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Comparative Evaluation of Different Commercial Probiotics on Growth Performance and Survival *Oreochromis niloticus* Fry

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Abstract

Nile tilapia (*Oreochromis niloticus*) is an important freshwater aquaculture commodity with strong development potential in Indonesia. Increasing market demand has encouraged farmers to improve production efficiency; however, several constraints remain, including aquaculture waste, low feed digestibility, and high feed costs One approach that has been widely studied is the use of feed additives such as probiotics, which have the potential to improve nutrient digestibility, feed utilization efficiency, growth performance, and health status of cultured aquatic organisms. This study aimed to evaluate the effect of different commercial probiotics applied in feed on the growth performance and survival of Nile tilapia fry. The experiment was conducted for 40 days using a Completely Randomized Design with three treatments and three replications: P0 (feed without probiotics), P1 (feed supplemented with a commercial probiotic containing *Bacillus subtilis* $\geq 10^6$ CFU/mL at a dose of 15 mL/kg feed), and P2 (feed supplemented with a commercial probiotic containing *Lactobacillus casei* $\geq 10^6$ CFU/mL and *Saccharomyces cerevisiae* $\geq 10^6$ CFU/mL at a dose of 20 mL/kg feed). The results showed that treatment P2 produced the best outcomes and differed significantly ($P<0.05$) from the other treatments. This treatment resulted in higher survival (94.43±7.35%), specific growth rate (8.44±0.30 %/day), average daily gain (5.32±0.86 g), and daily length gain (2.08±0.27 cm/day), along with improved feed conversion ratio (1.26±0.07), feed efficiency (81.34±0.36%), and protein efficiency ratio (8.53±0.36). These findings suggest that the application of the probiotic used in treatment P2 can improve feed utilization and support better growth and survival of Nile tilapia fry under culture conditions.

Keywords: bacteria, growth, Nile tilapia, probiotic, survival rate

I. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a growing freshwater aquaculture commodity and plays a vital role in Indonesia's aquaculture sector. Demand for tilapia in Indonesia is projected to reach 1.36 million tons in 2023 and increase to 1.38 million tons in 2024. Demand for tilapia is also increasing in the global market. This is reflected in the export value of tilapia in Indonesia, which reached USD 79 million in 2022 and increased to USD 82 million in 2023 (KKP, 2024). High demand in both local and global markets has prompted increased domestic tilapia production. These efforts still face several challenges, particularly related to feed efficiency (Vulla *et al.*, 2026). Feed remains one of the major constraints in tilapia aquaculture, accounting for approximately 30–70% of total production costs, thereby encouraging the development of strategies

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to improve feed utilization efficiency (Prameswari *et al.,* 2025; Langa *et al.,* 2026). Strategies are needed to increase feed efficiency without compromising fish growth performance. One approach that has been widely studied is the use of feed additives such as probiotics, which have the potential to increase the digestibility and utilization of feed nutrients by fish (Mohammed *et al.,* 2026).

Probiotics are live microorganisms that can provide benefits to the host by maintaining the balance of microbes in the digestive tract. Probiotics have also been reported to improve environmental quality, suppress opportunistic pathogenic bacteria, and enhance microbiological stability in aquaculture systems (Kurniawinata *et al.,* 2026). Probiotics are known to play a role in improving fish health, immune responses, digestive processes, and nutrient absorption more efficiently (Hisham *et al.,* 2026). Furthermore, probiotics have also been reported to suppress the growth of pathogenic microorganisms, including Aeromonas hydrophila bacteria, which often cause disease in farmed fish (Moustafa *et al.,* 2026). The use of probiotics is a safer alternative to antibiotics because they do not leave residues that could potentially harm human health or the environment (Vijayaram *et al.,* 2026). Therefore, the use of probiotics in feed is increasingly being implemented as a strategy to increase production success in aquaculture systems (Umasugi *et al.,* 2018).

Various commercial probiotic products are currently available and used by fish farmers in Indonesia with different microorganism compositions. Some contain lactic acid bacteria such as *Lactobacillus* sp. and the yeast *Saccharomyces cerevisiae*, which are known to improve digestive activity and balance the intestinal microbiota (Meilinasyiffa *et al.,* 2026). There are also probiotics containing bacteria from the Bacillus genus, such as *Bacillus subtilis* and *Bacillus megaterium*, which are known to produce various digestive enzymes and play a role in improving fish growth and immunity (Demirhan-Yazıcı *et al.,* 2025). Differences in microbial composition in these probiotics are thought to provide different responses to fish growth performance and survival rates (Fayed *et al.,* 2025). Based on this, this study was conducted to analyze the effect of different commercial probiotic applications in feed on the growth and survival of tilapia fry.

II. MATERIALS AND METHODS

2.1. Preparation of Grow Out Site

The experiment was conducted for 40 days and used a Completely Randomized Design (CRD) consisting of three treatments and three replications. The treatments were the addition of different commercial probiotics to the fish feed. The control treatment (P0) was feeding without the addition of probiotics. P1 was the feeding treatment with the addition of commercial probiotics containing *Bacillus subtilis* $\geq 10^6$ CFU/mL at a dose of 15 mL/kg (Hasan *et al.,* 2023), while P2 was the feeding treatment with the addition of commercial probiotics containing *Lactobacillus casei* $\geq 10^6$ CFU/mL and *Saccharomyces cerevisiae* $\geq 10^6$ CFU/mL at a dose of 20 mL/kg (Lestari *et al.,* 2024). The feeding trial was conducted for 40 days with a stocking density of 40 fish/m^2.

2.2 Rearing Procedure

2.2.1 Tank preparation

The maintenance tanks consisted of nine fiber tanks measuring 1.5 m in diameter and 0.3 m in height. The entire interior of the fiber tanks was cleaned using a sponge and then dried for one day. The fiber tanks were filled with clean water to a height of 25 cm, or a total volume of 112.5 L. The water was allowed to settle for 24 hours before being used for the test research. The aeration installation in each fiber tank consisted of an air stone and an aeration hose.

2.2.2 Fish seed preparation

The Nile tilapia (*O. niloticus*) fry used were 5–6 cm in size. They were sourced from the Cibalagung Fisheries Research Institute in Bogor, West Java. The fry were healthy, free of defects, and active. The fry were first acclimatized for 30 minutes. The fry were first acclimatized seven days prior to the experiment.

2.2.3 Fish feeding

The test feed used was commercial micropellets measuring 0.5–0.7 mm with a protein content of 39–41%. Feed was provided using the restricted method with a feeding rate of 5% per day. Feed preparation began by weighing the amount of feed needed using a digital scale. The probiotics used in each treatment were dissolved in distilled water at a dosage ratio of 1:1, after which the probiotic solution was mixed with the weighed feed. The feed was stirred evenly and allowed to stand for 15 minutes. Feeding was carried out

three times daily, at 8:00 AM, 1:00 PM, and 4:00 PM.

2.2.4 Water quality management

A 30% water change was performed twice a week. The water change technique was carried out simultaneously with the siphoning process and cleaning the tank with a sponge. Water quality parameters observed during the test maintenance included temperature, dissolved oxygen, pH, and ammonia. Temperature and dissolved oxygen parameters were measured using a YSI Pro 20 Dissolved Oxygen Meter (in-situ), pH using a LAMOTTE pH-5 Plus (in-situ), and ammonia using a Spectrophotometer (ex-situ). Observations of in-situ parameters (temperature, dissolved oxygen, and pH) were carried out at 07.00 AM and 04.00 PM, while ex-situ parameters (ammonia) were carried out once every 10 days.

2.3 Research Parameters

2.3.1 Growth performance

The growth parameters observed during the test maintenance were calculated using the formula survival rate (%) = Nt / No × 100 [Nt = Number of final fish population (fish); No = Initial fish population (fish)], specific growth rate (%/day) = ((ln Wt - ln Wo) / t) × 100 [Wt = fish weight at time t (g); Wo = fish weight at the beginning of the study (g); t = maintenance time (days)], average daily growth (g/day) = (Wt – Wo) / t [Wt = final weight of the test fish (g); Wo = initial weight of the test fish (g); t = interval period (days)], daily length gain (cm/day) = (Lt – Lo) / t [Lt = fish length at time t (cm); Lo = fish length at the beginning of the study (cm); t = nursery period (days)], feed conversion ratio = (F / ((Wt + D) - Wo) [F = amount of feed consumed (g); Wt = final fish biomass (g); Wo = initial fish biomass (g); D = fish mortality biomass (g)], and feed efficiency (%) = ((Wt + D) – Wo) / F) × 100 [Wt = final fish biomass (g); Wo = initial fish biomass (g); F = amount of feed consumed (g); D = fish mortality biomass (g)].

2.3.2 Bacterial abundance

Bacterial density in the probiotics and intestinal organs of the test fish was calculated using the Total Plate Count (TPC) method. Observations were made on D1 before treatment and D40 after treatment. A 0.01 g intestinal sample was collected from one representative fish per treatment, then crushed and diluted with 900 µL of PBS (Phosphate-Buffered Saline) solution, homogenized using a vortex, and serial dilutions were performed up to 10^8. At dilution levels of 10^6, 10^7, and 10^8, 25 µL of the sample was spread on TSA (Tryptic Soy Agar) media, then incubated at room temperature for 24 hours until bacterial colonies appeared.

The counting procedure for probiotics was carried out using similar steps: diluting the probiotic sample with PBS, homogenizing it, and plating it on TSA media at dilutions of 10^{-4}, 10^{-5}, and 10^{-6}, followed by 24 hours of incubation. The growing bacterial colonies were counted manually, and then a formula was used to determine the total bacterial abundance (CFU/g) = (average number of colonies / (dilution × spread volume).

2.3.3 Proximate analysis

Proximate analysis is carried out to identify the nutritional content of feed, including protein testing using the Kjeldahl method, fat testing using the Soxhlet method, carbohydrate testing using the Anthrone method, ash content testing using the ashing method, crude fiber analysis using acid-base treatment, and water content testing using the oven method (Janna *et al.,* 2022).

2.3.4 Business analysis

The analysis of the rearing test business is calculated using the formula total cost (IDR) = fixed costs + variable costs, depreciation (IDR) = (initial value – final value) / economic life, revenue (IDR) = production quantity × selling price, income (IDR) = revenue – total costs, r/c ratio = revenue / total costs, break-even point (IDR) = (fixed costs + variable costs) / production, and break-even point (unit) = (fixed costs + variable costs) / price.

2.4 Data analysis

Growth performance data and business analysis were tabulated in Microsoft Excel. Production performance data were analyzed using SPSS version 30.0 using the ANOVA method. Data with significant differences were further tested using Duncan's Multiple Range Test (DMRT) with a 95% confidence interval. Water quality, bacterial abundance, and proximate analysis data were analyzed descriptively.

III. RESULT

3.1 Growth Performance

The growth performance of nile tilapia (*O. niloticus*) nursery stock is presented in Table 1. The survival rate and specific growth rate of tilapia fry in the P2 treatment were significantly different from all other treatments ($P<0.05$). The specific growth rate and

survival rate were highest in the P2 treatment, reaching 8.44±0.30%/day and 94.43±7.35%, respectively. The results showed that the P2 treatment produced significantly different average daily gain and daily length growth values compared to the other treatments (P<0.05). The highest average daily gain and daily length gain values were achieved by P2, at 2.08±0.27 cm/day and 5.32±0.86 g/day, respectively.

The feed conversion ratio and feed efficiency in the P2 treatment were significantly different from the other treatments (P<0.05). Treatment P2 achieved the lowest feed conversion ratio (1.26±0.07), and the highest feed efficiency (81.34±0.26%). The protein efficiency ratios differed significantly between treatments (P<0.05). The highest feed efficiency ratio was achieved in treatment P2 at 8.53±0.36, while the lowest was achieved in treatment P0 at 5.74±0.24.

3.2 Bacterial Abundance

Based on the results of the TPC test on the intestines of tilapia, there were differences in bacterial abundance in each treatment during the 40-day test period. Bacterial abundance at H0 was 1.24 × 10^8 CFU/g for each treatment. An increase in bacterial abundance was only identified in the P2 treatment after 40 days of test maintenance, reaching 2.48 × 10^{10} CFU/g. The bacterial abundance in the intestines of tilapia, as seen in the TPC test, is shown in Table 2.

Bacterial observations showed that the same bacteria were identified in treatments P0 and P1, namely *Staphylococcus* sp., which is characterized by a coccus shape, gram-positive, non-motile, catalase-positive, and fermentative. In treatment P2, bacteria were presumptively identified as *Lactococcus* sp., characterized by a coccus shape, gram-positive, non-motile, catalase-negative, and fermentative. The bacterial identification results are listed in Table 3.

3.3 Proxymate Analysis

The results of the proximate analysis of the feed for each treatment showed a relative decrease in protein, ash, and carbohydrate content. An increase in fat and water content was observed for treatments P1 and P2 compared to P0. The proximate test results are presented in Table 4.

Table 1. Growth performance of tilapia (*O. niloticus*) nursery with the addition of different commercial probiotics to feed for 40 days of maintenance

Parameter	Treatments		
	P0	P1	P2
SR (%)	84.70±2.80^a	91.63±8.46^a	94.43±7.35^b
SGR (%/day)	5.03±0.98^a	7.31±1.06^b	8.44±0.30^c
ADG (g/day)	4.07±0.60^a	4.44±0.26^a	5.32±0.86^b
DLG (cm/day)	1.65±0.05^a	1.88±0.11^a	2.08±0.27^b
FCR	1.45±0.09^a	1.44±0.15^a	1.26±0.07^b
FE (%)	66.00±7.55^a	73.33±2.08^a	81.34±0.26^b
PER	5.74±0.24^a	6.79±0.73^b	8.53±0.36^c

Notes: Means in the same row with different superscripts differ significantly (P<0.05) at 95% confidence interval (DMRT); SR = survival rate, SGR = specific growth rate, ADG = average daily gain, DLG = daily length gain, FCR = feed conversion ratio, FE = feed efficiency, PER = protein efficiency ratio.

Table 2. Total abundance of bacteria in the intestines of tilapia (*O. niloticus*) during 40 days of culture

Treatment	Day	
	0 (CFU/g)	40 (CFU/g)
P0	1.24 × 10^8	1.12 × 10^8
P1	1.24 × 10^8	1.00 × 10^9
P2	1.24 × 10^8	2.48 × 10^{10}

3.4 Water Quality

Water quality data for the 40-day test culture period are presented in Table 5. Data for each parameter indicate values exceeding the recommended limits. However, the culture medium still supports tilapia production performance. This is evidenced by the high growth rate and survival rate of tilapia fry (Table 5).

3.5 Business Analysis

Analysis of Nile tilapia cultivation using different commercial probiotics showed varying results (Table

Table 3. Growth performance of tilapia (*O. niloticus*) nursery with the addition of different commercial probiotics to feed for 40 days of maintenance

Treatment	Treatments						
	Form	Gram	Motile	Indole	catalase	O/F	Genus
P0	Coccus	+	-	-	+	F	*Staphylococcus* sp.
P1	Coccus	+	-	-	+	F	*Staphylococcus* sp.
P2	Coccus	+	-	-	-	F	*Lactococcus* sp.

Table 4. Proximate test results of feed for tilapia (*O. niloticus*) nursery

Content	Treatments		
	P0	P1	P2
Protein (%)	40.50	40.22	40.29
Fat (%)	3.48	3.90	3.52
Ash (%)	10.25	9.36	8.42
Water (%)	6.24	17.71	14.05
Crude fiber (%)	0.38	0.51	0.12
Carbohydrate (%)	39.15	28.30	33.6
Total (%)	100	100	100

Table 5. Water quality of tilapia (*O. niloticus*) nursery during 40 days of culture

Parameter	Treatments			Optimal value*
	P0	P1	P2	
Temperature (°C)	24.6–28.3	24.6–28.3	24.5–28.4	20.0–30.0
pH	5.50−6.95	5.19−7.02	5.40−7.42	6.5–9.0
DO (mg/L)	3−7.60	3−7.40	3−7.42	>5.0
Ammonia (mg/L)	0.02−0.39	0.04−0.39	0.02−0.37	<0.025

Note: *Bhatnagar and Devi (2019)

Table 6. Analysis of tilapia (*O. niloticus*) nursery business with the application of different commercial probiotics to feed

Parameter	Treatments		
	P0	P1	P2
Total cost (IDR)	16,344,060	16,729,660	16,775,260
Revenue (IDR)	31,987,200	32,223,296	35,033,600
Profit (IDR)	15,643,140	15,493,636	18,258,340
R/C	1.96	1.93	2.09
Payback period	1.77	1.78	1.51
BEP (unit)	2875	2935	2943
BEP (Rp)	2,279,889	2,382,840	2,395,305
Cost of production (Rp/unit)	793	812	814

6). The highest profit was obtained in treatment P2 with a value of Rp18,258,340, while the lowest profit was obtained in treatment P1 with a value of Rp15,493,636. The R/C ratio for each treatment showed profitable results, with the highest value being 2.09 in treatment P2 and the lowest being 1.93 in treatment P1.

IV. DISCUSION

The application of different commercial probiotics to the feed affected the growth and survival of Nile tilapia (*O. niloticus*) fry. This is thought to be because the combination of *Lactobacillus casei* and *Saccharomyces cerevisiae* microbes in P2 is able to increase feed digestibility through enzymatic activity that helps break down complex nutrients into simpler forms that are more easily absorbed by the fish's digestive tract (Zhoua *et al.,* 2026). Lactic acid bacteria such as *Lactobacillus casei* are known to have proteolytic activity that can break down proteins into peptides and amino acids. *Lactobacillus casei* also plays a role in lowering the pH of the digestive tract, thereby supporting the activity of endogenous digestive enzymes (Zhang *et al.,* 2025). These conditions allow for more efficient feed utilization, which ultimately contributes to improved fish growth and physiological performance. Nutrients absorbed through the intestines will be distributed through the circulatory system to body tissues and play a role in increasing the metabolism and immune response of fish, thus supporting more optimal growth (Dawood, 2021).

High survival rate in P2 treatment is thought to be related to the role of lactic acid bacteria, which are able to inhibit the growth of pathogenic bacteria in the digestive tract through the production of antimicrobial compounds such as lactic acid, bacteriocins, and hydrogen peroxide (Loh *et al.,* 2017). This mechanism helps maintain the balance of the gut microbiota and improves fish health during cultivation. The results of this study are consistent with several previous studies that reported that the use of *Lactobacillus* sp.-based probiotics can increase the survival rate of cultured organisms (Fadri *et al.,* 2016; Telaumbanua *et al.,* 2023; Sutrisno *et al.,* 2026). The high survival rate proves that the microbial composition and the appropriate probiotic dosage play a crucial role in improving cultivation performance.

In contrast, treatment P1 containing *Bacillus subtilis* and *Bacillus megaterium* bacteria did not significantly improve growth performance or survival compared with the control. Bacillus sp. actually has the ability to produce digestive enzymes such as protease, amylase, and lipase, which play a role in the degradation of nutrient macromolecules (Ezraneti *et al.,* 2018). However, its effectiveness can be influenced by the microbial composition, the dose given, and the ability of the bacteria to colonize the fish's digestive tract (Ge *et al.,* 2025). According to Mariadi *et al.,* (2022), *Lactobacillus* sp.-based probiotics at certain doses can provide a better growth response than Bacillus sp.-based probiotics. This is thought to be related to the stability

of the intestinal microbiota and the fermentative activity of lactic acid bacteria.

Feed efficiency also showed a similar pattern to growth, where the P2 treatment resulted in a lower Feed Conversion Ratio (FCR) value and a higher feed efficiency and protein efficiency ratio. Feed fermented with Bacillus sp. and administered throughout the 78-day rearing period improved fish performance, resulting in a final body weight of 28.6–41.7 g/fish, survival rates of 80.87–98.87%, a specific growth rate of 2.7%/day, and a feed conversion ratio of 0.95 (Pratiwi *et al.,* 2020). In contrast to the results of research conducted by Usman (2025), the application of commercial probiotics (a combination of *Lactobacillus* cassei $\geq 10^6$ CFU/mL and *Saccharomyces cerevisiae* $\geq 10^6$ CFU/mL) at a dose of 20 mL/kg in the cultivation of catfish (Clarias sp.) seeds resulted in an FCR of 1.50. The low FCR value indicates that the feed provided can be utilized more effectively by the fish for biomass growth. This increase in feed efficiency is related to the activity of probiotics in improving the process of nutrient biosynthesis and feed digestibility in the digestive tract. Functionally, feed quality is better when combined with probiotics at the right dose (Adibrata *et al.,* 2026).

The results of the analysis of bacterial abundance in the intestines also supported these findings, with the P2 treatment showing the highest bacterial count at 2.48×10^{10} CFU/g. This indicates that the microorganisms derived from the probiotics are able to adapt and thrive in the digestive tract of tilapia. The presence of stable bacterial colonies in the intestines plays a crucial role in the digestive process, competing with pathogenic microbes, and maintaining the balance of the gut microbiota (Deng *et al.,* 2022). The presence of lactic acid bacteria such as *Lactococcus* sp. indicates a more favorable gut microbiota condition because these bacteria are able to produce organic acids and bacteriocins that can suppress the growth of pathogenic microorganisms and support the health of the fish's digestive tract (Huang *et al.,* 2024).

In addition to probiotics, water quality conditions during the study also played a role in supporting fish growth performance. The water quality in each treatment decreased over time. While water quality occasionally fell outside the optimal range, this was still tolerable for tilapia and did not significantly impact their appetite. This finding echoes the research findings of Rahman *et al.,* (2025) on eel (Anguilla bicolor bicolor) nursery farms, which found that water quality declines beyond optimal limits still supported fish survival and growth.

The business analysis showed that treatment P2 was the most profitable compared to the other treatments, with the highest profit of Rp18,258,340, a R/C ratio of 2.09, and a shorter payback period of 1.51. This shows that the use of commercial probiotics containing *Lactobacillus* cassei ≥10zzzzzzzzzz CFU/mL and *Saccharomyces cerevisiae* $\geq 10^6$ CFU/mL at a dose of 20 mL/kg feed not only improves the biological performance of tilapia but also provides economic benefits in cultivation activities.

V. CONCLUSION

The application of commercial probiotics in feed significantly enhanced the growth and survival of Nile tilapia (*O. niloticus*) fry. The optimal performance was achieved through a supplementation dosage of 20 mL/kg, which synergistically utilized *Lactobacillus casei* and *Saccharomyces cerevisiae* ($\geq 10^6$ CFU/mL) to optimize digestive efficiency and stabilize intestinal microbiota. Consequently, this probiotic intervention represents an effective strategy for maximizing aquaculture productivity and ensuring the robust development of tilapia fry.

CONFLICT OF INTEREST

We certify that there are no conflicts of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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