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GROWTH AND SURVIVAL OF TILAPIA FISH (Oreochromis mossambicus) WITH THE PROVISION OF PROBIOTIC ISOLATES OF PAKOBA FRUIT (Syzygium luzonense)

Nadia Patiung[1*], Helen Joan Lawalata[1], Livana Detris Rawung[1], Johanna Zusye Wantania[1], Rievo Handry Djarang[1], Masje Wurarah[1], Christny Ferdina Evie Rompas[1]

[1]Biology Departement, Faculty of Matehematics,Natural, and Earth Science, Universitas Negeri Manado, Indonesia.

**Corresponding author:* nadiapatiung@gmail.com

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Abstract

Tilapia (*Oreochromis mossambicus*) is a freshwater fish that is extensively cultured because of its strong adaptability to fluctuating environmental conditions and its relatively rapid growth performance. Current strategies to enhance fish growth increasingly involve the incorporation of biological feed supplements, particularly probiotics. This research was designed to evaluate the influence of probiotic isolates Lactiplantibacillus plantarum derived from pakoba fruit (*Syzygium luzonense*) on the specific growth rate (SGR), length increment, and survival rate of tilapia. The study applied a Completely Randomized Design (CRD) consisting of four treatments with five replications each: P0 (control), P1 (10 mL/kg feed), P2 (20 mL/kg feed), and P3 (30 mL/kg feed). Observed parameters included SGR, absolute length growth, and survival rate. The results demonstrated that Probiotic supplementation significantly influenced both SGR and survival rate ($p < 0.05$), with treatment P3 (30 mL/kg feed) yielding the most favorable outcomes for both parameters. In contrast, the data for length growth were normally distributed and subsequently analyzed using one-way ANOVA. The analysis revealed that probiotic supplementation did not produce a statistically significant effect on the length growth of tilapia ($p > 0.05$).

Key words: Growth, Oreochromis mossambicus, Probiotics, Survival, Syzygium luzonense, Water Quality.

INTRODUCTION

Tilapia, particularly Mozambique tilapia (Oreochromis mossambicus), is widely cultivated due to its adaptability to diverse environmental conditions, efficient feed utilization, and relatively fast growth. These characteristics allow the species to be reared in multiple aquaculture systems, from traditional earthen ponds to intensive systems with high stocking densities. Nevertheless, the intensification of aquaculture frequently results in environmental stress, reduced water quality, and increased susceptibility to infectious diseases. Elevated stocking density and organic waste accumulation may alter key water parameters such as dissolved oxygen and ammonia, which negatively affect fish physiology and immune competence. Chronic stress conditions are known to impair growth performance and survival, thereby threatening

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production sustainability (FAO, 2022; Limbu et al., 2022). Therefore, effective fish health management has become a central component of sustainable aquaculture development. Poor feed conversion not only limits growth but also increases feed costs, which represent the largest operational expense in aquaculture.Therefore, the application of probiotics as beneficial biological agents offers a viable solution.

Probiotic microorganisms are living organisms that can positively impact the health and growth of their hosts. Their primary role is to maintain biological balance and help restore the host's health and nutritional needs. Furthermore, probiotics play a crucial role in improving water quality by decomposing compounds such as ammonia, nitrite, nitrate, and other organic matter, and by suppressing the growth of blue-green algae. The use of probiotics is an alternative solution in controlling pathogenic microorganisms, because it can work through the mechanism of nutrient competition, production of antibacterial compounds, and improving the host's immune system, thus providing a safer protective effect than antibiotics (Newaj-Fyzul et al., 2014; Ringø et al., 2018).

In addition to their direct benefits, probiotics also have the potential to improve water quality in aquaculture ponds by reducing ammonia and nitrite levels. According to research by Pratama et al. (2013), probiotics function by breaking down organic matter in water, ultimately reducing pollution and improving water quality. This provides benefits for fish health while supporting sustainable aquaculture practices. Although the use of probiotics in fish farming has been extensive, most research still focuses on commercial probiotics or commonly used bacteria. The development of probiotics based on local natural ingredients is still relatively limited. The use of probiotics based on natural ingredients is expected to be an alternative in reducing dependence on antibiotics in aquaculture activities. Probiotic microorganisms contribute to maintaining the balance of intestinal microflora, improving nutrient absorption, and enhancing the immune competence of cultured fish (Hai, 2015). One of the potential probiotic bacteria is Lactiplantibacillus plantarum, a member of the lactic acid bacteria group known for its ability to produce antimicrobial substances, including organic acids and bacteriocins, which can suppress the proliferation of pathogenic microorganisms. In addition, this bacterium has been reported to promote digestive processes, improve feed utilization, and increase the resistance of aquatic organisms to stress and disease challenges (Ringø et al., 2018). These characteristics support the potential application of Lactiplantibacillus plantarum as a probiotic candidate to enhance the growth and health performance of Nile tilapia.

Pakoba fruit is a local biological resource known to contain potential compounds and microorganisms. Research conducted by(Lawalata et.al.,2023) found that pakoba fruit contains various bioactive components that have the potential to support the growth and activity of beneficial microorganisms. However, this study was limited to identifying the contents of pakoba fruit and did not examine the use of probiotic isolates from pakoba fruit in aquaculture applications, particularly as a fish feed mixture.Therefore, their potential role in environmental management and fish health enhancement requires experimental verification.

Despite the increasing use of probiotics in aquaculture, most commercial products are based on

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widely distributed or imported bacterial strains. The exploration of locally isolated probiotic bacteria, particularly in tropical regions, remains limited. Indigenous bacterial isolates may offer better adaptability to local environmental conditions and culture systems. Several bacterial genera commonly associated with plant environments, such as Bacillus and lactic acid bacteria, have been reported to improve growth performance enhance immune responses, and contribute to water quality management in tilapia culture. However, scientific evaluation of locally isolated bacterial strains in Oreochromis mossambicus culture systems is still limited. Therefore, the identification and assessment of indigenous probiotic bacteria are essential to support sustainable and cost-effective aquaculture practices (Dawood et al., 2020; El-Saadony et al., 2021; Kuebutornye et al., 2020).

Based on this background, the present study aimed to evaluate the effectiveness of probiotic isolates derived from pakoba fruit (Syzygium luzonense) when incorporated into feed on the growth performance and survival rate of tilapia (Oreochromis mossambicus) during the rearing period.

RESEARCH METHODS

Location and Research Period

This research was conducted from September to October 2025 and the experiment was conducted over a 30-day period in the Laboratory of the Biology Department, Faculty of Mathematics, Natural Sciences and Earth Sciences, Universitas Negeri Manado

Experimental Design

This research was an experimental study using a Completely Randomized Design (CRD) consisting of four treatments with five replications. These treatments included:

Table 1. Treatment groups of tilapia fish *(Oreochromis mossambicus)*

Group	Treatment
PO	Normal control or without probiotics
P1	Administration of probiotics at a concentration of 10 mL/kg feed
P2	Administration of probiotics at a concentration of 20 mL/kg feed
P3	Administration of probiotics at a concentration of 30 mL/kg feed

Experimental Fish

The experimental fish used in this study were Mozambican tilapia *(Oreochromis mossambicus),* taken from the Tatelu Freshwater Aquaculture Center with an average initial body weight of 3–5 g and a total length of 3–5 cm. Each experimental unit consisted of four fish stocked in a 10 L plastic bucket containing 7 L of settled well water. Fish were fed commercial pellets twice daily at 07:00 and 17:00 WITA. The feeding rate was set at 3% of the total biomass per day.

Probiotic Bacteria

The probiotic used in this study was *Lactiplantibacillus plantarum*, a lactic acid bacterium isolated

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from *Syzygium luzonense* (Lawalata et.al .,2023 ;Lawalata et.al.,2025).

Research Procedure

All rearing containers were first cleaned with detergent and rinsed thoroughly. The containers were then filled with water and sterilized using 10 ppm chlorine solution (Body, 1990). The water was left for four days without aeration to ensure complete sterilization. After this period, the sterilized water was discarded, and each container was refilled with 7 L of settled fresh well water. An aeration system was installed in each container to maintain adequate dissolved oxygen levels. The aeration equipment was also pre-soaked in 10 ppm chlorine solution before use. Four tilapia juveniles were stocked into each bucket. The fish were first acclimated and then reared for a period of 30 days. Feeding was performed twice a day at 3% of total biomass (NCR, 2011). Water was replaced every three days prior to feeding. At each water renewal, the feed was supplemented with probiotic suspension based on the respective treatment dosage before being given to the fish.

During the experiment, observations on growth and survival were carried out regularly, while water quality was consistently managed to maintain optimal and stable culture conditions.

The specific growth rate (SGR) of tilapia was measured by weighing the fish every seven days using an analytical balance. The growth rate was calculated using the formula (Muchlisin et al., 2016):

$$\text{SGR } (\%) = \frac{(Ln\ Wt - Ln\ Wo)}{} \times 100\%$$

Description:

SGR: Specific Growth Rate

Ln Wo: Average initial weight of fish at the start of cultivation (grams)

Ln Wt: Average final weight of fish after cultivation (grams)

T: Length of cultivation period (days)

The growth of fish length was measured every seven days using a ruler. Growth was calculated using the formula (Effendie, 1997).

$$P = Lt\text{-}Lo$$

Description:

P = Length growth (cm)

Lt = Average fish length at the end of the rearing period (cm)

Lo = Average fish length at the beginning of rearing (cm)

The number of dead fish was recorded daily, and the survival rate was calculated using the formula. (Muschlisin et.al., 2017)

$$\text{KH} = \frac{(No - Nt)}{No} \times 100$$

Description:

KH: Survival rate (%)

Nt: Number of fish that died during the rearing period (fish)

No: Number of fish at the start of rearing (fish)

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The observed water quality parameters included temperature, pH, TDS, and ammonia. Temperature was measured using a thermometer, pH was measured using a pH meter, TDS was measured using a TDS meter, and ammonia was measured using the *EBI* NH_3 - NH_4 Ammonia Water Test Kit.Measurements of temperature, pH, and TDS were taken daily before feeding, while ammonia was measured before each water replacement.

Data Analysis

The data on growth performance, survival rate, and physicochemical water quality parameters were subjected to Analysis of Variance (ANOVA) to evaluate the effect of probiotic supplementation derived from pakoba fruit on tilapia (*Oreochromis mossambicus*). When significant differences among treatments were observed, further analysis using a post hoc test was performed to determine specific differences between treatment groups.

RESULTS AND DISCUSSION

The results of measurements of specific growth rate, fish length, survival rate, and water quality are presented in tabular form

Specific Growth Rate

The specific growth rate (SGR) of tilapia fish with the administration of probiotic isolates *Lactiplantibacillus plantarum* of pakoba fruit *(Syzygium luzonense)* is presented in Table 2.

Table 2. Average Specific Growth Rate (SGR) of tilapia *(Oreochromis mossambicus)*

Treatment	SGR
	30 Days of Probiotics
P0	4,13 ± 0,99 (a,b)
P1	3,62 ± 0,48 (a)
P2	4,80 ± 0,62 (b,c)
P3	5,14 ± 0,12 (c)

Description: PO (Control), P1 (Probiotic 10 mL/kg feed), P2 (Probiotic 20 mL/kg feed), P3 (30 mL/kg feed). Descriptive values (mean ± standard deviation). Values in the same column followed by the same superscript letter are not significantly different ($p > 0.05$)).

Fish Length

The length (P) of tilapia fish given probiotic isolate *Lactiplantibacillus plantarum* of pakoba fruit *(Syzygium luzonense)* is presented in Table 3.

Table 3. Average Fish Length (P) of tilapia *(Oreochromis mossambicus)*

Treatment	P
	30 Days of Probiotics
PO	12,5 ± 2,98
P1	12,4 ± 4,50
P2	18,5 ± 5,34
P3	20,9 ± 3,14

Description: PO (Control), P1 (10 mL/kg feed), P2 (20 mL/kg feed), P3 (30 mL/kg feed)

Survival Rate

Survival rate (KH) of tilapia fish with probiotic isolate *Lactiplantibacillus plantarum* of pakoba fruit (*Syzygium luzonense)* is presented in Table 4.

Table 4. Average Survival Rate (KH) of tilapia *(Oreochromis mossambicus)*

Treatment	KH 30 Days of probiotic
PO	50 ± 17,6 (a,b)
P1	35 ± 13,6 (a)
P2	50 ± 17,6 (a,b)
P3	60 ± 13,6 (b)

Description: PO (Control), P1 (10 mL/kg feed), P2 (20 mL/kg feed), P3 (30 mL/kg feed). Descriptive values (mean ± standard deviation). Descriptive values (mean ± standard deviation)

Water Quality

The water quality parameters in the maintenance media for tilapia fish *(Oreochromis mossambicus)* with the provision of probiotic isolates *Lactiplantibacillus plantarum* of pakoba fruit *(Syzygium luzonense)* are presented in Table 5.

Table 5. Range of Water Quality Parameters in Tilapia Fish *(Oreochromis mossambicus)* Maintenance Media During Maintenance.

Treatment	TDS (ppm)	pH	Temperatur (ºC)	NH_3 (mg/l)
PO	124-281	7,02-8,12	21,8-22,7	0,1- (>0,2)
P1	121-256	6,06-8,20	20,9-22,7	0,1- (>0,2)
P2	121-281	7,03-8.20	21,8-22,3	0,1- (>0,2)
P3	118-243	7,09-8,23	20,9-22,7	0,1- (>0,2)
Recommended Range (References)	<1000***	6,5-8,5**	25-30*	<0,20*

Description : * : Amri *et al*. (2005)

** : Pramleonita *et al*.(2018)

*** : PP No.82 (2001)

Discussion

Spesific Growth Rate

Based on the analysis of the Specific Growth Rate (SGR) of tilapia reared for 30 days and treated with pakoba fruit probiotic isolates, the highest SGR was recorded in treatment P3 (30 mL probiotic), reaching 5.14 ± 0.12. In contrast, the lowest SGR was observed in treatment P1 (10 mL), with a value of 3.62 ± 0.48. These findings indicate that higher probiotic dosages contribute to a significant improvement in the growth performance of tilapia. According to Saraswati et al. (2020), incorporating probiotic isolates into fish feed enhances growth by improving nutrient utilization efficiency and maintaining the balance of intestinal microflora in fish.

The increased growth of fish fed probiotic isolates is influenced by the activity of these microorganisms in degrading organic matter and producing digestive enzymes. This has an impact on increasing feed utilization efficiency. According to Widanarni et al. (2018), probiotic microbes produce protease, amylase, and lipase enzymes that accelerate the process of breaking down nutrients into forms that are easily absorbed by the fish's body.

In this study, the probiotic isolate Lpb. Plantarum producing Exopolysaccharides (EPS) isolated from pakoba fruit (Syzygium luzonense) (Lawalata et al., 2023; Lawalata et al., 2025), showed that Pakoba

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fruit has functional potential as a probiotic agent that can provide benefits to the host organism (Data not yet published). One of the main roles of EPS is to increase the ability of bacteria to attach and combine with the surface of the intestinal mucosa, so that probiotic bacteria can survive longer in the fish digestive system (Yang et al., 2023). In addition, exopolysaccharides are known to function as a protective layer that helps bacteria face unfavorable environmental conditions such as changes in pH, environmental stress, or the presence of certain compounds in the digestive tract. This protection allows probiotic bacteria to remain active so that they can help the process of nutrient breakdown and increase the efficiency of feed utilization (Liu et al., 2025). By using this isolate in tilapia fish feed, enzyme activity and colonization of beneficial intestinal microbiota are thought to increase, thus directly accelerating the specific growth rate (SGR).

The difference in SGR values between treatments also indicates that increasing the appropriate probiotic dosage can balance the microbial community in the digestive tract, helping fish better utilize feed and maintaining their overall health. Too high a probiotic dosage can cause an imbalance in the gut microflora. Ringo et al. (2010) found that excessive probiotic administration can inhibit growth due to competition between microorganisms in the fish's intestines. Therefore, optimal dosages, such as those in treatment P3, are considered highly effective in increasing the growth of tilapia.Overall, the results of this study demonstrate that the administration of probiotic isolates in feed significantly affects the specific growth rate of tilapia.

Fish Length

The results of the study showed that the average body length of tilapia fish increased with the addition of the probiotic dose of Lpb. plantarum in the feed. The control treatment (P0) had the lowest average length of 12.15 ± 2.98 cm, while the treatment with the highest dose (P3) reached 20.9 ± 3.14 cm. This increase indicates that the probiotic isolate Lpb. plantarum mixed into the feed is able to create more optimal physiological conditions for the growth of fish structures. The administration of probiotics such as Lactobacillus has been reported to enhance intestinal villi length, width, and absorptive surface area (Ringo et al., 2016; Salton et al., 2016; Yassine et al., 2021). Furthermore, probiotic supplementation in fish feed has been shown to positively influence growth performance, intestinal microbiota composition, immune response, and resistance against pathogenic microorganisms.

The ANOVA results for body length data indicated that different probiotic dosages incorporated into the feed did not produce a statistically significant effect ($P > 0.05$) on the linear growth of tilapia during the 30-day rearing period. This means that increasing the probiotic dose to a certain level has not been able to produce a statistically significant difference in the linear growth of the fish. However, the increasing effect remains clearly visible from P0 to P3, indicating that the probiotics have a positive biological effect, although not yet statistically significant. This phenomenon is thought to be due to the relatively short maintenance period not being sufficient to show significant changes in length growth, which generally occurs slower than weight growth. Research by Rahman et al. (2020) also found similar results, indicating

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that probiotic administration to tilapia had no significant effect on body length (p>0.05) but showed a tendency to increase in the treatment with the highest dose.

The increase in fish body length due to the administration of the probiotic isolate Lpb. plantarum is primarily associated with increased efficiency of nutrient utilization and metabolic activity in the fish's body. Probiotics play a role in increasing the number of beneficial bacteria such as Lactobacillus which can produce digestive enzymes (Protease, Lipase and Amylase). The activity of these enzymes accelerates the digestion process and nutrient absorption which are then used for the formation of elongated muscle and bone tissue. This mechanism is in accordance with the explanation of Oliveira et al., (2023) who found that administering probiotics to tilapia in a biofloc system increased digestive enzyme activity and improved feed ratio, so that more energy can be directed to body growth.

Length growth occurs through the gradual development of bone and cartilage tissues, a process that progresses more slowly than weight gain. Consequently, within a 30-day rearing period, changes in body length may not yet reach statistical significance, even though an increasing trend is observable. Sirbu et al. (2022) also reported that body length parameters generally require a longer period to exhibit significant responses to nutritional treatments compared to body weight, as the formation of structural tissues occurs at a slower rate than muscle tissue accumulation.

Survival Rate

The survival rate data demonstrated clear variation among treatments. The highest survival was observed in P3 (60 ± 13.6%), whereas P1 showed the lowest value (35 ± 13.6%). Treatments P0 and P2 presented similar survival rates of 50 ± 17.6%. The normality test indicated $p < 0.05$, meaning that the survival data were not normally distributed. Therefore, a non-parametric Kruskal–Wallis test was applied. The analysis yielded $p < 0.05$, indicating statistically significant differences among treatments. These results indicate that probiotic doses affect survival in a tiered manner. The highest dose (P3) appears to provide an adaptive advantage for fish, while the lowest dose (P1) even lowers survival compared to the control treatment. This aligns with the dose-response principle often reported in fish probiotic studies: a minimum dose threshold is required for colonization and immunomodulatory effects, and doses below this threshold are sometimes not only ineffective but also temporarily disrupt the microbiota balance (Zabidi et al., 2021).

The increase in survival in the P3 treatment is thought to be related to the modulation of the non-specific immune system by probiotics, for example increased lysozyme activity and plasma antibacterial activity which strengthens the body's defense against infection. Certain probiotics, such as Bacillus sp., have also been reported to stimulate the expression of immune-related genes and reinforce intestinal epithelial integrity, thereby improving the fish’s resistance to pathogenic challenges (Shija et al., 2023). Although probiotics have been reported to influence immune responses and intestinal microbial balance, these mechanisms were not directly assessed in the current study. Consequently, the proposed explanations remain hypothetical and are based on findings from previous research. Additional

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investigations involving immunological and microbiological analyses are needed to confirm the mechanisms underlying the observed responses.In addition to direct immunological effects, probiotics may indirectly benefit fish by improving water quality. Certain probiotic microorganisms have the ability to lower ammonia and nitrite levels through microbial activity, which helps reduce environmental stress and supports higher survival rates. Therefore, the role of the environment in interpreting these results is equally important, as treatment with a high-dose probiotic (P3) may benefit both immune and environmental pathways (Nashiroh et al., 2024). The result that treatment P1 showed the lowest survival (35 ± 17.6%) compared to the control indicates that a probiotic dose that is too low may not provide optimal protection. In some studies, suboptimal doses can actually cause temporary imbalances in the microbiota because the probiotic has not reached a stable colonization level or competes less effectively with commensal/pathogenic microbiota. This explains why P1 can be worse than the control under certain conditions. Previous research has shown that the benefits of probiotics are highly dependent on the minimum effective dose and environmental conditions (Waiyamitra et al., 2020).

Water Quality

Water quality is a critical factor in aquaculture practices, as cultured species and other aquatic organisms depend entirely on the aquatic environment for survival. Therefore, water conditions must be continuously monitored and properly managed to maintain stability. In intensive or closed culture systems with high stocking densities, water quality may deteriorate due to the accumulation of uneaten feed and fish metabolic waste (Hapsari et al., 2020).In this study, the evaluated water quality parameters included Total Dissolved Solids (TDS), acidity (pH), temperature, and ammonia concentration. Throughout the experimental period, water temperature ranged from 20.9 to 22.7 °C. The pH values varied between 6.6 and 8.23, TDS levels ranged from 118 to 281 ppm, and ammonia concentrations were recorded between 0.1 and >2.0 mg/L.

During the 30-day experimental period, TDS values ranged from 118 to 281 ppm. Referring to the study on water quality fluctuations in Mozambique tilapia (Oreochromis mossambicus) Ismail (2025), TDS levels below 1000 ppm are considered suitable for freshwater aquaculture. These results indicate that the rearing environment remained within acceptable conditions and that probiotic supplementation did not lead to excessive accumulation of dissolved residues beyond normal thresholds.

The temperature recorded during the rearing period was relatively consistent across all treatments, ranging from 20.9°C to 22.7°C, which is slightly below the recommended optimal temperature range for tilapia culture, Amri et al., (2005).Extreme temperatures can induce stress in fish and may ultimately lead to mortality. The lower temperature values recorded during the study were likely associated with prevailing climatic conditions and the geographic setting of the research area. According to Hendratta et al. (2024), the Tondano region experiences consistent rainfall throughout the year, with mean monthly temperatures ranging between 21.9°C and 22.5°C.

Ammonia levels observed in this study varied from 0.1 mg/L to more than 2.0 mg/L. The accumulation of ammonia was presumably linked to leftover feed and fish metabolic waste, and this condition may have been further intensified by the absence of a filtration system during the experimental period. In intensive aquaculture systems with high stocking densities, the buildup of organic waste is recognized as a primary contributor to water quality deterioration (Hapsari et al., 2020).In addition, elevated ammonia levels may have been influenced by the relatively high stocking density and the comparatively low environmental temperatures, which can reduce the activity of nitrifying bacteria responsible for ammonia conversion. Daniel & Negeswar. (2017).,Robles-Porchas et al. (2020) stated that probiotics can stimulate increased nitrifying microbial activity in biofloc systems. This increased microbial activity helps suppress ammonia accumulation, although the degree of change did not show a statistically significant difference.

CONCLUSION

Feeding enriched with the probiotic isolate Lpb. plantarum isolated from pakoba fruit affected the growth performance and survival rate of tilapia (Oreochromis mossambicus). Increasing the probiotic dose tended to be followed by an increase in the specific growth rate (SGR) and survival percentage, with a dose of 30 mL showing the best results. However, the treatment did not significantly affect the length increase of the fish. These findings indicate that the Lpb. plantarum isolate from pakoba fruit has the potential to be developed as a natural probiotic source in tilapia cultivation, especially in supporting increased production performance and fish survival as well as an alternative that supports more sustainable aquaculture practices.

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