, and P3. On day 1, the K+ group showed the highest phagocytic activity of 18.08±3.92%, which was significantly different (p<0.05) compared to the other treatment groups. Meanwhile, phagocytic activity in groups K−, P1, P2, and P3 ranged from 8.14% to 14.73%, with no significant differences between each other. On day 3, no significant differences were found between all groups, with relatively even phagocytic activity values, namely between 10.20% and 15.82%. However, a different pattern was

Table 3. Average values of water quality parameters during rearing period

Water quality parameters	**Treatment**			
	k+	**P1**	**P2**	**P3**
Temperature (ºC)	27,0	26,8	27,0	27,1
pH	7,7	7,7	7,6	7,6
DO (mg/L)	5,3	5,5	5,5	5,7

Table 4. Phagocity Activity (PA), Respiratory Burst (RB), Leukocyte Differentiation (Lymphocytes, Neutrophils, Monocytes), Total Erythrocytes in catfish observed after *A. hydrophilla* challenge test after 30 days of rearing with feed supplemented with Minaraya probiotics.

Parameter	Observation Time (days)	Treatment				
		K+	K-	P1	P2	P3
Phagocytic Activity (%)	1	18.08±3.92^{a}	11.66 ±7.16^{ab}	8.14 ±0.41^{ab}	11.94 ±2.76^{ab}	14.73 ±6.73^{ab}
	3	15.82±1.13^{a}	10.59±1.61^{a}	10.20±3.78^{a}	10.85±1.96^{a}	10.90±4.86^{a}
	5	7.43±1.04^{a}	19.95±3.62^{b}	28.23±5.99^{b}	18.94±7.06^{b}	25.44±4.53^{b}
Respiratory Burst	1	0.80±0.15^{b}	0.29±0.29^{a}	0.28±0.02^{a}	0.17±0.01^{a}	0.12±0.02^{a}
	3	0.39±0.08^{c}	0.19±0.01^{a}	0.30±0.01^{bc}	0.24±0.07^{ab}	0.39±0.02^{c}
	5	0.13±0.04^{a}	0.19±0.01^{ab}	0.22±0.01^{b}	0.20±0.02^{b}	0.31±0.06^{c}
Lymphocytes (%)	1	52,85	74,60	59,34	59,68	52,55
	3	48,57	55,27	46,26	47,99	55,76
	5	58,83	54,51	57,49	44,24	55,03
Neutrophils (%)	1	19,76	14,62	22,28	20,72	21,50
	3	25,11	20,33	22,00	25,41	20,18
	5	16,16	25,40	19,37	21,24	22,56
Monocytes (%)	1	27,38	10,78	18,38	19,60	25,95
	3	26,32	24,40	31,74	26,61	24,06
	5	25,01	20,08	23,14	34,52	22,41
Erythrocytes (Cells/mm^3)	1	8,5 × 10^6	6,5 × 10^6	4,8 × 10^6	7,2 × 10^6	7,5 × 10^6
	3	9,6 × 10^6	5,9 × 10^6	9,1 × 10^6	5,8 × 10^6	6,5 × 10^6
	5	9,3 × 10^6	6,8 × 10^6	9,2 × 10^6	7,1 × 10^6	5,8 × 10^6

evident on day 5, where phagocytic activity in treatment groups P1 (28.23±5.99%), P2 (18.94±7.06%), and P3 (25.44±4.53%) experienced a significant increase compared to K+ (7.43±1.04%) and K− (19.95±3.62%). The highest increase occurred in group P1, which showed the strongest phagocytic response. These results indicate that the addition of Minaraya probiotics has the potential to significantly increase phagocytic activity, especially on day 5 post-treatment, which may reflect the immunostimulant role of each treatment. The value of catfish phagocytic activity during rearing period can be seen in Table 4.

Respiratory Burst (RB)

Observations on respiratory burst activity showed significant differences between treatments and observation times. On day 1, the positive control group (K+) showed the highest activity of 0.80±0.15, which was significantly different ($p<0.05$) compared to all other treatments (K−, P1, P2, and P3). Values in the treatment groups ranged from 0.12 to 0.29 and were not statistically significantly different from each other, indicating that the initial response to stimulation was not optimal. On day 3, there was an increase in respiratory burst activity in groups P1 (0.30±0.01), P2 (0.24±0.07),

and P3 (0.39±0.02), with P3 showing a value equivalent to K+ (0.39±0.08) and significantly different from K−. This indicates that the addition of the Minaraya probiotic treatment, especially in the P3 treatment, began to significantly stimulate the activation of the innate immune system on day 3. Meanwhile, on the 5th day, respiratory burst activity decreased in all groups, but the P3 treatment still showed the highest value (0.31±0.06) and was significantly different compared to the other groups. The values in P1 (0.22±0.01) and P2 (0.20±0.02) were also higher than K+ (0.13±0.04), which decreased drastically. Overall, these results indicate that the addition of Minaraya probiotics in the feed after A. hydrophylla injection can increase respiratory burst activity, with the most optimal effect seen on the 3rd to 5th day after treatment, indicating its potential as an immunostimulant. The Respiratory Burst values of catfish during rearing period can be seen in Table 4.

Leukocyte Differential

Observations of leukocyte differentiation in catfish during three observation periods (1, 3, and 5) showed changes in the composition of lymphocytes, neutrophils, and monocytes in response to treatment. The highest lymphocyte percentage was recorded in the negative control group (K−) on day 1 at 74.60%, while the treatment groups showed lower values, with P3 the lowest (52.55%), approaching the positive control (K+) value. Over time, the lymphocyte percentage fluctuated, with a decrease on day 3 and an increase again on day 5 in most groups, except for P2, which decreased significantly to 44.24%. Conversely, the neutrophil percentage increased on day 3, particularly in the K+ (25.11%) and P2 (25.41%) groups, indicating a phagocytic response to treatment. Despite a decrease on day 5 in some groups, neutrophil values remained relatively high in the treatment groups, particularly P2 and P3. Monocytes also showed an increasing trend, with the highest value in the P2 group on day 5 at 34.52%, reflecting the activation of the non-specific immune system through increased phagocytic cells. Overall, these data indicate that the addition of Minaraya probiotics, especially the P2 treatment, can stimulate the fish's immune system by increasing neutrophils and monocytes, which play an important role in innate immune defense. The leukocyte differentiation values of catfish during rearing period can be seen in Table 4.

Number of Erythrocytes

Observations on the total number of erythrocytes showed variations between

treatment groups during the three observation periods. On day 1, the positive control group (K+) had the highest number of erythrocytes, namely 8.5 × 10^6 cells/mm^3, while the P1 treatment group showed the lowest number, namely 4.8 × 10^6 cells/mm^3. The number of erythrocytes in the negative control group (K−) was also relatively low (6.5 × 10^6 cells/mm^3), indicating that the absence of infection or stimulation did not increase erythrocyte production. On day 3, there was an increase in the number of erythrocytes in the K+ group (9.6 × 10^6 cells/mm^3) and P1 (9.1 × 10^6 cells/mm^3), indicating a response to treatment or infection. Meanwhile, the number of erythrocytes in P2 and K− decreased to around 5.8–5.9 × 10^6 cells/mm^3. Interestingly, on day 5, the P1 group still showed a high erythrocyte count (9.2 × 10^6 cells/mm^3), approaching the K+ value (9.3 × 10^6 cells/mm^3), indicating that addition of Minaraya probiotics at the P1 dose was able to maintain or increase erythrocyte production during the recovery process. In contrast, the number of erythrocytes in the P3 group decreased on day 5 (5.8 × 10^6 cells/mm^3), indicating that a higher probiotic dose does not necessarily provide better hematological effects. Overall, treatment with Minaraya probiotics, especially at the P1 dose, appears effective in maintaining erythrocyte counts and supporting the physiological conditions of catfish infected with *A. hydrophila*.

Discussion

Probiotics are live microorganisms added to feed and can provide benefits to the host animal by improving the balance of intestinal microflora (Farzanfar, 2006). This study evaluated the effect of adding the probiotic Minaraya on the survival, growth, and immunity of catfish. According to the results, catfish survival rates were relatively high, between 95–98.89%, even though no statistically significant differences were found ($p > 0.05$). This is thought to be related to the probiotic bacteria and herbs contained in Minaraya, which play a role in maintaining fish health. Minaraya contains a consortium of microorganisms such as *Bacillus* sp., *Bifidobacterium longum, Bifidobacterium bifidum*, and *Saccharomyces cerevisiae*. The consortium of several probiotic bacterial strains synergistically improves digestive efficiency, nutrient absorption, and disease resistance (Andriani & Pratama, 2022). The presence of a stable and resilient gut microbial community contributes to better feed conversion, optimal growth, and a stronger immune system (Favetta *et al.,* 2024). These findings align with research by Hadijah *et al.* (2023) who reported an 85–

90% increase in fish survival through EM4 probiotic supplementation, and by Ferdous *et al.* (2024) who noted a 79–87% increase in *Labeo rohita* survival following multi-strain probiotic addition. Generally, the increased survival of fish fed probiotics can be attributed to the role of probiotics in improving digestibility, boosting the immune system, and producing antimicrobial compounds effective against pathogens.

The application of Minaraya probiotics in catfish feed significantly increased growth and feed efficiency, as demonstrated by increases in growth parameters such as Specific Growth Rate (SGR) and Daily Growth Rate (DGR), as well as feed efficiency measured by Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER). The results showed that the best treatment was obtained at a dose of 10 mL/2.5 kg feed (P3), which significantly increased the SGR and DGR values of catfish. This increase is attributed to the probiotic's ability to enhance digestive efficiency and nutrient absorption by producing digestive enzymes and balancing the digestive tract microflora (Amenyogbe *et al.,* 2024). Additionally, probiotics also contribute to improving the fish's immune response, thereby reducing stress levels and the risk of disease infections that can inhibit growth. With a more stable physiological condition and higher feed absorption efficiency, catfish exhibit faster and optimal growth (Fachri *et al.,* 2024; Nathanailides *et al.,* 2021). On the other hand, water quality during rearing period is also a supporting factor in increasing growth. During the study, water quality parameters were within the optimal range, specifically temperature 26.8–27.1°C, pH 7.6–7.7, and DO 5.3–5.7 mg/L. According to Caesar *et al.* (2021), the optimal range of water quality parameters for catfish growth is temperature 23–30°C, pH 6.5–8.5, and DO >3 mg/L.

The application of Minaraya probiotics in this study was proven to increase feed efficiency in catfish, as indicated by a low Feed Conversion Ratio (FCR) and a high Protein Efficiency Ratio (PER) value. The best treatment was obtained at a dose of 10 mL/2.5 kg of feed (P3), with an FCR value of 1.02 ± 0.08 and a PER of 3.14 ± 0.24. A low FCR value reflects the fish's ability to efficiently convert feed into biomass, resulting in less feed requirement for weight gain (Elvy *et al.,* 2022). This efficiency not only increases productivity but also contributes to reducing production costs and supporting the sustainability of the aquaculture system (Boyd, 2023). On the other hand, a high PER value indicates that

the protein in the feed is optimally utilized for body tissue formation, which has implications for better fish growth and development (Muin & Taufek, 2024). These results are consistent with the findings of Hartono & Barades, (2021), who reported that administering commercial probiotics such as APB, LOB, and MSC to catfish resulted in an FCR of 0.87–1.01, lower than the control treatment. Similarly, Redhwan *et al.* (2024) reported that adding AquaStar® Pond probiotics to tilapia resulted in an FCR of 1.20–1.35, also lower than the control. In general, the use of probiotics in feed has been shown to increase feed efficiency by reducing FCR values and increasing PER values, as probiotics can improve digestion and nutrient absorption in fish (Puvanasundram *et al.,* 2021). Probiotics such as *Bacillus* sp., *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Saccharomyces cerevisiae* are known to possess extracellular enzyme activity that helps break down complex compounds in feed into simpler forms, making them more easily absorbed and utilized by the fish's body (Andriani & Pratama, 2022; Latif *et al.,* 2023).

The results of this study indicate that the addition of Minaraya probiotics significantly increased the immunity of catfish (*Clarias gariepinus*), as indicated by increased phagocytic activity, respiratory burst, leukocyte differentiation, and total erythrocyte count. Phagocytic activity gradually increased during the observation period on days 1, 3, and 5, especially in the group supplemented with Minaraya probiotics in the feed, indicating the role of probiotics as immunostimulants. Furthermore, the probiotic-treated group showed a faster recovery of phagocytic activity compared to the control group, indicating the contribution of probiotics in stabilizing and strengthening the immune system after infection (Seviana *et al.,* 2023). The positive effect of probiotic bacteria is caused by their ability to stimulate immune cells, increase cytokine production, and release extracellular vesicles that facilitate cell-to-cell communication. Increased phagocytic activity promotes the development of a stronger immune system, providing more optimal protection against pathogenic bacteria (Jaffar *et al.,* 2018; Rocha-Ramírez *et al.,* 2017).

The results of the respiratory burst test showed that probiotic addition was able to stimulate phagocytic activity by increasing the production of oxygen radicals by immune cells. On the 5th day, all treatments with probiotics showed high respiratory burst values, including the third treatment (P3),

which even exceeded the positive control group in terms of respiratory burst values. These findings indicate that the addition of Minaraya probiotics in the feed after *Aeromonas hydrophila* injection was able to increase respiratory burst activity, with the most optimal effect observed on the 3rd to 5th day post-treatment, which also strengthens the potential of probiotics as immunostimulant agents. The increase in respiratory burst activity reflects an increased oxidative response as one of the main defense mechanisms against pathogens, which is characterized by the rapid production of reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide by phagocytic cells, including neutrophils and macrophages (Lulijwa *et al.,* 2019; Srivastava & Pandey, 2015). This ROS production is an important part of the fish's non-specific defense system against invasive microorganisms. Additionally, probiotic applications, both in monospecies and multispecies forms, have been reported to increase phagocytic activity, lysozyme, complement, respiratory burst, and the expression of various immune cytokines in fish (Nayak, 2010). For example, research conducted by Amit *et al.* (2022) showed that administering Lactobacillus plantarum at a dose of 10^9 CFU/g was able to increase the non-specific immune response, as indicated by increased lysozyme activity and respiratory burst, thus contributing to an improvement in the general health condition of fish.

Observations of leukocyte differentiation in catfish over three time points (days 1, 3, and 5) revealed changes in the composition of lymphocytes, neutrophils, and monocytes in response to *Aeromonas hydrophila* infection. These findings suggest that the addition of the Minaraya probiotic, particularly in the P2 treatment group, stimulates the fish's immune system by increasing the numbers of neutrophils and monocytes, which are key components of the innate immune defense. The observed changes in leukocyte composition indicate the role of probiotics in modulating the immune response to bacterial infection. This increase is believed to be associated with the mechanism by which probiotics activate the innate immune system through interactions between microbe-associated molecular patterns (MAMPs) and pattern recognition receptors (PRRs) on phagocytic cells such as macrophages, natural killer cells, and dendritic cells. These interactions subsequently trigger phagocyte activation and cytokine production, which enhance both cellular and humoral immune responses

(Seviana *et al.,* 2023; Sumon *et al.,* 2022). Furthermore, the herbal components in the probiotic such as ginger (*Zingiber officinale*), Java turmeric (*Curcuma xanthorrhiza*), black turmeric (*Curcuma aeruginosa*), turmeric (*Curcuma domestica*), and aromatic ginger (*Kaempferia galanga*) also contribute to enhancing fish immunity, as all of these ingredients possess immunostimulant properties that have been shown to strengthen immune responses, including leukocyte differentiation, respiratory burst activity, and phagocytosis (Ahmad *et al.,* 2024; Pridayem *et al.,* 2022).

The observation results showed that the total number of erythrocytes in catfish ranged from 4.8×10^6 to 9.6×10^6 cells/mm³, indicating an active physiological condition in response to *A. hydrophila* infection. On the 5th day, group P1 showed a high erythrocyte count (9.2×10^6 cells/mm³), approaching the positive control (9.3×10^6 cells/mm³), indicating that Minaraya probiotics at this dose were effective in maintaining erythrocyte production during recovery. On the other hand, group P3 reported a decrease in erythrocyte count, suggesting that higher doses do not necessarily result in better hematological results. This finding is in line with Fazio, (2019), who stated that an increase in erythrocyte count may reflect increased metabolic and immunological activity in fish. Additionally, Nayak, (2010) explained that probiotics can play a role in stimulating hematopoiesis by improving the health and immune status of fish. Furthermore, Minaraya probiotics contain several types of probiotic bacteria, one of which is *Bacillus* sp., which is widely used as a probiotic due to its beneficial properties. According to Hamka *et al.* (2021), the use of *Bacillus* sp. can help improve growth and the immune response of catfish, as well as stimulate erythrocyte production. This stimulation of erythrocyte production is related to optimal digestion, which ultimately has a positive effect on the fish's immune system.

Conclusion

The addition of Minaraya probiotics to catfish feed (*Clarias gariepinus*) improved growth, feed efficiency, and non-specific immune function. Survival rates remained high across all treatments, ranging from 95% to 98.89%, despite no statistically significant differences in survival. Minaraya probiotics significantly improved growth parameters (SGR and DGR) and feed efficiency (FCR and PER), especially in treatment 3 (P3) with a dose of 10 mL/2.5 kg of feed. On the other hand, the non-specific immune response also increased, as indicated by increased

phagocytic activity, respiratory burst, and the proportion of lymphocytes, neutrophils, and monocytes, as well as an increase in the total number of erythrocytes after *Aeromonas hydrophila* infection. Thus, the application of Minaraya probiotics can be recommended as a strategy to improve the health and productivity of catfish in sustainable aquaculture systems.

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