

EFFECTIVENESS OF DIETARY SYNBIOTICS FOR CONTROLLING *Aeromonas hydrophila* AND *Pseudomonas* sp. INFECTIONS IN PATIN (*Pangasianodon hypophthalmus*)

Efektivitas Sinbiotik dalam Pakan untuk Mengendalikan Infeksi Bakteri *Aeromonas hydrophila* dan *Pseudomonas* sp. pada Ikan Patin (*Pangasianodon hypophthalmus*)

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ABSTRACT

Infections caused by *Aeromonas hydrophila* and *Pseudomonas* sp. are a major challenge in patin aquaculture. This study aimed to evaluate the effect of a synbiotic consisting of *Lactobacillus plantarum* combined with orange peel powder, dragon fruit peel, or banana peel on the health status of patin following a challenge test with *A. hydrophila* and *Pseudomonas* sp. The study used a completely randomized design with four treatments and three replicates. The treatments consisted of no synbiotic as the control; synbiotic feed with orange peel powder; synbiotic feed with dragon fruit peel powder; and synbiotic feed with banana peel powder. The synbiotic feed was prepared for each treatment by adding 2,5 g/kg combined with 0,1 mL/g of *L. plantarum* bacteria at a concentration of 10⁸ CFU/mL. A total of 15 patin were reared in aquariums. The synbiotic feed was administered for 30 days, and a challenge test was conducted on day 33 using *A. hydrophila* and *Pseudomonas* sp. The fish were then observed until day 45. The results showed that synbiotic administration alleviated clinical symptoms, accelerated recovery, maintained hematological profiles, and improved fish survival. The best results were observed in the *L. plantarum* synbiotic feed treatment with orange peel flour, which resulted in faster recovery, a survival rate of 95,55%, and the best hematological profile, its potential as a strategy to control *A. hydrophila* and *Pseudomonas* sp. bacterial infections in patin.

Keywords: *Aeromonas hydrophila*, fruit peel powder, patin, *Pseudomonas* sp., synbiotic

ABSTRAK

Infeksi *Aeromonas hydrophila* dan *Pseudomonas* sp. merupakan tantangan utama dalam budidaya ikan patin. Penelitian ini bertujuan untuk mengevaluasi pengaruh sinbiotik berupa *Lactobacillus plantarum* yang dikombinasikan dengan tepung kulit jeruk, kulit buah naga, atau

kulit pisang terhadap status kesehatan ikan patin setelah uji tantang bakteri *A. hydrophila* dan *Pseudomonas* sp. Penelitian menggunakan Rancangan Acak Lengkap dengan empat perlakuan dan tiga ulangan. Perlakuan tersebut terdiri dari: tanpa sinbiotik sebagai kontrol; pakan sinbiotik dengan tepung kulit jeruk; pakan sinbiotik dengan tepung kulit buah naga; dan pakan sinbiotik dengan tepung kulit pisang. Pakan sinbiotik dicampur sesuai perlakuan masing-masing dengan penambahan tepung kulit buah 2,5 g/kg yang dikombinasikan dengan 0,1 mL/g bakteri *L. plantarum* berkonsentrasi 10^8 CFU/mL. Ikan patin sebanyak 15 ekor dipelihara dalam akuarium. Pemberian pakan sinbiotik dilakukan selama 30 hari dan diuji tantang pada hari ke-33, menggunakan *A. hydrophila* dan *Pseudomonas* sp. Ikan kemudian diamati hingga hari ke-45. Hasil penelitian menunjukkan bahwa pemberian sinbiotik mampu meringankan gejala klinis, mempercepat pemulihan, mempertahankan profil hematologi, serta meningkatkan kelangsungan hidup ikan. Hasil terbaik ditunjukkan oleh perlakuan pakan sinbiotik *L. plantarum* dengan penambahan tepung kulit jeruk menghasilkan pemulihan lebih cepat, tingkat kelangsungan hidup sebesar 95,55%, dan profil hematologi terbaik sehingga berpotensi sebagai strategi untuk mengendalikan infeksi bakteri *A. hydrophila* dan *Pseudomonas* sp. pada ikan patin.

Kata Kunci: *Aeromonas hydrophila*, patin, *Pseudomonas* sp., sinbiotik, tepung kulit buah

INTRODUCTION

Patin (*Pangasianodon hypophthalmus*) is a leading freshwater commodity in Indonesia because of its rapid growth, strong environmental adaptability, and high economic value. Rising demand has driven aquaculture intensification through high stocking densities and the use of high-protein commercial feed. However, this system also increases organic matter accumulation, which can degrade water quality and cause physiological stress in fish (Zaki et al., 2023). These conditions can disrupt metabolism and increase the fish's susceptibility to infectious diseases (Vaishali et al., 2024).

Bacterial diseases are a major challenge in Patin aquaculture because they can reduce productivity, cause crop failure, and lead to economic losses. Suboptimal environmental conditions in intensive aquaculture systems can weaken fish immune responses and promote the proliferation of opportunistic pathogenic bacteria (Samsing & Barnes, 2024; Irshath et al., 2023). Two common pathogens that infect fish are *Aeromonas hydrophila* and *Pseudomonas* sp. (Bakiyev & Bissenbaev, 2021). Infection with *A. hydrophila* can cause Motile Aeromonas Septicemia (MAS), which is characterized by bleeding on the skin and fins. *Pseudomonas* sp. infections can result in internal bleeding and tissue necrosis (Abdella et al., 2024).

Disease problems become more complex when *A. hydrophila* and *Pseudomonas* sp. infect fish simultaneously. Interactions between bacteria under co-infection conditions are thought to produce synergistic effects that increase virulence, accelerate tissue damage, suppress the immune response, and expand disease spread within aquaculture environments (Abdel-Latif et al., 2020). In intensive systems with suboptimal environmental management, mixed infections have been reported to cause 70–90% mortality in Patin within a relatively short period (Okon et al., 2023). Such high mortality rates not only reduce production success but also threaten the sustainability of the aquaculture industry (Dien et al., 2022).

Aquaculture still relies heavily on antibiotics to control bacterial diseases because they can rapidly suppress pathogen growth (Rodrigues et al., 2025). However, excessive or improper antibiotic use can promote antimicrobial resistance, reduce treatment effectiveness, and increase the risk of emerging bacterial strains that are harder to control (Milijasevic et al., 2024). Antibiotic residues can also accumulate in fish tissues and be released into the aquatic environment, thereby disrupting the balance of the natural microbiota and spreading resistance

genes (Yuan et al., 2023). There is a need for safer and more sustainable alternative control strategies, including the supplementation of synbiotics in feed.

Synbiotics combine probiotics beneficial live microorganisms with prebiotics indigestible substrates that support the growth and activity of probiotics. This combination can restore the balance of the gut microbiota and enhance the innate immune system by activating macrophages and increasing lysozyme activity, thereby improving fish resistance to pathogen invasion (Amenyogbe, 2024; Hardi et al., 2021). However, the effectiveness of synbiotics depends on the compatibility of the probiotic strains and the prebiotic used, and their ability to control co-infections of *A. hydrophila* and *Pseudomonas* sp. in Patin has not yet been comprehensively studied. Therefore, this study aims to evaluate synbiotic supplementation in feed as an effective, safe, and sustainable biocontrol strategy against infections caused by these two bacteria.

RESEARCH AND METHOD

Time and Place

This study was conducted from November 2025 to January 2026. The test feed preparation for the experiment took place at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Science, Mulawarman University. Meanwhile, the experimental animals were raised at the Microbiology and Aquaculture Biotechnology Laboratory, also part of the Faculty of Fisheries and Marine Science, Mulawarman University.

Experimental Design

This study used a completely randomized design (CRD) with four treatments and three replicates over a 45-day rearing period. Differences between treatments were based on prebiotics derived from fruit peel powder: orange peel, dragon fruit peel, and banana peel each added to the feed formulation at 2,5 g/kg. The use of these three types of fruit peel powders was based on previous research findings that demonstrated the potential of orange peel powder (Agustina et al., 2025), dragon fruit peel powder (Susanti et al., 2026), and banana peel powder (Agustina et al., 2024) as functional ingredients in feed. In addition, each prebiotic source was synergistically combined with the probiotic *Lactobacillus plantarum* at a concentration of 10^8 CFU/mL, administered at a dosage of 0,1 mL/g of feed. This strategic combination was designed as a synbiotic treatment.

P0 : Feed without synbiotic/commercial supplement (control)

P1 : Feed synbiotic with *L. plantarum* and orange peel powder

P2 : Feed synbiotic with *L. plantarum* and dragon fruit peel powder

P3 : Feed synbiotic with *L. plantarum* and banana peel powder

Research Procedures

1. Production of Fruit Peel Flour

The process of producing flour from fruit peel waste begins with collecting peels from oranges, dragon fruits, and bananas. Once gathered, the peels are meticulously sorted and rinsed with clean water to remove impurities. To speed evaporation and reduce spoilage risk, the peels are sliced into thin strips, approximately 2-3 cm. The peels are dried in an oven at a constant temperature of 50°C for 3 hours, ensuring the moisture content falls below 10%. After drying, the material is ground and sifted to produce a fine powder. To protect against physical and chemical degradation caused by hygroscopic properties and to minimize the risk of fungal contamination, the flour is stored in tightly sealed, airtight containers within a low-humidity environment.

2. Culture of *Lactobacillus plantarum*

Lactobacillus plantarum was obtained from a laboratory slant agar stock with a concentration of 10^{12} CFU/mL. The microorganisms were activated by inoculating the

bacterial isolate into Tryptic Soy Broth (TSB) and incubating for 24 hours. The bacterial suspension was diluted serially until a concentration of 10^8 CFU/mL was reached.

3. Preparation of Test Diets

The diet used in this study was based on a commercial formulation. Preparation involved reducing the commercial pellets to a fine powder. To incorporate prebiotic supplementation, fruit peel powder was mixed into the commercial diet at 2,5 g/kg. The mixture was then homogenized and extruded into pellets with size 2-3 mm. These extruded pellets were dried in an oven at 40-50°C for 3-4 hours, until the moisture content fell below 10%.

Test feed samples weighing 50 g were prepared for each replicate. Probiotic inoculation was performed by creating a 5 mL suspension of *L. plantarum* at 10^8 CFU/mL, combined with 1 mL of egg albumin as a binder. This mixture was homogenized using a vortex mixer, then evenly applied to the feed surface via spraying. The feed was dried at room temperature and stored in an airtight container in a refrigerator at 4°C to maintain bacterial viability before administration to the test fish.

4. Cultures of *Aeromonas hydrophila* dan *Pseudomonas* sp.

The pathogenic bacterial isolates used in this study were obtained from the Laboratory of Microbiology and Aquaculture Biotechnology, Faculty of Fisheries and Marine Science, Mulawarman University. Before use, the isolates were revitalized by inoculating them onto Tryptic Soy Agar (TSA) medium using the streak plate technique. Subsequently, all inoculated petri dishes were incubated at 28-30°C for 24 hours to achieve optimal bacterial growth.

5. Test to Patin

Patin were reared for 45 days in aquariums at a density of 15 fish for replicates. During the first 30 days, fish were fed according to their treatment group to satiation twice a day, then fed without synbiotic supplementation until the end of the rearing period. The quality of the rearing medium was maintained through periodic water changes. The challenge test used a 1:1 ratio of *A. hydrophila* and *Pseudomonas* sp. The bacterial were administered by intramuscular injection at a concentration of 10^6 CFU/mL and a dose of 0,1 mL/fish.

Data Collection

Data in this study included clinical symptoms, survival rates, hematology parameters (total red blood cell count, total white blood cell count, white blood cell differential), and water quality.

1. Clinical Symptoms

Clinical signs were observed following challenge tests with *A. hydrophila* and *Pseudomonas* sp. Observed clinical signs included passive movement, frequently staying at the bottom, decreased appetite, and reduced reflex movement.

2. Survival Rate

Survival rates were calculated at the end of the rearing period using the formula by Effendie (2002):

$$SR = \frac{N_t}{N_0} \times 100$$

Notes:

SR : Survival rate (%)

N_t : Number of live fish at the end of the study (individuals)

N_0 : Number of fish at the beginning of the study (individuals)

3. Total Red Blood Cells

Total red blood cells count was performed according to the method of Blaxhall and Daisley (1973). A blood sample was drawn using a red blood cell pipette to the 0,5 mark,

then Hayem's solution was added to the 101 mark. The mixture was then gently homogenized until uniform.

The diluted blood solution was then slowly introduced into the counting chamber of the hemocytometer, which had been covered with a cover slip. The preparation was then observed under a microscope. Red blood cells were counted within a designated counting field, and the count was converted to the number of red blood cells per volume of blood using the formula:

$$\text{Total red blood cell} = n \times 10^4 \text{ cells/mm}^3$$

Notes:

n = number of red blood cells counted.

4. Total White Blood Cells

White blood cells are counted using the method described by Blaxhall and Daisley (1973). A blood sample is drawn using a white blood cell pipette up to the 0,5 mark and mixed with Turk's solution until the 11 mark is reached. Gently homogenize the blood-diluent mixture by swirling the pipette in a figure-eight motion for approximately 3-5 minutes.

Discard the first few drops of the solution and then place the next drop into the counting chamber of a hemocytometer covered with a coverslip. Observe the hemocytometer under a microscope. White blood cells are counted in the four large squares of the hemocytometer, and the total white blood cells count is determined using the formula:

$$\text{Total white blood cells} = n \times 50 \text{ cells/mm}^3$$

Notes:

n = number of white blood cells counted.

5. White Blood Cell Differential

The method for determining white blood cell differential parameters is based on Blaxhall and Daisley (1973). A blood sample is placed on the first slide, and then place a second slide over the blood droplet at a 45° angle. Slide the second slide until the blood spreads evenly along its edges, then push it quickly and steadily to form a thin, even blood smear. Air-dry the specimen at room temperature. The blood smear is fixed using methanol. After drying, stain with Giemsa solution for 15–20 minutes. The specimen is then rinsed with distilled water and air-dried. Observation is performed using a microscope to identify and count each type of white blood cell using the formula:

$$\% \text{ Monocytes} = \frac{\text{Monocytes}}{100} \times 100\%$$

$$\% \text{ Lymphocytes} = \frac{\text{Lymphocytes}}{100} \times 100\%$$

$$\% \text{ Neutrophils} = \frac{\text{Neutrophils}}{100} \times 100\%$$

6. Water Quality

Water quality data includes temperature, pH, dissolved oxygen, and ammonia levels. A multi-parameter water quality meter measures temperature, pH, and dissolved oxygen (DO). Ammonia levels are determined using the spectrophotometric method.

Data Analysis

Survival rate, total red blood cell count, total white blood cell count, and white blood cell differential were statistically analyzed using Analysis of Variance (ANOVA) at a 95% confidence level. If the results showed a significant effect, the analysis continued with Duncan's Multiple Range Test (DMRT). Meanwhile, clinical symptoms and water quality data were presented descriptively.

RESULTS

Clinical Symptoms

Clinical symptoms following the challenge test (Table 1) showed that the control exhibited more severe clinical symptoms compared to the other treatments. This was evident through day 45, as the control exhibited a greater number of indicator symptoms. This suggests slower recovery in the control. In contrast, the synbiotic feed treatments showed a reduce infection symptoms intensity.

Table 1. Clinical symptoms in patin following a challenge test.

Treatment	Indicator	Day		
		30	40	45
P0	Lack of movement	++++	++++	+
	Often lies at the bottom	+++	+++	+
	Decreased appetite	+++	++	++
	Reduced reflexes	++++	+++	++
P1	Lack of movement	+++	++	+
	Often lies at the bottom	+++	+++	+
	Decreased appetite	++++	++	++
	Reduced reflexes	++	++	++
P2	Lack of movement	+++	++	+
	Often lies at the bottom	++	++	+
	Decreased appetite	+++	+++	+
	Reduced reflexes	++++	+	+
P3	Lack of movement	++++	+	+
	Often lies at the bottom	+++	++	+
	Decreased appetite	++	++	++
	Reduced reflexes	++	++	++

Note: (-) no clinical signs observed, (+) 1-25%, (++) 26-50%, (+++) 51-75%, dan (+++++) 76-100%. (The treatment of P0 : control; P1 : feed synbiotic with *L. plantarum* and orange peel powder; P2 : feed synbiotic with *L. plantarum* and dragon fruit peel powder; P3 : feed synbiotic with *L. plantarum* and banana peel powder).

Survival Rate

The survival rate of patin after the challenge test (Figure 1) differed significantly among treatment groups. The control group had the lowest survival rate 46,5%, which differed significantly lower than all synbiotic treatments. The synbiotic with orange peel powder had the highest survival rate at 95,55%, followed by synbiotic with dragon fruit peel powder at 93,33%, and synbiotic with banana peel powder at 82,38%. Although, numerical differences were observed among the three synbiotic treatments, they were not statistically significant.

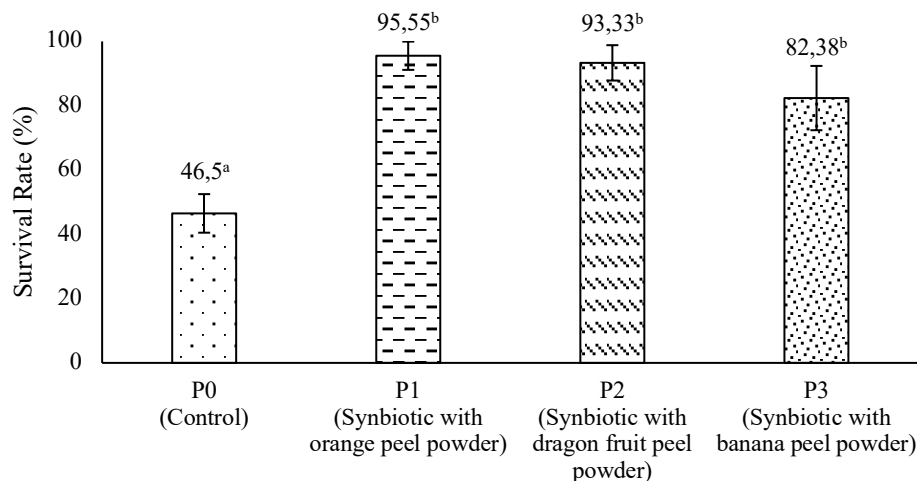


Figure 1. Survival rate of patin after the challenge test. Mean values marked with the same letters did not differ significantly between treatments at the 5% significance level ($P > 0,05$).

Hematology Parameters

The hematological parameters in this study including total red blood cells, total white blood cells, monocyte count, lymphocyte count, and neutrophil count are shown in Table 2.

Table 2. Results of the count of total red blood cells, total white blood cells, monocyte count, lymphocyte count, and neutrophil count.

Treatment	Day			
	0	30	40	45
Total Red Blood Cells ($\times 10^6$ cells/mm³)				
P0	2,10 $\pm$ 0,07 ^a	2,21 $\pm$ 0,03 ^a	2,04 $\pm$ 0,03 ^a	2,10 $\pm$ 0,02 ^a
P1	2,15 $\pm$ 0,05 ^a	2,54 $\pm$ 0,04 ^b	2,47 $\pm$ 0,03 ^b	2,55 $\pm$ 0,04 ^b
P2	2,07 $\pm$ 0,06 ^a	2,50 $\pm$ 0,04 ^b	2,43 $\pm$ 0,04 ^b	2,49 $\pm$ 0,04 ^b
P3	2,15 $\pm$ 0,08 ^a	2,62 $\pm$ 0,03 ^c	2,48 $\pm$ 0,04 ^b	2,54 $\pm$ 0,04 ^b
Total White Blood Cells ($\times 10^4$ cells/mm³)				
P0	5,55 $\pm$ 0,15 ^a	5,72 $\pm$ 0,07 ^a	7,17 $\pm$ 0,07 ^c	6,60 $\pm$ 0,05 ^c
P1	5,52 $\pm$ 0,07 ^a	6,27 $\pm$ 0,07 ^b	6,50 $\pm$ 0,05 ^a	6,07 $\pm$ 0,03 ^a
P2	5,58 $\pm$ 0,07 ^a	6,20 $\pm$ 0,10 ^b	6,65 $\pm$ 0,05 ^b	6,20 $\pm$ 0,05 ^b
P3	5,51 $\pm$ 0,15 ^a	6,47 $\pm$ 0,07 ^c	6,60 $\pm$ 0,05 ^b	6,20 $\pm$ 0,05 ^b
Monocyte Count (%)				
P0	6,67 $\pm$ 1,53 ^a	9,33 $\pm$ 0,58 ^b	12,67 $\pm$ 0,58 ^b	11,67 $\pm$ 0,58 ^b
P1	7,00 $\pm$ 1,73 ^a	7,67 $\pm$ 0,58 ^a	9,67 $\pm$ 0,58 ^a	9,00 $\pm$ 0,00 ^a
P2	6,67 $\pm$ 2,08 ^a	7,67 $\pm$ 0,58 ^a	10,33 $\pm$ 0,58 ^a	9,33 $\pm$ 0,58 ^a
P3	5,67 $\pm$ 1,15 ^a	7,33 $\pm$ 0,58 ^a	10,33 $\pm$ 0,58 ^a	8,67 $\pm$ 0,58 ^a
Lymphocyte Count (%)				
P0	76,67 $\pm$ 5,03 ^a	75,67 $\pm$ 0,58 ^a	64,00 $\pm$ 1,00 ^a	65,67 $\pm$ 0,58 ^a
P1	73,33 $\pm$ 4,16 ^a	78,33 $\pm$ 0,58 ^b	72,33 $\pm$ 0,58 ^c	75,33 $\pm$ 0,58 ^c
P2	77,33 $\pm$ 4,16 ^a	73,33 $\pm$ 0,58 ^a	70,00 $\pm$ 1,00 ^b	73,00 $\pm$ 1,00 ^b
P3	72,67 $\pm$ 2,51 ^a	79,33 $\pm$ 0,58 ^b	71,00 $\pm$ 1,00 ^b	74,00 $\pm$ 1,00 ^b
Neutrophil Count (%)				
P0	3,51 $\pm$ 0,36 ^{ab}	3,73 $\pm$ 0,32 ^{ab}	8,40 $\pm$ 0,60 ^b	7,43 $\pm$ 0,51 ^b
P1	3,30 $\pm$ 0,28 ^a	3,40 $\pm$ 0,30 ^a	6,93 $\pm$ 0,55 ^a	6,33 $\pm$ 0,58 ^a
P2	4,13 $\pm$ 0,41 ^b	4,33 $\pm$ 0,45 ^b	8,00 $\pm$ 0,00 ^b	7,17 $\pm$ 0,29 ^b
P3	3,83 $\pm$ 0,30 ^{ab}	4,01 $\pm$ 0,25 ^{ab}	8,33 $\pm$ 0,58 ^b	7,00 $\pm$ 0,00 ^{ab}

Note: Mean values followed by the same letter indicate no significant difference at the 95% confidence level ($P > 0,05$). (The treatment of P0 : control; P1 : feed synbiotic with *L. plantarum* and orange peel powder; P2 : feed synbiotic with *L. plantarum* and dragon fruit peel powder; P3 : feed synbiotic with *L. plantarum* and banana peel powder).

Hematological parameters assessed at the start of treatment showed no significant differences. After 30 days of synbiotic treatment, total red blood cells counts in the group receiving synbiotic with orange peel powder and synbiotic with dragon fruit peel powder were significantly higher than control, but there was no significant difference between both of them. Notably, synbiotic with banana peel powder had the highest total red blood cells count at $2,62 \pm 0,03$ cells/mm³, which differed significantly from the others. On day 40 after the bacterial challenge, total red blood cells count in fed with synbiotics were significantly higher than in control. However, the three synbiotic groups did not differ significantly from each other. The same pattern persisted through the end of experiment period on day 45. At that time, the total red blood cells counts in fed with synbiotic each $2,55 \pm 0,04$ cells/mm³, $2,49 \pm 0,04$ cells/mm³, $2,54 \pm 0,04$ cells/mm³. All values were significantly higher than control group at $2,10 \pm 0,02$ cells/mm³, yet no significant differences were detected among the treatment groups.

The white blood cells count after 30 days of treatment, synbiotic with banana peel powder produced the highest white blood cells count at $6,47 \pm 0,07$ cells/mm³, which was significantly different from all other groups. The white blood cells counts in synbiotic with orange peel powder at $6,27 \pm 0,07$ cells/mm³ and synbiotic with dragon fruit peel powder at $6,20 \pm 0,10$ cells/mm³ were not significantly different from each other, but both were significantly higher than control at $5,72 \pm 0,07$ cells/mm³. After the bacterial challenge test on day 40, control showed the highest total white blood cells count at $7,17 \pm 0,07$ cells/mm³ and differs significantly from other synbiotic treatments. Synbiotic with dragon fruit peel powder and synbiotic with banana peel powder were not significantly different from each other, but both were significantly higher than synbiotic with orange peel powder at $6,50 \pm 0,05$ cells/mm³. The same pattern of differences remained evident at the end of the rearing period on day 45, namely, control had the highest total white blood cells count at $6,60 \pm 0,05$ cells/mm³, synbiotic with orange peel powder the lowest at $6,07 \pm 0,03$ cells/mm³, while synbiotic with dragon fruit peel powder at $6,20 \pm 0,05$ cells/mm³ and synbiotic with banana peel powder at $6,20 \pm 0,05$ cells/mm³ showed values that were not significantly different.

The percentage of monocytes after synbiotic administration in the control at $9,33 \pm 0,58\%$ was significantly higher than in the other treatments. After the bacterial challenge test on day 40, the percentage of monocytes increased in all groups, but the value in control at $12,67 \pm 0,58\%$ remained significantly higher than the others. The same condition was still observed at the end of the maintenance period on day 45, when the percentage of monocytes in control at $11,67 \pm 0,58\%$ remained significantly higher than the others.

The highest lymphocyte percentage on day 30 was found in synbiotic with banana peel powder at $79,33 \pm 0,58\%$, followed by synbiotic with orange peel powder at $78,33 \pm 0,58\%$, control at $75,67 \pm 0,58\%$, and synbiotic with dragon fruit peel powder at $73,33 \pm 0,58\%$. After the bacterial challenge test on day 40, synbiotic with orange peel powder at $72,33 \pm 0,58\%$ showed the highest lymphocyte percentage, while control at $64,00 \pm 1,00\%$ showed the lowest. At the end of the rearing period on day 45, the lymphocyte percentage in the treatment groups remained higher than that control at $65,67 \pm 0,58\%$, with the highest value observed in synbiotic with orange peel powder at $75,33 \pm 0,58\%$.

The percentage of neutrophils on day 30 was lower in synbiotic with orange peel powder at $3,40 \pm 0,30\%$ than the others. After the bacterial challenge test on day 40, synbiotic with orange peel powder at $6,93 \pm 0,55\%$ showed a significantly lower value compared to the other

groups. At the end of the maintenance period, neutrophil counts decreased numerically, synbiotic with orange peel powder at $6,33 \pm 0,58\%$ continuing to show the lowest.

Water Quality

Water quality measurements during the rearing period (Table 3) showed that temperature, pH, dissolved oxygen, and total ammonia remained relatively stable and did not differ significantly among treatments. Temperature ranged from 26,1-28,8°C, pH from 6,8-6,9, dissolved oxygen from 4,55-5,28 mg/L, and total ammonia from 0,0073-0,0140 mg/L. All parameters stayed within ranges suitable for supporting freshwater fish.

Table 3. Results of water quality parameters.

Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Ammonia (mg/L)
26,1 - 28,8	6,80 - 6,97	4,55 - 5,28	0,0073 - 0,0140

DISCUSSION

Clinical symptoms in patin showed differences in severity and recovery rates among the treatment groups. Fish in the group fed the synbiotic tended to exhibit milder clinical symptoms and recover more quickly than the control group. The observed differences in responses are thought to be related to the role of synbiotics in maintaining gut microbiota balance, enhancing nutrient utilization, and supporting fish immune function (De Marco et al., 2023). By the end of the rearing period, the group receiving a synbiotic formulated from dragon fruit peel powder showed relatively better clinical conditions, primarily evidenced by lower lethargy. Nevertheless, some clinical signs persisted, including a tendency for the fish to remain at the bottom of the holding tanks, reduced appetite, and diminished reflex responses. Dragon fruit peel, rich in polyphenols, can serve as a source of natural antioxidants that reduce oxidative stress while supporting fish immune responses and physiological resilience against environmental disturbances and pathogen attacks (Fadillah et al., 2024). This suggests that the type of prebiotic in each synbiotic formulation can result in varying levels of protection and physiological recovery (Amenyogbe et al., 2024; Abualreesh, 2026).

The control treatment had the lowest survival rate. This indicates that fish not fed the synbiotic diet have a limited ability to maintain physiological functions when facing bacterial infection. In contrast, the orange peel powder synbiotic had the highest survival rate. The high survival rate in the synbiotic group indicates that synbiotics boost patin's immune system through interactions between probiotics and prebiotics in the digestive tract. Prebiotics function as substrates that promote the growth and activity of beneficial microorganisms, thereby contributing to overall health. Probiotics play a crucial role in promoting gut health by inhibiting harmful pathogens. They do this by competing for space and essential nutrients, helping beneficial bacteria thrive while keeping harmful ones in check. Additionally, probiotics produce various antimicrobial substances that directly combat pathogens, further reducing their chances of colonization. A well maintained balance of the gut microbiota allows probiotics to create an environment that supports overall digestive health and infection resistance (Rai et al., 2025; Hardi et al., 2021).

Feeding synbiotics increased the total red blood cells count. This increase suggests that synbiotic supplementation has the potential to maintain or reduce red blood cells damage during a challenge test. The stability of red blood cells counts in the synbiotic group may reflect the patin's ability to maintain physiological homeostasis and demonstrate better adaptive capacity during infections with *A. hydrophila* and *Pseudomonas* sp. (Yang et al., 2020). Adequate red blood cells counts support oxygen distribution to tissues, thereby helping to maintain metabolism, bodily functions, tissue repair, and the fish's immune response (Bai et al., 2024).

The white blood cell count in fish that received the synbiotic treatment was generally higher than in the control group. This increase indicates stimulation of the immune response before the challenge test, as white blood cells are essential components of the body's defense system, responsible for recognizing, engulfing, and eliminating pathogens (Speirs *et al.*, 2024). After pathogen exposure, a lower white blood cell count in the synbiotic group does not necessarily indicate reduced immune function. This finding may indicate that the immune response occurs in a more controlled manner, particularly because it is accompanied by improved clinical status and higher survival rates than in the control group (Ghaly *et al.*, 2022).

Changes in the proportion of monocytes further support the findings regarding total white blood cell count. Monocytes play a role in phagocytosis, clearing damaged tissue, and differentiating into macrophages that produce inflammatory mediators. Higher monocyte levels indicate an increased or prolonged phagocytic and inflammatory response due to uncontrolled infection (Li *et al.*, 2023). This is believed to be the body's mechanism for combating bacteria and repairing tissue damage (Mokhtar *et al.*, 2023). Conversely, the decrease in monocyte percentage in the synbiotic treatment group suggests that synbiotics can trigger a regulated immune response, as shown by clinical recovery and improved survival, thereby preventing excessive monocyte mobilization (Li *et al.*, 2023).

Lymphocyte levels through the end of the maintenance phase in the synbiotic with orange peel powder group showed the highest percentage of lymphocytes and differed significantly from the other groups. Lymphocytes play a role in humoral and cellular immune responses through antigen recognition, antibody production, and the formation of specific responses to pathogens (Chan *et al.*, 2024). The high percentage of lymphocytes in the synbiotic group with orange peel powder, along with the highest survival rate and milder clinical symptoms, indicates that this treatment better maintained adaptive immunity (Speirs *et al.*, 2024). Similarly, it has been reported Riefard *et al.* (2025) that orange peel can improve fish health by stimulating lactic acid bacteria, maintaining a balanced gut microbiota, and strengthening the immune response. Conversely, the low percentage of lymphocytes in the control suggests a greater reliance on the innate immune response, while adaptive immunity was relatively less well maintained. Furthermore, lymphocyte levels are associated with the dominance of monocytes and neutrophils during the infectious response (Nayan *et al.*, 2026).

The increase in neutrophil percentages across all groups after the challenge test indicates that the innate immune system is activated in response to bacterial infection. Neutrophils are first line defense cells that migrate to the site of infection, perform phagocytosis, release antimicrobial compounds, and generate an oxidative burst to kill pathogens (Ferreira *et al.*, 2023). The relatively high percentage of neutrophils in the control suggests the infection triggered a stronger or harder to control acute inflammatory response. In contrast, the synbiotic with orange peel powder had the lowest neutrophil percentage after the challenge test and at the end of the maintenance period, which may reflect a more controlled immune response and potential benefit of the synbiotic intervention in modulating inflammation.

Water quality parameters, including temperature, pH, dissolved oxygen, and ammonia remained within ranges that supported Patin survival throughout the study (SNI, 2002). Relatively stable temperature helps maintain metabolic activity, nutrient utilization, and the fish's physiological functions and immune responses. Appropriate pH levels also indicate a relatively stable rearing environment that does not cause significant physiological stress. Adequate dissolved oxygen concentrations supported respiration, aerobic metabolism, and the fish's energy requirements, particularly during recovery following the bacterial challenge test. Meanwhile, low ammonia concentrations helped reduce the risk of impaired gill function, disrupted osmoregulation, and increased physiological stress. The stability of these water quality parameters suggests that rearing environment conditions were not a major limiting

factor for fish health during the study. Thus, the observed variations in fish health responses are more likely related to the treatments administered than to changes in rearing-medium quality (Menon et al., 2023).

CONCLUSION

Adding synbiotics to the diet increased Patin resistance to infections caused by *Aeromonas hydrophila* and *Pseudomonas* sp., as shown by milder clinical symptoms, a more stable hematological response, and a higher survival rate than the control group. The combination of *Lactobacillus plantarum* with orange peel powder yielded the best response, as evidenced by the highest survival rate and a more controlled immune response. These findings suggest that this combination could be developed as a feed based strategy to safely and sustainably enhance Patin resistance to bacterial infections.

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