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Effects of Probiotic Red Water System on Growth, Tissue Histology, and Water Quality of Mutiara Catfish

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Abstract

Mutiara catfish (Clarias gariepinus) is one of the freshwater fish species with high economic value and increasing aquaculture production each year. The success of catfish farming largely depends on proper water quality management and appropriate feeding practices. Poor water quality management can lead to tissue damage in fish, deterioration of environmental conditions, and suboptimal growth performance. This study evaluated the effect of probiotic application using the red water system technique on fish growth performance, kidney tissue condition, and intestinal histology in catfish culture ponds. The research employed an experimental method using a completely randomized design. The study was conducted from November 2022 to February 2023. The results showed that probiotic treatment produced a positive allometric growth pattern with a survival rate of 87%, relative growth of 53%, and a feed conversion ratio of 1.6. Histopathological scoring indicated that the highest level of tissue damage was necrosis in the intestinal tissue of the non-probiotic treatment (45.83%, classified as severe), while the lowest damage score was hemorrhage in the kidney tissue of the probiotic treatment (20.83%, classified as moderate). Water quality analysis showed that ammonia, nitrate, and phosphate concentrations were associated with fish growth, indicated by coefficients of determination (R^2) of 97.4% for ammonia, 97.8% for nitrate, and 79.9% for phosphate, showing positive linear trends. These findings indicate that probiotic application through the red water system has the potential to improve water quality conditions, support fish growth performance, and reduce tissue damage in cultured Mutiara catfish.

Keywords**:** *Clarias gariepinus; probiotics; red water System; fish growth; histology*

1. Introduction

Freshwater aquaculture, particularly catfish farming, is a strategic subsector because it plays a role in providing animal protein, increasing community incomes, and creating jobs. Catfish (*Clarias gariepinus*) excels due to its rapid growth, adaptability, resilience to environmental changes, and high market demand. Catfish production has been also reported in Asian countries (Thailand, China, Vietnam, Bangladesh, Malaysia, Indonesia and India), achieving for 30% of the global catfish production in 2022 [1]. At the regional level, catfish production in East Java increased significantly, from 65.08 tons in 2020 to 1.06 million tons in 2021, thus confirming catfish as a leading freshwater aquaculture commodity [2,3].

However, quantitative increases in production are not always accompanied by improvements in quality and sustainability of aquaculture systems. Numerous studies have indicated that intensive catfish farming systems frequently encounter challenges such as declining water quality, increased organic waste loads, and high pathogen pressure, which ultimately affect fish health and growth performance [3,4]. Water quality parameters such as temperature, potential of hydrogen (pH), total dissolved solids (TDS), total suspended solids (TSS), ammonia, nitrate, and phosphate are limiting factors in cultivation because they directly affect the metabolism, immune system, and survival of fish [5,6]. Poorly managed environmental conditions can trigger physiological stress and increase fish susceptibility to disease, thereby reducing cultivation productivity.

The main challenge in intensive catfish farming is not only reduced growth rates, but also increased disease and damage to vital organs. Uncontrolled growth of pathogenic bacteria can cause infections and changes in the morphology and function of fish tissues [7]. Kidney and intestinal tissue damage, such as necrosis, edema, and hemorrhage, often occurs in fish raised in poor water quality. These changes disrupt essential physiological functions such as excretion, osmoregulation, and digestion, resulting in stunted fish growth and reduced survival rates [8].

Various efforts have been made to overcome this problem, such as mechanical and chemical water quality management, the use of antibiotics, and regulating feed management [6,9]. However, this approach has several limitations, particularly related to operational costs, the risk of pathogen resistance, and negative impacts on aquatic ecosystems. Therefore, more sustainable and environmentally friendly solutions are needed that can enhance fish growth while improving tissue health and water quality. One approach that has received considerable attention is the use of probiotics in aquaculture systems [10].

Various studies have shown that probiotics, live microorganisms that benefit their hosts, have significant potential to improve aquaculture performance. Microorganisms such as *Lactobacillus* sp., *Bacillus subtilis*, and *Nitrobacter* are known to increase feed digestibility through enzyme production, suppress pathogenic bacteria through competition and inhibitory compounds, and improve water quality through the biodegradation of organic matter [11]. In addition, the use of probiotics has been reported to be able to increase the immune response of fish and reduce stress levels, thus having a positive impact on the growth and survival of farmed fish [12,13].

Probiotics not only improve growth and health, but also maintain vital organs such as the kidneys and intestines. Probiotics can improve intestinal structure (increasing villi height and reducing epithelial damage) for optimal nutrient absorption, as well as reducing kidney necrosis and hemorrhage caused by toxins [14]. In addition, probiotic-based systems such as the red water system can reduce ammonia and nitrate levels in aquaculture ponds [15]. However, research on probiotics has generally focused on growth and feed efficiency, while integrated studies of growth, organ histopathology, and water quality in catfish have been limited. Therefore, this study comprehensively analyzes the effects of probiotics using an integrative approach, and is expected to support the development of sustainable catfish farming.

2. Materials and Methods

2.1. Experimental Design

This study used an experimental method with a quantitative approach to test the effects of probiotics on fish growth, kidney and intestinal tissue conditions, and water quality. The design used was a randomized controlled trial (CRD) with two main treatments: a pond with probiotics and a pond without probiotics as a control, which were monitored periodically throughout the culture period.

Fish rearing and probiotic treatments were carried out at the aquaculture ponds owned by Pokdakan Roi Lele, located in Bringin Village, Wajak District, Malang Regency, East Java, Indonesia. Water quality analyses were performed at the Freshwater Fisheries Laboratory of

Sumberpasir, while histopathological analyses of kidney and intestinal tissues were conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya.

The research object was Mutiara catfish (*Clarias gariepinus*) in intensive ponds, with probiotic application as the independent variable. Observed variables included fish growth (length, weight, growth rate, and survival), kidney and intestinal histopathology (necrosis, edema, hemorrhage), and water quality (temperature, TDS, TSS, pH, ammonia, nitrate, and phosphate).

2.2. Probiotic Treatment Procedure

Probiotic application is carried out using the red water system (RWS), a microorganism-based recirculation system to maintain water quality. RWS media is made through the fermentation of *Lactobacillus casei* (which increases nutrient absorption) and *Saccharomyces cerevisiae* (which supports growth and stabilizes microbes and water quality).

RWS maintains water quality without water changes, supports high stocking densities, and reduces aeration requirements. The probiotics used (*Lactobacillus* sp., *Bacillus subtilis*, and *Nitrobacter*) play a role in organic matter degradation and nitrification. Applications were carried out periodically through water and commercial feed, with a dose of 100 ml in the morning, while controls were treated without probiotics.

2.3. Fish Rearing and Growth Measurement

During the experimental period, Mutiara catfish were maintained under uniform aquaculture management across all experimental units, including stocking density, feed type and quantity, and feeding frequency. Ten samples of fish from each pond were collected fro measurement. Fish growth measurements were conducted periodically by recording increases in fish length and body weight. These growth data were used to calculate relative growth rate, total growth, and the length–weight relationship pattern. Survival rate was also calculated to determine the percentage of fish that survived throughout the rearing period.

Growth parameters are important indicators for evaluating the biological response of fish to probiotic treatments in aquaculture systems. The length–weight relationship and growth pattern were calculated using the Eq. (1)[16].

$$W = aL^b \quad \ldots\ldots \quad (1)$$

Where: W = fish weight (g)
L = fish length (cm)
a and b = constants

When the value of *b* = 3, growth is considered isometric, indicating that the fish body shape remains constant and length increases proportionally with weight. When *b* is not equal to 3, the growth pattern is allometric. If *b* > 3, the growth is positive allometric, indicating that weight increases faster than length. Conversely, if *b* < 3, the growth is negative allometric, indicating that length increases faster than weight.

Relative growth rate was calculated using the Eq. (2) and (3)[17].

$$RG_L = \left(\frac{Lt - L0}{L0}\right) \times 100\% \quad \ldots\ldots \quad (2)$$

$$RG_W = \left(\frac{Wt - W0}{W0}\right) \times 100\% \quad \ldots\ldots \quad (3)$$

Where: RG_L = relative length growth rate (%)
L_0 = initial length (cm)
L_t = final length (cm)
RG_W = relative weight growth rate (%)

W0 = initial weight (g)
Wt = final weight (g)

Total growth was calculated using the Eq. (4) [18].

$$Lm = Lt - L0 \quad \ldots \quad (4)$$

Where: L_m = total length or weight growth
L_t = final length or weight
L_0 = initial length or weight

Fish survival rate was calculated using the Eq. (5) [19].

$$SR = \left(\frac{Nt}{N0}\right) \times 100\% \quad \ldots \quad (5)$$

Where: SR = *survival rate* (%)
N_0 = number of fish at the beginning of the rearing period
N_t = number of fish at the end of the rearing period

2.4. Histopathological Analysis of Kidney and Intestinal Tissues

Kidney and intestinal tissue were analyzed histopathologically to identify structural changes resulting from probiotic treatment and environmental conditions. Samples were taken at the end of the culture, prepared as slides, and then observed under a microscope to identify damage such as necrosis, edema, and hemorrhage, which are the symptoms of the fish's desease [20].

2.5. Water Quality Measurement and Analysis

Water quality in the culture ponds was measured periodically throughout the study to determine the dynamics of physical and chemical parameters influencing fish growth and health. The physical parameters measured included temperature, TDS, and TSS, while the chemical parameters included pH, ammonia, nitrate, and phosphate. Water quality measurements were conducted using appropriate instruments and analytical methods according to laboratory standards. These water quality data were used to evaluate the effects of probiotic application on the aquaculture environment, as water quality is a critical factor influencing the success of catfish farming.

2.6. Data Analysis

Growth, histopathology, and water quality data were analyzed quantitatively using an independent t-test ($p < 0.05$) to compare treatments with and without probiotics. Furthermore, a correlation analysis of water quality and fish growth was conducted to explain the effects of probiotics scientifically and data-drivenly.

3. Results and Discussion

3.1. Effect of Probiotic on the Fish Growth

Growth performance was used to assess the effectiveness of probiotics, by measuring the length and weight of fish at the beginning and end of maintenance to compare the probiotic and control treatments, as presented in Table 1. The results showed that probiotic application significantly increased the growth of pearl catfish, with an average increase in length of 10.1 cm and weight of 160.7 g, as well as a positive allometric growth pattern. Statistical tests showed significant differences, with final length ($p < 0.001$) and final weight ($p < 0.05$) higher than the control. This is in line with Mahmud et al. who stated that positive allometric growth reflects good environmental conditions and adequate nutrient availability [21], as shown in Fig. 1.

Table 1. Average length and weight measurements of Mutiara catfish cultured with probiotic and non-probiotic treatments

Parameter	Probiotic		Non-Probiotic	
	Length (cm)	SD	Length (cm)	SD
Initial average	19	2.65	14.5	1.86
Final average	29.1	3.85	21.3	2.5
Total increase	10.1		6.8	
	Weight (g)	SD	Weight (g)	SD
Initial average	100	37.01	76.3	20.69
Final average	260.7	59.55	193.2	45.34
Total increase	160.7		116.9	

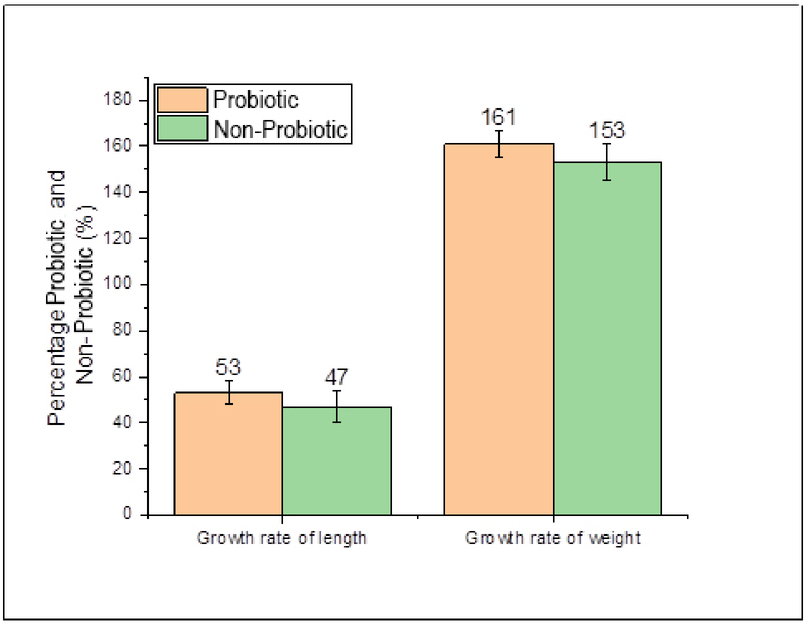

Fig. 1. Comparison of average length and weight growth of Mutiara catfish cultured in probiotic and non-probiotic ponds.

Based on the calculation of the relative growth rate (RGR), fish cultured in probiotic ponds showed a relative length growth of 53%, while fish in non-probiotic ponds showed 47%. The presence of probiotic bacteria can stimulate the production of digestive enzymes that enhance the digestibility of feed substrates. Improved digestibility allows fish to utilize nutrients more efficiently, resulting in increased growth performance [22]. Similarly, the relative weight growth rate reached 161% in probiotic ponds and 153% in non-probiotic ponds. Probiotics are known to break down complex feed components into simpler nutrients that can be readily absorbed by fish. Consequently, fish can utilize feed nutrients more efficiently, leading to improved growth and better water quality in aquaculture ponds [23].

The increase in relative growth rate indicates that probiotics contribute to enhancing the efficiency of nutrient utilization. Relative growth rate is a key indicator reflecting the ability of fish to convert available energy sources into biomass within a specific time period. In addition to growth performance, survival rate also differed between treatments. The highest survival rate was observed in the probiotic treatment (87%), whereas the lowest survival rate occurred in the non-probiotic treatment (65%). Several factors influence fish survival, including water quality, feed composition, and fish health management. The higher survival rate observed in probiotic ponds indicates that environmental conditions and fish health were more stable during the culture period. Mustofa et al. stated that fish survival is strongly influenced by water quality and the physiological condition of fish. Therefore, improved survival in probiotic ponds may be associated with enhanced water quality and improved immune response stimulated by probiotic microorganisms [24].

3.2. Length–Weight Relationship Analysis

The relationship between fish length and weight is widely used to evaluate fish growth patterns and physiological condition. In this study, length–weight analysis was conducted to determine the growth characteristics of Mutiara catfish under probiotic treatments, with results as shown in Fig. 2.

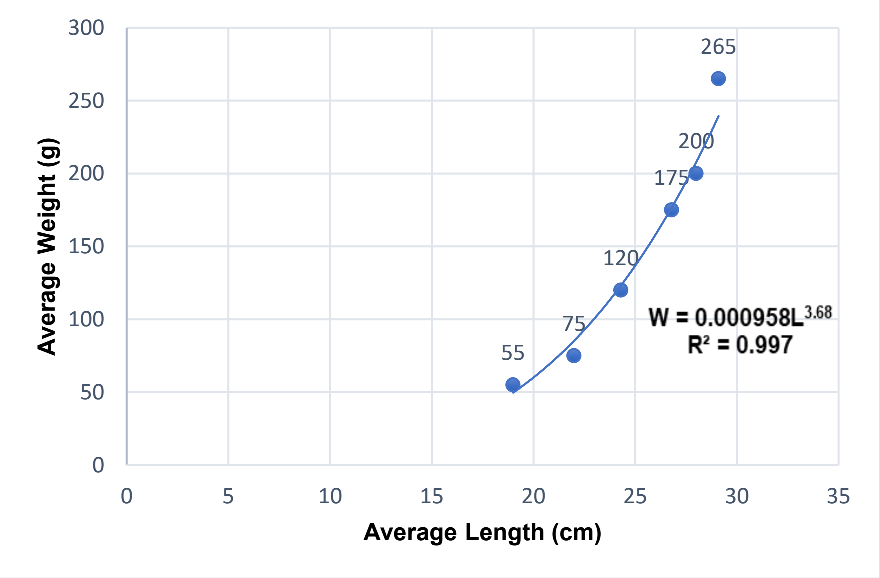

Fig. 2. Length–weight relationship of Mutiara catfish cultured in probiotic ponds.

Analysis showed a very strong relationship between fish length and weight ($R^2 = 0.997$), with a positive allometric growth pattern ($b = 3.68 > 3$) indicating that weight increased faster than length [25,26]. Higher growth coefficients in probiotic ponds indicate better environmental conditions and nutrient efficiency, supported by the role of probiotics in improving fish metabolism and growth [27].

3.3. Histopathological Status of Kidney and Intestinal Tissues

Histopathological analysis was conducted to evaluate the physiological condition of fish organs in response to probiotic application and environmental conditions in the culture ponds. Kidney and intestinal tissues were examined using hematoxylin–eosin (HE) staining and observed under a microscope at 400× magnification, as depicted in Fig. 3.

Histopathological analysis showed clear differences between the treatments, with the pond without probiotics experiencing higher tissue damage (necrosis, edema, and hemorrhage), particularly in the kidneys, which exhibited more severe necrosis due to stress and poor water quality. Conversely, the pond with probiotics showed less damage, with the lowest hemorrhage at 20.83% (moderate category), indicating a protective role for probiotics in maintaining kidney tissue integrity (Table 2).

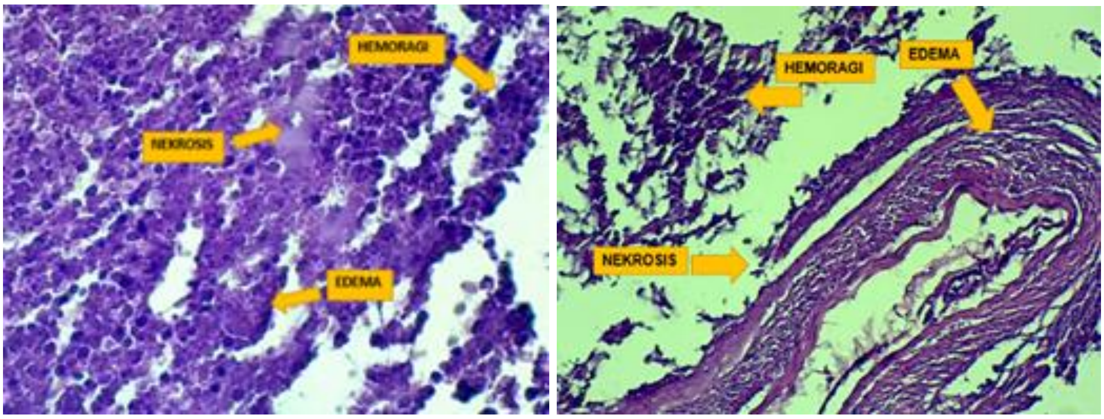

Fig. 3. The histopathological characteristics of the kidney tissue (left) and intestine (right) of Mutiara catfish. At M.400×, It show lesions such as necrosis, edema, and hemorrhage which indicate cell degeneration and vascular disorders.

Table 2. Average length and weight measurements of Mutiara catfish cultured with probiotic and non-probiotic treatments

Kidney Tissue

Lesion	Treatment	Replication 1	2	3	4	5	6	Total Damage	Average	Score	Damage Level
Necrosis	Probiotic	20	20	25	20	20	20	125	20.83	2	Moderare
	Non-Probiotic	65	50	40	25	25	55	260	43.33	3	Severe
Edema	Probiotic	20	20	25	20	20	20	125	20.83	2	Moderare
	Non-Probiotic	65	50	40	25	25	55	260	43.33	3	Severe
Hemorrhage	Probiotic	82	30	30	25	30	20	217	36.17	2	Moderare
	Non-Probiotic	45	55	40	60	40	35	275	45.83	3	Severe

Intestinal Tissue

Lession	Treatment	Replication 1	2	3	4	5	6	Total Damage	Average	Score	Damage Level
Necrosis	Probiotic	25	20	25	30	20	30	150	25	2	Moderare
	Non-Probiotic	50	40	45	30	50	60	275	45.83	3	Severe
Edema	Probiotic	15	20	25	20	30	20	130	21.67	2	Moderare
	Non-Probiotic	45	45	60	30	30	40	250	41.67	3	Severe
Hemorrhage	Probiotic	25	20	25	20	25	10	125	20.83	2	Moderare
	Non-Probiotic	35	45	30	35	30	25	200	33.33	3	Severe

The kidneys are organs that are very sensitive to changes in water quality and exposure to toxic substances, so improving water quality by probiotics plays a role in maintaining the fish health. Histopathological observations of the intestines showed that the ponds without probiotics experienced severe damage, predominantly necrosis, at 45.83%, which disrupted digestion and nutrient absorption. Conversely, the ponds with probiotics showed healthier intestines with less damage, as probiotics maintained microbial balance and intestinal epithelial integrity, thereby increasing nutrient absorption efficiency and supporting fish growth [28].

3.4. Water Quality Analysis

Water quality is very important for the success of cultivation because it affects the metabolism, growth, and survival of fish, so this study measured physical and chemical parameters to assess the effect of probiotics, with results is shown in Table 3.

Table 3. Water quality parameters in probiotic and non-probiotic Mutiara catfish culture ponds

Water Quality Parameter	Unit	Probiotic	Non-Probiotic
Temperature	^{O}C	25.98 ± 0.29	25.56 ± 0.24
TSS	mg/L	77.80 ± 4.15	93.29 ± 3.26
TDS	mg/L	424.23 ± 22.81	379.97 ± 29.99
pH	-	6.31 ± 0.12	6.41 ± 0.10
Ammonia	mg/L	3.30 ± 0.21	4.19 ± 0.30
Nitrate	mg/L	0.512 ± 0.106	1.105 ± 0.233
Orthophospate	mg/L	0.991 ± 0.263	2.79 0.371

The water temperature during the study ranged between 25–26.9°C, which falls within the optimal range for Mutiara catfish culture. TDS ranged from 260 to 488.5 mg/L, while TSS ranged from 60 to 105 mg/L. Although fluctuations occurred, these values remained within tolerable limits for catfish culture. Chemical parameters showed more pronounced differences between treatments. The pH values ranged from 6.0 to 6.55, which is still suitable for catfish aquaculture. However, ammonia, nitrate, and phosphate concentrations differed significantly between treatments. Ammonia levels were lower in probiotic ponds compared to control ponds, although still within the range of 2.5–5.1 mg/L. This reduction indicates the role of probiotic bacteria, particularly Nitrobacter, in the nitrification process that converts ammonia into less toxic nitrate [29]. Similarly, nitrate and phosphate concentrations were more stable in probiotic ponds than in non-probiotic ponds. High nitrate concentrations can trigger eutrophication and reduce water quality. Therefore, the stabilization of nitrate and phosphate concentrations in probiotic ponds suggests that probiotics help maintain nutrient balance within aquaculture systems [30].

Probiotics enhance fish growth by improving digestion (enzymes), improving gut structure and function, and balancing the microbiota, which suppresses pathogens. Furthermore, probiotics strengthen the immune system, reduce oxidative stress, and maintain tissue integrity. Their role in improving water quality through the degradation of organic matter and reducing nitrogenous waste also creates a more optimal environment, thus improving overall fish growth performance.

Overall, probiotics provide an integrated effect of improving fish growth, organ tissue health, and water quality stability. Improved water quality reduces stress, improves organ health, and enhances nutrient absorption, resulting in optimal fish growth. This demonstrates that probiotics are an effective strategy for increasing productivity while maintaining fish health and the sustainability of the aquaculture environment [31,32]. Although probiotics enhance growth, several water quality parameters require attention, such as increased ammonia due to organic and metabolite loads, and increased phosphate, which can potentially lead to eutrophication. This suggests that probiotic use must be carefully managed to avoid negative impacts on fish health and system sustainability.

4. Conclusions

Based on the results of this study, the application of probiotics in the culture of Mutiara catfish (*Clarias gariepinus*) significantly improved fish growth performance. Fish cultured in probiotic-treated ponds exhibited a total length increase of 10.1 cm and a weight gain of 160.7 g. The growth pattern observed was positive allometric, indicating that body weight increased faster than body length. In addition, the probiotic treatment resulted in a survival rate of 87%

and a relative growth rate of 53%, demonstrating that probiotic supplementation positively influences the growth and survival of Mutiara catfish. Histopathological observations of kidney and intestinal tissues revealed several types of tissue damage, including necrosis, edema, and hemorrhage. The highest level of tissue damage was observed as necrosis in the intestinal tissue (45.83%) in the non-probiotic treatment, indicating impaired digestive function and physiological stress. In contrast, the lowest level of damage was hemorrhage in kidney tissue (20.83%) in the probiotic treatment, suggesting that probiotic application contributed to maintaining organ integrity. Overall, the use of probiotics containing Lactobacillus sp. and Bacillus sp. was effective in improving fish growth performance, enhancing survival rate, and reducing the severity of tissue damage. These beneficial effects are likely associated with the ability of probiotic microorganisms to improve nutrient digestion, stabilize microbial balance, and suppress the growth of pathogenic bacteria in the aquaculture environment. Therefore, probiotic application can be considered a promising strategy to improve the productivity and health of Mutiara catfish culture systems.

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Author Declaration

Data availability statement: The data supporting the findings of this study are available upon reasonable request.

CRediT authorship contribution statement: Dewa Sukma Trinanda Adhytia: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. Nico Rahman Caesar: Methodology, Supervision, Validation, Writing – review and editing. Reval Pahlevi: Resources, Project administration, Data Curation.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Artificial Intelligence Statement: The authors declare that generative AI tools were used solely to assist with language editing and grammar improvement. All scientific content, data analysis, interpretation, and conclusions presented in this manuscript were developed entirely by the authors.

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