Effects of water-applied commercial *Lactobacillus rhamnosus* on growth performance, feed efficiency, and survival of *Oreochromis niloticus* (Linnaeus, 1758) fingerlings

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Abstract. Probiotics are beneficial microbes that are being studied more in aquaculture, as they offer an environmentally sustainable alternative to antibiotics while improving fish growth and feed efficiency. The present study investigated the effects of water-applied commercial *Lactobacillus rhamnosus* on the growth performance, feed efficiency, and survival rate of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fingerlings. The experiment ran for 60 days of culture from September 07 to November 05, 2025, and was conducted at the Multi-Species hatchery, Bongao, Tawi-Tawi, Philippines. A total of 450 *O. niloticus* fingerlings were randomly distributed in 15 tanks. Fish were fed proportionally at 10% of their average body weight (ABW) twice daily. The experiment used a completely randomized design which included three replications and five treatments that tested different *L. rhamnosus* concentrations through control: 0 mg L^{-1}; T1: 1 mg L^{-1}; T2: 2 mg L^{-1}; T3: 3 mg L^{-1}; T4: 4 mg L^{-1}. Data were analyzed using JASP Version 20, with normality and homogeneity tested. The results show that specific growth rate (SGR), weight gain (WG), and total length gain (TLG) were significantly increased at 4 mg L^{-1} of *L. rhamnosus* ($p < 0.05$). Whereas feed efficiency and survival rate did not differ significantly across the treatments ($p > 0.05$). A quadratic regression estimated that the optimum concentration level for maximizing SGR was 3.63 mg L^{-1}. Water-applied *L. rhamnosus* improved tilapia growth, which may indicate its potential as a water additive in aquaculture systems.

Keywords: feces, feed intake, Nile tilapia, optimum inclusion, probiotic.

Introduction. Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is one of the most economically important freshwater fish species globally (Munguti et al., 2022), valued for its rapid growth (Omasaki et al., 2017), adaptability to culture conditions (Santos et al., 2013), and high nutritional quality (Ibrahim et al., 2022). In the Philippines, it serves as a primary source of affordable animal protein (El-Sayed & Fitzsimmons, 2023) and a key commodity for small-scale and commercial aquaculture (Jamandre et al., 2011). Tilapia ranked 5th among the top ten produced fisheries commodities by volume in 2023, with a total production of 307.878.28 MT and accounting for 8.9% of the total production value (Bureau of Fisheries and Aquatic Resources, 2023). Due to its wide distribution and stable populations status, the species is classified as Least Concern on the IUCN Red List (Rusdianto et al., 2025).

While *O. niloticus* culture has shown promising growth and contributed to the global food supply (Ashouri et al., 2023), the aquaculture industry still faces challenges that could affect its long-term sustainability (Samaddar, 2022), such as disease outbreaks, environmental degradation (Munguti et al., 2022), and overuse of antibiotics (Ntakirutimana et al., 2025). Antibiotics are frequently used in intensive aquaculture operations as prophylactic and therapeutic agents to control and prevent bacterial infections (Romero et al., 2012). However, their excessive use has led to the presence of antimicrobial-resistant microorganisms, contributing to environmental pollution and food safety concerns (Cabello, 2006; Santos & Ramos, 2018). Handling, high stocking densities, and varying water quality are some of the stressors in aquaculture related to the culture process that can negatively impact fish (Portz et al., 2006; Zhang et al., 2025).

Lactobacillus rhamnosus is a Gram-positive, lactic acid-producing bacterium widely recognized as a safe and effective probiotic strain (Shah et al., 2024), with established use in both terrestrial and aquatic animal production (Amenyogbe, 2023). It has been implemented in aquaculture as an environment-friendly approach to improve fish health and culture performance (Dawood et al., 2016a). Its importance lies in its ability to inhibit pathogenic bacteria including *Aeromonas* and *Streptococcus* through the production of organic acids and bacteriocins, while simultaneously stimulating the immune response and reducing disease risk (Ringo et al., 2018). In particular, *L. rhamnosus* has been reported to benefit cultured species by promoting intestinal microbiota stability, defense against pathogens, and enhanced growth and development performance (Liang et al., 2023).

However, despite this proven potential, most studies on water-applied probiotics focused largely on multi-strain commercial mixtures or *Bacillus* species in *O. niloticus*, *Centropomus undecimalis*, and *Clarias* sp. (Tarnecki et al., 2019; Naiel et al., 2022; Afriansyah et al., 2025). These reports also use inconsistent dosing units, including mg L^{-1}, ppm, g m^{-3}, and volumetric rates per tank-hindering cross study comparison and standardized field use (Larasati et al., 2021; Amin et al., 2023; Monier et al., 2023). Notably, no published work has yet evaluated water applied single-strain commercial *L. rhamnosus* for *O. niloticus* fingerlings, leaving this promising approach unvalidated. We hypothesized that water-applied of commercially available probiotic capsules containing *L. rhamnosus* would significantly improve growth performance, feed utilization efficiency, and survival rate of *O. niloticus* fingerlings compared to an untreated control.

Therefore, this study aimed to evaluate the effects of water-applied commercial *L. rhamnosus* on the growth, feed efficiency, and survival rate of *O. niloticus* fingerlings. The findings may contribute positively to the development of sustainable probiotic-based management method that supports a healthier and more productive tilapia culture.

Material and Method

Study area. The present study was conducted at the Multi-Species Hatchery, College of Oceanography, Fisheries, Environmental Science, and Technology (COFEST), Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Bongao, Tawi-Tawi, Philippines (05°02'13.71'' N 119°44'34.98" E). The feeding experiment was observed over a period of 60 days, from September 07, 2025, to November 05, 2025.

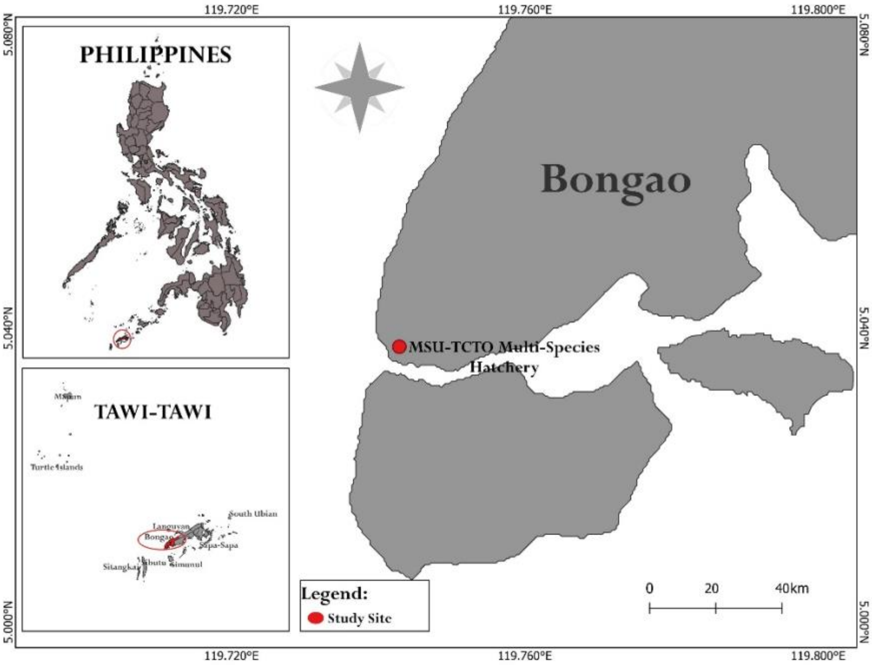

Figure 1. Map Location of the study site (QGIS Version 3.0).

Conditioning and stocking. Healthy *O. niloticus* fingerlings of similar size were collected from the Sanga-Sanga swamp located in Bongao, Tawi-Tawi, Philippines, using a standard seine net. Fish were transported to the Multi-Species Hatchery by motorbike in oxygenated plastic bags that were securely sealed to prevent oxygen loss and water leakage during transport. The transportation time was approximately 10 minutes. Upon arrival, the sealed bags were placed in a concrete tank for 20 minutes of acclimation, depending on temperature and water conditions. This was followed by a 15-day conditioning period, during which fish were gradually released into the water column to adapt to experimental conditions. The fish were then distributed into 20L capacity plastic experimental tanks supplied with continuous aeration. The study used a Completely Randomized Design (CRD) with five treatments, each replicated three times. A total of 450 fingerlings were stocked in 15 tanks at a density of 30 fish per tank.

Probiotic preparation. A commercially available probiotic capsule containing *Lactobacillus rhamnosus* GG (LGG) was used as the probiotic source. The product was manufactured by i-Health, Inc., Cromwell, Connecticut, USA. According to the manufacturer, each capsule contained guaranteed minimum of 1×10^{10} colony-forming units (CFU) of *L. rhamnosus* GG and 200 mg inulin. The capsule contents were removed and dissolved directly in the rearing water following the method of Larasati et al. (2021) and Monier et al. (2023), with minor modifications. Based on the manufacturer, each milligram of probiotic product was estimated to contain 5×10^7 CFU. Experimental treatments were therefore prepared at 0, 1, 2, 3, and 4 mg L^{-1}, respectively (Table 1).

Table 1

Concentration of probiotic

Treatment	*Dose (mg L^{-1})*	*CFU L^{-1}*	*CFU L^{-20} Tank*
Control	0	0	0
T1	1	5×10^7	1×10^9
T2	2	1×10^8	2×10^9
T3	3	1.5×10^8	3×10^9
T4	4	2×10^8	4×10^9

Note: T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water; CFU: colony-forming units.

Figure 2. Experimental set-up. Completely Randomized Design (CRD) showing treatments with varying doses of *Lactobacillus rhamnosus* in 20L culture tanks.

Feeding regime. Fish were fed twice daily at 10% average body weight (ABW). Before feeding, water was gently siphoned to remove feces and uneaten feed; the removed water was collected, then filtered five (5) times using filter cloth (100-150 μm) to ensure the quality of the water before gradually returned to the tank to maximize the usage of water-applied commercial *L. rhamnosus*. Every 7 days, 50% of the water was replaced to maintain water quality, and the corresponding half-dose of probiotic was replaced to maintain the intended concentration in the water. Collected feces were dried under sunlight and were weighed using a digital weighing scale with an accuracy of 0.5 grams. Physico-chemical water parameters such as salinity, temperature, and pH were measured using a 9-in-1 pH meter (TDS, EC).

Growth measurements. Sampling for growth was conducted every 15 days throughout the 60-day experimental period. The total length of individual fish was measured using a standard digital Vernier caliper and measuring board, while body weight was recorded using a digital weighing scale with an accuracy of 0.5 g. The parameters were computed using the following formula:

Growth Performance was evaluated using the following equations (Ricker, 1979; Hopkins, 1992):

$$SGR\ (\%\ day) = In\ Wf - In\ Wi/DOC \times 100$$

Where:
Wi - initial weight/length
Wf - final weight/length
DOC - days of culture
In - natural log

$$\text{Weight gain} = \text{Final weight} - \text{Initial weight}$$

Feed efficiency was calculated using the following equations (Aanyu et al., 2020):

$$\text{Feed conversion efficiency} = \text{Weight gain} / \text{Feed intake}$$

$$\text{Feed conversion ratio} = \text{Feed intake} / \text{Weight gain}$$

Survival rate was calculated using the given formula (Bautista-Teruel et al., 2016):

$$\text{Survival rate} = (\text{Total number of species} / \text{Initial number of species stock}) \times 100$$

Data analysis. The study used JASP version 20 to analyze the data. Data normality and homogeneity of variance were assessed prior to ANOVA using Shapiro-Wilk and Levene's tests, respectively. Post Hoc (Duncan) tests were performed to determine whether there were significant differences among the treatments.

Results

Growth performance. Fish exposed to 4 mg L^{-1} and 1 mg L^{-1} of *L. rhamnosus* exhibited significantly higher specific growth rate (SGR) compared to the control group ($p < 0.05$), while 2 mg L^{-1} and 3 mg L^{-1} of *L. rhamnosus* were intermediate and not statistically different from any group ($p > 0.05$). Weight gain (WG) showed the same trend. Total length gain (TLG) was significantly higher in 4 mg L^{-1} of *L. rhamnosus*, than in the control group, 1 mg L^{-1}, and 2 mg L^{-1} ($p < 0.05$), but not statistically different from 3 mg L^{-1} ($p > 0.05$).

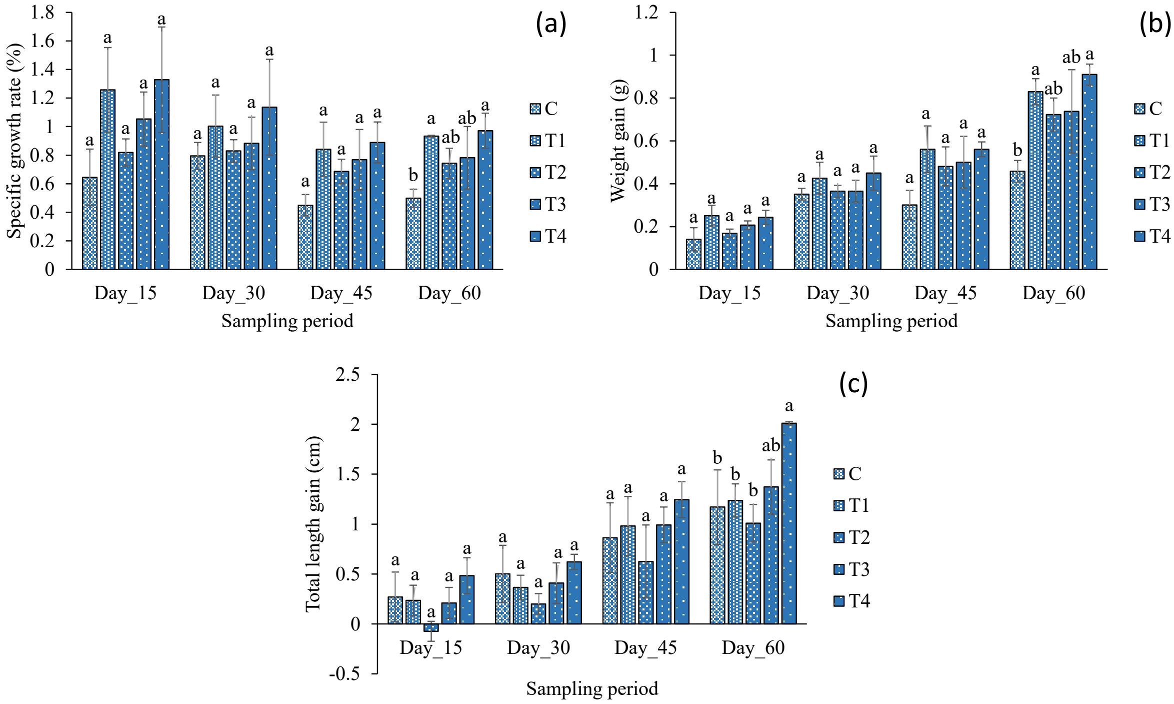

Figure 3. Growth performance of *Oreochromis niloticus* culture fed with varying concentrations of probiotic *Lactobacillus rhamnosus.* Experiment C: 0 mg L^{-1}, T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water.

Feed efficiency. Feed intake (FI), feed conversion ratio (FCR), feed conversion efficiency (FCE), and feces production did not differ significantly among fish exposed to 0 (control), 1, 2, 3, or 4 mg L^{-1} *L. rhamnosus* at any sampling period during the 60-day experiment ($p > 0.05$; Figure 4). FI increased progressively with culture duration in all treatments, whereas FCR generally showed a declining trend over time. Although fish receiving *L. rhamnosus* tended to exhibit lower FCR and slightly higher FCE values than the control group, these differences were not statistically significant. Likewise, feces production remained comparable among treatments throughout the study.

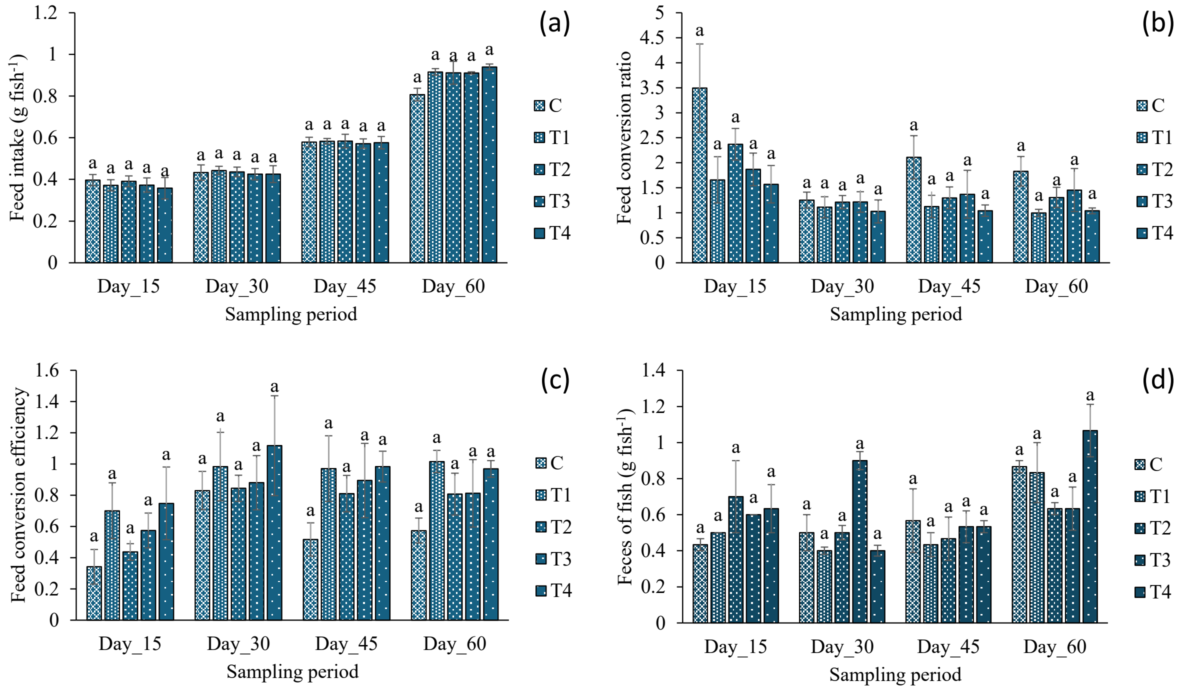

Figure 4. Feed efficiency of *Oreochromis niloticus* culture fed with varying concentrations of probiotic *Lactobacillus rhamnosus.* Experiment C: 0 mg L^{-1}, T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water.

Estimated optimum concentration level. The fitted regression model indicates a non-linear (quadratic) relationship between probiotic concentration and specific growth rate, described by the equation: $y = -0.0243x^2 + 0.1766x + 0.5868$. The positive linear coefficient and negative quadratic coefficient indicate that SGR increases with increasing probiotic concentration up to a certain point. The coefficient of determination ($R^2 = 0.5149$) indicates that approximately 51.49% of the variation in SGR may be explained by differences in probiotic concentration. Based on the vertex of the quadratic equation, the estimated optimum concentration level for maximizing SGR was at 3.63 mg L^{-1}.

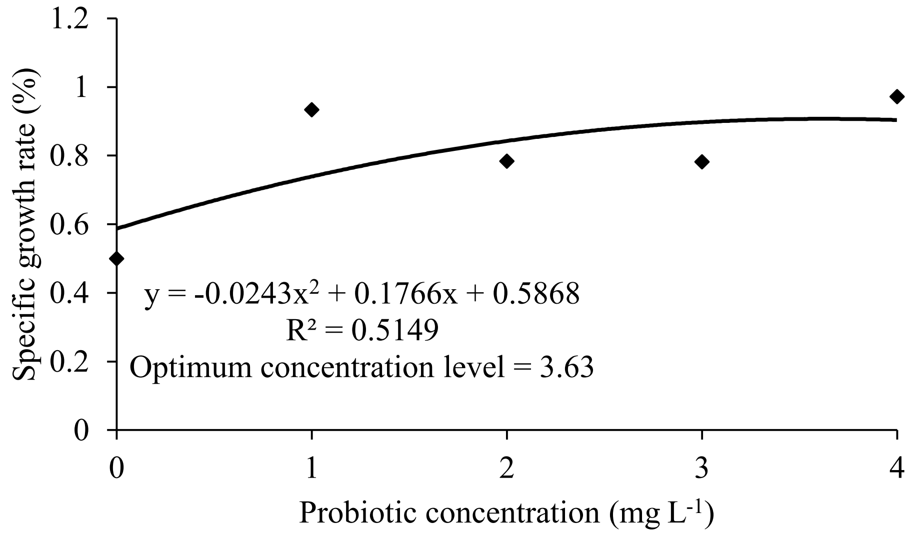

Figure 5. Estimated optimum concentration level of *Oreochromis niloticus* culture fed with varying concentrations of probiotic *Lactobacillus rhamnosus.* Experiment C: 0 mg L^{-1}, T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water.

Survival rate and water parameters. The survival rate of *O. niloticus* cultured with varying concentrations of *L. rhamnosus* such as control group: 0 mg L^{-1}, T1: 1 mg L^{-1}, T2: 2 mg L^{-1}, T3: 3 mg L^{-1}, and T4: 4 mg L^{-1} of *L. rhamnosus* were showed no significant difference throughout the treatments ($p > 0.05$) after 60 days of culture period. The water parameters such as temperature, pH, and salinity remained within acceptable and optimal ranges for Nile tilapia culture (Table 2). No extreme fluctuations were observed among treatments. The highest ammonia level was recorded in the control group (5.33±0.77) and 1 mg L^{-1} (5.67±0.51); however, it lowered numerically as *L. rhamnosus* concentration increased, reaching the lowest value at 3 mg L^{-1} (1.61±0.31).

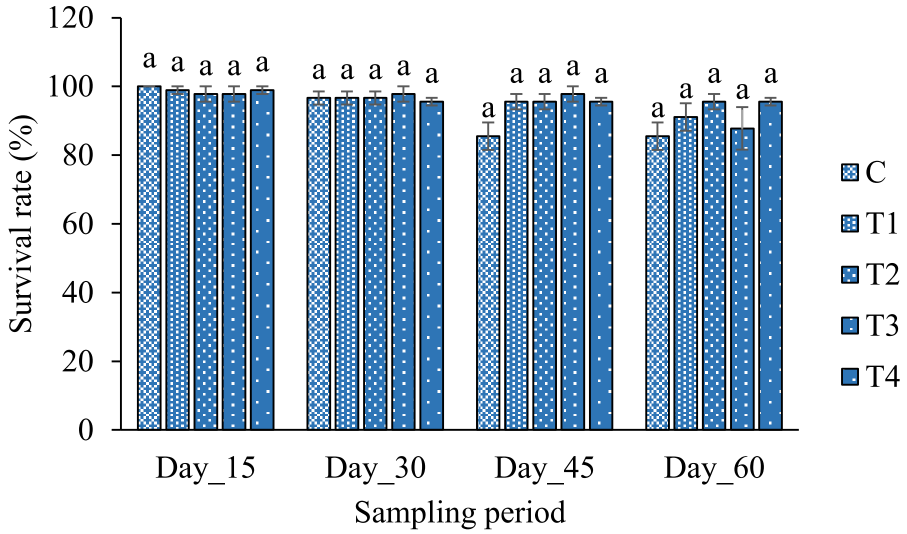

Figure 6. Survival rate (%) of *Oreochromis niloticus* culture fed with varying concentrations of probiotic *Lactobacillus rhamnosus.* Experiment C: 0 mg L^{-1}, T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water.

Table 2

Water parameters (Means±SD) during the 60-day trial of *Oreochromis niloticus* in varying concentrations of probiotic *Lactobacillus rhamnosus* in cultured water

Treatments	*Temp (%)*	*pH*	*Salinity (g L^{-1})*	*Ammonia (mg L^{-1})*
C	28.78±0.06	7.70±0.06	0.07±0.00	5.33±0.77
T1	28.72±0.07	7.71±0.03	0.08±0.02	5.67±0.51
T2	28.78±0.03	7.79±0.03	0.07±0.01	3.56±1.60
T3	28.74±0.04	7.77±0.05	0.07±0.01	1.61±0.31
T4	28.75±0.07	7.71±0.02	0.07±0.00	3.72±1.60

Note: C: 0 mg L^{-1}, T1: 1 mg L^{-1} water, T2: 2 mg L^{-1} water, T3: 3 mg L^{-1} water, and T4: 4 mg L^{-1} water.

Discussion. The present study showed that *O. niloticus* fingerlings exposed to water-applied *L. rhamnosus* at a concentration of 4 mg L^{-1} exhibited higher SGR, WG, and TLG. The higher SGR and WG observed in the probiotic-treated group may be associated with improved intestinal microbial balance and enhanced digestive processes. Similar to the previous studies of Haraz et al. (2023), it is suggested that supplementation of probiotics may enhance the growth of fish under controlled application rates. The growth improvements observed in probiotic-treated groups may be attributed to the interaction of probiotics with the intestinal microbiota (Zuo et al., 2019). Bacteria like *Lactobacillus* spp. can be beneficial to help maintain a balanced microbial gut community, control harmful pathogens, and increase the production of digestive enzymes (Fachri et al., 2024). These attributes may enhance the breakdown of dietary components, increase absorption of nutrients across the intestinal epithelium, and improve energy metabolism, which may provide a greater proportion of consumed nutrients to be directed to the growth rather than maintenance (Abdel-Tawwab et al., 2008; Aly et al., 2008). Similar to the result of greater TLG recorded in higher probiotic treatments, this may result in enhanced skeletal and somatic development associated with improved nutrient assimilation (Ringø et al., 2010). Previous studies have shown that probiotic supplementation supports normal intestinal development and microbial balance, which may regulate more efficient utilization of dietary proteins, lipids, and minerals needed for growth (Ringø et al., 2010; Dawood et al., 2016b). These findings may provide a plausible biological explanation for the increase in body size that was observed in the present study.

Although no significant differences were observed in feed efficiency indices, probiotic-treated groups showed a tendency towards lower FCR values, suggesting a possible improvement in nutrient utilization. This trend agrees with the previous study reporting that *Lactobacillus reuteri* changed the composition of digestive enzyme activity, promotes nutrient retention, improve gut health, and microbiota (Li et al., 2022). The study results showed that probiotics do not alter tilapia feeding patterns because they, with enhance nutrient absorption rather than increase FI (Kuebutornye et al., 2020). The probiotics, which include *Lactobacillus* spp. help animals use their feed more efficiently by producing digestive enzymes and boosting intestinal enzyme production (Zuo et al., 2019). In tilapia, application of *L. rhamnosus* has been documented to modulate intestinal microbiota, promote the breakdown of complex nutrients, and reduce energy losses through excretion (Herbing & Anderson, 2025), which may explain the slight reduction in FCR observed in the present study. These findings indicate that a lower FCR value requires less feed to achieve growth because their body mass increases at a better rate. The absence of statistical significance may be attributed to the trial duration and dosage range, suggesting that longer culture periods or higher concentrations could yield clearer differences.

The estimated optimum concentration level of *L. rhamnosus* for maximizing SGR was estimated at 3.63 mg L^{-1}. However, given the moderate R^2 value, this estimate should be interpreted with caution and requires further validation. Under the present conditions, the highest SGR was observed at the tested concentration of 4 mg L^{-1}. Mohammed et al. (2025) showed that fish respond to probiotics with dose-dependent effects while optimal probiotic levels produce their maximum benefits before they start to decline. Quadratic models often provide a useful theoretical estimate (Yao and Muller, 2010), but the recommendation is better supported by the best-performing treatment in the tested range, especially when the model fit is only moderate. This finding suggests that while 3.63 mg L^{-1} represents the mathematical optimum, 4 mg L^{-1} is the most effective dosage observed under the present culture conditions.

Survival rates remained high across all treatments and did not differ significantly ($p > 0.05$). The consistently high survival observed in probiotic-treated groups suggests that water-applied probiotic supplementation was well tolerated and did not negatively affect the fish health. The research study tested Nile tilapia farming using multiple methods, but only one element failed to meet standard water quality requirements, causing Ammonia levels to change in control and 1 mg L^1 groups. Probiotic treatments led to lower ammonia levels because they improved nitrogen usage and decreased waste production. The improvement of water quality through probiotics is due to the enhancement of microbial activities that can break down both organic matter and compounds containing nitrogen (Hassan et al., 2022). The finding suggested that the use of *L. rhamnosus* in fish food or water contributes to the development and feed efficiency of Nile tilapia without negatively impacting mortality rates and water quality levels. The use of probiotics proves to be an environmentally sustainable alternative to antibiotics in aquaculture settings.

Conclusions*.* The present study demonstrated that water application of *Lactobacillus rhamnosus* conducted under laboratory conditions positively influenced the growth performance of *Oreochromis niloticus* fingerlings, particularly at higher concentrations within 60 days of culture period. However, no significant effects were observed on feed efficiency indices or survival rate. The estimated optimum concentration level for maximizing SGR was 3.63 mg L^{-1}. These findings suggest that water-applied commercial probiotics may serve as a potential supplementary strategy for improving tilapia culture performance.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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