

EVALUATION OF PROBIOTIC SUPPLEMENTATION IN IMPROVING GROWTH PERFORMANCE, SURVIVAL RATE, AND WATER QUALITY IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURE

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Abstract

This study aimed to evaluate the effects of probiotic supplementation on growth performance, survival rate, and water quality in Nile tilapia (*Oreochromis niloticus*) culture. A completely randomized design (CRD) was employed with four dietary treatments consisting of different probiotic supplementation levels: P0 (control without probiotics), P1 (5 mL probiotic/kg feed), P2 (10 mL probiotic/kg feed), and P3 (15 mL probiotic/kg feed), with three replicates per treatment. A total of 240 Nile tilapia fingerlings with an initial average body weight of 10.25 ± 0.48 g were reared for 60 days in fiberglass tanks under controlled culture conditions. The evaluated parameters included final body weight, weight gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Efficiency (FE), Survival Rate (SR), and water quality parameters, including temperature, pH, dissolved oxygen, ammonia, and nitrite. Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$. The results demonstrated that probiotic supplementation significantly improved all evaluated parameters compared with the control treatment. The P2 treatment produced the best performance, with a final body weight of 68.57 g, weight gain of 58.33 g, SGR of $3.15\% \text{ day}^{-1}$, FCR of 1.24, feed efficiency of 80.37%, and a survival rate of 98.33%. Moreover, probiotic application effectively reduced ammonia concentration to 0.11 mg/L and nitrite concentration to 0.05 mg/L, indicating a substantial improvement in water quality. These findings demonstrate that dietary supplementation with 10 mL probiotic/kg feed is the optimal dosage for improving growth performance, feed utilization, fish survival, and environmental conditions in intensive Nile tilapia culture. Therefore, probiotic supplementation represents a sustainable and environmentally friendly strategy for enhancing aquaculture productivity while reducing dependence on antibiotics and synthetic chemical inputs.

Keywords: *Oreochromis niloticus*; probiotics; growth performance; survival rate; water quality.

INTRODUCTION

Aquaculture has become one of the fastest-growing food production sectors worldwide, playing a pivotal role in ensuring global food security, improving nutrition, and supporting rural livelihoods. Among freshwater aquaculture commodities, Nile tilapia (*Oreochromis niloticus*) has emerged as one of the most economically important fish species because of its rapid growth rate, high adaptability to diverse environmental conditions, efficient feed utilization, and strong market demand. The expansion of intensive tilapia farming has significantly contributed to fish production in many developing countries, including Indonesia. However, increasing production intensity is frequently accompanied by deteriorating water quality, elevated stocking densities, disease outbreaks, and reduced production efficiency, all of which threaten the sustainability of aquaculture operations (Food and Agriculture Organization [FAO], 2022; El-Sayed, 2020).

The intensification of aquaculture systems often results in excessive accumulation of organic waste originating from uneaten feed, fish feces, and metabolic by-products. These materials contribute to elevated concentrations of ammonia and nitrite, which adversely affect fish physiology, suppress immune function, and reduce growth performance. Conventional disease management has relied heavily on antibiotics and chemical treatments; however, their prolonged use has raised serious concerns regarding antimicrobial resistance, environmental contamination, and residual compounds in aquatic products. Consequently, environmentally friendly and sustainable management strategies have become increasingly important to maintain productivity while minimizing ecological impacts (Boyd et al., 2020; El-Saadony et al., 2021).

Probiotics have gained considerable attention as one of the most promising biological approaches for improving aquaculture performance. According to the FAO/WHO, probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. In aquaculture, probiotic bacteria such as *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Pseudomonas* species have been widely applied through feed supplementation or water treatment. These beneficial microorganisms improve intestinal microbial balance, stimulate digestive enzyme secretion, inhibit pathogenic bacteria through competitive exclusion, and enhance both innate and adaptive immune responses. In addition, probiotics contribute to the biodegradation of organic matter and improve nutrient cycling within the culture environment, thereby promoting healthier production systems (Hai, 2015; Ringø et al., 2018).

Growth performance is one of the primary indicators of aquaculture productivity because it directly influences production costs and economic returns. Several studies have demonstrated that dietary probiotic supplementation enhances nutrient digestibility, increases feed efficiency, and improves specific growth rate by promoting digestive enzyme activities and optimizing intestinal morphology. Furthermore, probiotics stimulate the synthesis of essential vitamins, amino acids, and other bioactive metabolites that support metabolic processes and tissue development. These physiological improvements ultimately result in better feed conversion efficiency and higher biomass production, making probiotics an attractive alternative to synthetic growth promoters (Merrifield & Ringø, 2014; Dawood et al., 2019).

In addition to promoting growth, probiotics play a significant role in improving fish survival and maintaining water quality. Enhanced immune competence enables cultured fish to better resist pathogenic microorganisms and environmental stressors, leading to lower mortality rates during the production cycle. Simultaneously, probiotic microorganisms participate in the decomposition of organic wastes and the transformation of nitrogenous compounds, reducing toxic ammonia and nitrite concentrations while maintaining favorable dissolved oxygen and pH levels. The synergistic effects of improved fish health and enhanced environmental conditions contribute substantially to the sustainability and profitability of intensive Nile tilapia culture systems (Newaj-Fyzul et al., 2014; Hoseinifar et al., 2018).

Although numerous studies have reported the beneficial effects of probiotics in aquaculture, considerable variation remains regarding probiotic strains, application methods, dosage levels, and environmental conditions, resulting in inconsistent production outcomes. Therefore, further evaluation is required to identify effective probiotic supplementation strategies for Nile tilapia culture under intensive farming conditions. This study aims to evaluate the effects of probiotic supplementation on growth performance, survival rate, and water quality in Nile tilapia (*Oreochromis niloticus*) culture. The findings are expected to provide scientific evidence supporting the adoption of sustainable probiotic-based management practices that improve production efficiency while reducing dependence on antibiotics and other chemical additives. The contribution of this study is to provide experimental evidence on the effectiveness of different probiotic dosages and to identify the dosage that provides the most favorable balance between fish growth, survival, and water quality.

LITERATURE REVIEW

Nile Tilapia (*Oreochromis niloticus*)

Nile tilapia (*Oreochromis niloticus*) is one of the most extensively cultured freshwater fish species worldwide due to its remarkable adaptability to diverse environmental conditions, rapid growth rate, and efficient feed conversion. Originally native to Africa, Nile tilapia has been successfully introduced into tropical and subtropical regions, becoming a major aquaculture commodity because of its high economic value and consumer acceptance. The species exhibits excellent tolerance to fluctuations in temperature, salinity, dissolved oxygen, and water quality, making it highly suitable for both semi-intensive and intensive farming systems. Furthermore, its high reproductive capacity and resistance to environmental stress contribute significantly to sustainable fish production (El-Sayed, 2020).

The productivity of Nile tilapia farming is influenced by several factors, including seed quality, feed management, stocking density, water quality, and disease control. Although intensive culture systems substantially increase production

efficiency, they also elevate the risks of environmental deterioration, physiological stress, and infectious disease outbreaks. Consequently, the implementation of sustainable aquaculture technologies that simultaneously improve fish health and maintain environmental stability has become essential for enhancing production performance and ensuring long-term sustainability (Food and Agriculture Organization [FAO], 2022).

Probiotics in Aquaculture

Probiotics are defined as live microorganisms that provide health benefits to the host when administered in adequate amounts. In aquaculture, probiotics are commonly applied through feed supplementation or direct water application to improve intestinal microbial balance, enhance digestive efficiency, stimulate immune responses, and maintain environmental quality. The probiotic microorganisms most frequently used in fish culture belong to the genera *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Enterococcus*, *Nitrosomonas*, and *Nitrobacter*, all of which have demonstrated beneficial effects on fish health and production performance (Ringø et al., 2018).

The beneficial mechanisms of probiotics involve competitive exclusion of pathogenic bacteria, production of antimicrobial compounds, enhancement of digestive enzyme activity, and modulation of both innate and adaptive immune systems. Moreover, probiotics improve the composition of intestinal microbiota, thereby increasing nutrient digestion and absorption. Compared with antibiotics, probiotic supplementation represents a safer and more sustainable strategy because it does not produce harmful residues in cultured fish or the surrounding environment and does not contribute to antimicrobial resistance (Hai, 2015).

Effects of Probiotics on Fish Growth Performance

Growth performance is one of the primary indicators of aquaculture productivity because it directly affects production efficiency and economic profitability. Fish growth is influenced by genetic characteristics, dietary quality, environmental conditions, physiological status, and metabolic efficiency. Dietary probiotic supplementation enhances the activity of digestive enzymes, including protease, amylase, and lipase, thereby improving the digestion and utilization of proteins, carbohydrates, and lipids. Enhanced digestive efficiency increases nutrient availability for tissue development and overall fish growth (Merrifield & Ringø, 2014).

Numerous studies have reported that probiotic supplementation significantly improves weight gain, Specific Growth Rate (SGR), feed utilization efficiency, and reduces the Feed Conversion Ratio (FCR). In addition, probiotic microorganisms contribute to the synthesis of essential vitamins, amino acids, and various bioactive metabolites that support metabolic activities and physiological development. These combined effects ultimately result in superior growth performance compared with fish cultured without probiotic supplementation (Dawood et al., 2019).

Effects of Probiotics on Survival Rate

Survival rate is a critical indicator reflecting the ability of cultured fish to withstand environmental stress and disease challenges throughout the production cycle. Survival is influenced by environmental conditions, nutritional status, management practices, and the presence of pathogenic microorganisms. Intensive aquaculture systems frequently expose fish to chronic stress due to high stocking densities, which suppress immune responses and increase susceptibility to infectious diseases (Newaj-Fyzul et al., 2014).

Probiotic supplementation has been shown to enhance immune defense mechanisms by stimulating phagocytic cell activity, increasing lysozyme production, promoting immunoglobulin synthesis, and strengthening intestinal mucosal integrity. Furthermore, probiotics produce antimicrobial substances that inhibit the colonization and proliferation of pathogenic bacteria such as *Aeromonas hydrophila* and *Vibrio* spp. Consequently, dietary probiotics effectively reduce mortality and improve the survival rate of cultured Nile tilapia (El-Saadony et al., 2021).

Effects of Probiotics on Water Quality

Water quality is one of the most important environmental factors affecting the success of Nile tilapia culture. Essential water quality parameters include temperature, pH, dissolved oxygen (DO), ammonia, nitrite, nitrate, and organic matter concentration. Poor water quality disrupts fish respiration, metabolism, feed utilization, and growth while increasing physiological stress and disease susceptibility (Boyd et al., 2020).

Probiotic microorganisms play a significant role in degrading organic waste through enzymatic activities that accelerate the decomposition of uneaten feed and fish feces. Moreover, nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* convert toxic ammonia into nitrite and subsequently into the less toxic nitrate, thereby improving the overall water environment. These biological processes contribute to healthier culture conditions, enhanced fish welfare, greater production efficiency, and improved sustainability of intensive aquaculture systems (Hoseinifar et al., 2018).

Conceptual Framework

The conceptual framework of this study is based on the hypothesis that probiotic supplementation improves the balance of beneficial microorganisms in both the gastrointestinal tract and the culture environment. Improved microbial balance enhances nutrient digestion and absorption, strengthens immune responses, and accelerates the biodegradation of organic waste. These physiological and environmental improvements subsequently promote better growth performance, higher survival rates, and superior water quality throughout the culture period. Therefore, evaluating the effectiveness of probiotic supplementation is essential for developing sustainable and environmentally friendly management strategies for intensive Nile tilapia culture.

RESEARCH METHODOLOGY

This study employed an experimental research design with a quantitative approach to evaluate the effects of probiotic supplementation on growth performance, survival rate, and water quality in Nile tilapia (*Oreochromis niloticus*) culture. The experiment was conducted over a 60-day culture period at a freshwater aquaculture facility using a Completely Randomized Design (CRD). Nile tilapia fingerlings with an initial average body weight of 10.25 ± 0.48 g were stocked in **12 fiberglass tanks** with a capacity of **200 L**, each containing **150 L** of freshwater. Twenty fish were randomly distributed into each tank, resulting in a total experimental population of **240 fish**. Before the experiment commenced, all fish were acclimatized for **seven days** to minimize handling stress and allow adaptation to the experimental conditions.

The experiment consisted of four dietary treatments based on probiotic supplementation levels: **P0** (control, without probiotic supplementation), **P1** (5 mL probiotic/kg feed), **P2** (10 mL probiotic/kg feed), and **P3** (15 mL probiotic/kg feed). Each treatment was replicated three times, producing a total of twelve experimental units. The commercial probiotic preparation contained beneficial bacterial strains, including *Bacillus subtilis*, *Lactobacillus plantarum*, and *Bacillus licheniformis*. The probiotic solution was uniformly sprayed onto commercial floating pellets containing **30% crude protein** and air-dried for approximately **30 minutes** before feeding. Fish were fed daily at **3% of their total biomass**, divided into three feeding sessions conducted in the morning, afternoon, and evening.

The evaluated parameters included growth performance, feed utilization, survival rate, and water quality. Growth performance was assessed through final body weight, weight gain, and Specific Growth Rate (SGR), while feed utilization was evaluated using Feed Conversion Ratio (FCR) and Feed Efficiency (FE). Fish survival was determined using the Survival Rate (SR). Water quality parameters consisted of temperature, pH, dissolved oxygen (DO), ammonia (NH₃), nitrite (NO₂⁻), and nitrate (NO₃⁻). Fish body weight and total length were measured every **14 days** using a digital balance with **0.01 g precision** and a measuring board with **1 mm accuracy**. Water quality measurements were performed weekly using portable digital instruments, while ammonia, nitrite, and nitrate concentrations were analyzed following standard laboratory procedures for aquaculture water quality assessment.

Growth parameters were calculated using standard aquaculture formulas. Weight gain was determined by subtracting the initial body weight from the final body weight. Specific Growth Rate (SGR) was calculated based on the natural logarithm of body weight over the culture period. Feed Conversion Ratio (FCR) was determined by dividing the total feed consumed by the total biomass gain, whereas Feed Efficiency (FE) was expressed as the percentage of biomass gain relative to feed intake. Survival Rate (SR) was calculated as the percentage of fish remaining alive at the end of the experiment relative to the initial stocking number. All observations were systematically recorded throughout the experimental period to evaluate the effectiveness of probiotic supplementation on Nile tilapia culture performance.

The collected data were statistically analyzed using **IBM SPSS Statistics version 26.0**. Prior to hypothesis testing, data normality was examined using the **Shapiro–Wilk test**, while homogeneity of variance was evaluated using **Levene's test**. Differences among treatment means were analyzed using **One-Way Analysis of Variance (ANOVA)** at a **95% confidence level ($\alpha = 0.05$)**. Statistical significance was determined at $P < 0.05$, and differences among treatment means were interpreted based on the results of the statistical analysis. When significant differences were detected, **Duncan's**

Multiple Range Test (DMRT) was performed to identify significant differences among treatment groups. The results were subsequently presented in tables and figures to facilitate interpretation of the effects of probiotic supplementation on growth performance, survival rate, and water quality in Nile tilapia culture.

RESULTS AND DISCUSSION

Results and Discussion

Dietary probiotic supplementation for 60 days significantly improved the growth performance of Nile tilapia compared with the control treatment. Fish receiving probiotic-supplemented diets exhibited higher final body weight, greater weight gain, and increased Specific Growth Rate (SGR). Among the treatments, **P2 (10 mL probiotic/kg feed)** produced the best growth performance, whereas increasing the probiotic dosage to **15 mL/kg feed (P3)** did not result in a substantial improvement over P2. These findings suggest that probiotic supplementation has an optimal dosage at which digestive efficiency and metabolic activity are maximized without causing excessive microbial competition within the gastrointestinal tract.

Table 1. Effect of Probiotic Supplementation on the Growth Performance of Nile Tilapia

Parameter	P0 (0 mL/kg)	P1 (5 mL/kg)	P2 (10 mL/kg)	P3 (15 mL/kg)
Initial body weight (g)	10.25 ± 0.31	10.27 ± 0.35	10.24 ± 0.28	10.26 ± 0.30
Final body weight (g)	52.84 ± 2.16	60.92 ± 2.41	68.57 ± 2.84	66.83 ± 2.53
Weight gain (g)	42.59 ± 2.05	50.65 ± 2.27	58.33 ± 2.60	56.57 ± 2.35
Specific Growth Rate (% day ⁻¹)	2.72 ± 0.08	2.94 ± 0.07	3.15 ± 0.06	3.09 ± 0.08
Feed Conversion Ratio (FCR)	1.61 ± 0.07	1.42 ± 0.05	1.24 ± 0.04	1.29 ± 0.05

The enhanced growth performance observed in probiotic-treated fish can be attributed to the increased activity of beneficial microorganisms that produce digestive enzymes such as protease, amylase, and lipase. These enzymes improve the digestion and absorption of dietary nutrients, enabling fish to utilize feed more efficiently while reducing metabolic energy losses. The lowest Feed Conversion Ratio (FCR) recorded in treatment P2 indicates superior feed utilization efficiency, allowing a greater proportion of consumed feed to be converted into body biomass. Similar findings have been reported in previous studies demonstrating that probiotics improve nutrient digestibility by maintaining a balanced intestinal microbiota and enhancing digestive physiology.

Besides improving growth performance, probiotic supplementation also exerted a positive effect on fish survival throughout the experimental period. The highest mortality was observed in the control treatment, where fish were more susceptible to environmental stress and fluctuations in water quality. In contrast, probiotic-treated groups exhibited considerably higher survival rates, indicating that probiotics not only improve nutrient utilization but also strengthen physiological resilience against disease challenges and adverse environmental conditions.

Table 2. Effect of Probiotic Supplementation on Survival Rate and Feed Efficiency

Parameter	P0	P1	P2	P3
Survival Rate (%)	88.33 ± 2.89	93.33 ± 1.67	98.33 ± 1.67	96.67 ± 1.67
Feed Efficiency (%)	62.18 ± 2.40	70.45 ± 2.16	80.37 ± 2.25	77.84 ± 2.11
Mortality (fish)	7	4	1	2

The increased survival rate observed in probiotic-supplemented fish is likely associated with enhanced immune responses and improved intestinal health. Beneficial bacteria inhibit pathogenic microorganisms through competitive exclusion, bacteriocin production, and stimulation of innate immune mechanisms. Furthermore, probiotics reinforce intestinal mucosal integrity, reducing the likelihood of pathogen colonization and infection. Consequently, probiotic supplementation provides dual benefits by simultaneously increasing fish productivity and improving overall health status during intensive culture.

Another important finding of this study was the improvement in water quality following probiotic application. Throughout the experimental period, all measured water quality parameters remained within the acceptable range for Nile

tilapia culture. Nevertheless, probiotic-treated tanks exhibited lower concentrations of ammonia and nitrite than the control treatment, indicating a more favorable aquatic environment for fish growth and survival.

Table 3. Water Quality Parameters During the Experimental Period

Parameter	P0	P1	P2	P3	Optimum Range
Temperature (°C)	28.4	28.5	28.6	28.5	26–30
pH	7.32	7.38	7.41	7.40	6.5–8.5
Dissolved Oxygen (mg/L)	5.18	5.64	6.11	5.95	>5
Ammonia (mg/L)	0.28	0.19	0.11	0.14	<0.20
Nitrite (mg/L)	0.12	0.08	0.05	0.06	<0.10

The reduction in ammonia and nitrite concentrations demonstrates that probiotic microorganisms effectively accelerated the decomposition of organic wastes and enhanced the nitrification process within the culture system. Improved water quality minimized physiological stress, promoted normal feeding activity, and created more favorable conditions for fish growth. Additionally, the higher dissolved oxygen concentration observed in probiotic treatments indicates a healthier aquatic environment with reduced organic matter accumulation and lower oxygen demand for microbial decomposition.

Overall, the results clearly demonstrate that probiotic supplementation substantially improves the biological performance of Nile tilapia cultured under intensive conditions. Among all treatments, **P2 (10 mL probiotic/kg feed)** was identified as the optimum dosage, producing the highest growth performance, the lowest Feed Conversion Ratio, the greatest feed efficiency, a survival rate of **98.33%**, and superior water quality. These findings indicate that appropriate probiotic supplementation represents an effective and environmentally sustainable strategy for enhancing aquaculture productivity while reducing dependence on antibiotics and other synthetic chemical inputs.

CONCLUSION

The findings of this study demonstrate that probiotic supplementation significantly enhances the culture performance of Nile tilapia (*Oreochromis niloticus*). Fish receiving probiotic-supplemented diets exhibited superior growth performance, as reflected by higher final body weight, greater weight gain, increased Specific Growth Rate (SGR), and improved feed utilization compared with the control treatment. These results indicate that probiotics enhance nutrient digestion and metabolic efficiency, thereby promoting more effective biomass production.

Probiotic supplementation also significantly improved the survival rate of Nile tilapia throughout the 60-day culture period. The higher survival observed in probiotic-treated groups is likely associated with enhanced immune responses, improved intestinal microbial balance, and greater resistance to environmental stress and pathogenic microorganisms. Consequently, probiotics contribute not only to improved production performance but also to better fish health and welfare.

In addition to their physiological benefits, probiotics positively influenced water quality by reducing ammonia and nitrite concentrations while maintaining suitable levels of temperature, pH, and dissolved oxygen. These improvements created a more stable aquatic environment, minimized physiological stress, and supported optimal fish growth and survival under intensive culture conditions.

Among the evaluated treatments, supplementation with **10 mL probiotic/kg feed (P2)** proved to be the most effective dosage. This treatment produced the highest growth performance, the lowest Feed Conversion Ratio (FCR), the greatest feed efficiency, the highest survival rate, and the best overall water quality. Increasing the probiotic dosage beyond this level did not result in additional improvements, indicating the existence of an optimal supplementation level for Nile tilapia culture.

Overall, probiotic supplementation can be considered an effective, environmentally friendly, and sustainable management strategy for intensive Nile tilapia farming. The application of probiotics has considerable potential to enhance aquaculture productivity while reducing dependence on antibiotics and synthetic chemicals. Future studies should investigate different probiotic strains, combinations of beneficial microorganisms, longer culture periods, and various farming systems to further optimize probiotic application in commercial Nile tilapia production.

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