



Harnessing Biotechnological Probiotic Applications to Improve Fish Health and Aquaculture Sustainability

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Abstract

The effects of *Lactobacillus plantarum* and *Bacillus subtilis* probiotics were investigated for individual application and in combination of the two on the growth, water quality of the catfish (*Clarias gariepinus*) and their survival. Four categories of the experimental design diet (control, *B. subtilis*, *L. plantarum*, and mixed probiotics) were adopted, using a total of 180 fingerlings for observation period of 60 days. Results showed that probiotic-fed groups outperformed the control in all parameters, where the mixed probiotic group achieved the highest final weight (**46.8 g vs. 35.2 g control**), survival (**94 % vs. 81 %**), and feed efficiency (**FCR 1.34 vs. 1.82**). the water quality was also improved, with higher dissolved oxygen (**6.2 mg/L**) and reduced ammonia (**0.89 mg/L**) compared to the control. These findings proved that probiotics, particularly combined strains, enhance catfish production while promoting sustainable aquaculture.

Keywords: Probiotic, catfish, sustainable aquaculture, diet types, biotechnology technique

INTRODUCTION

Aquaculture has been established as one of the fastest-growing food production sectors, contributing over 50 % of global fish consumption (pereira *et al.*, 2022); however, the intensification of farming systems has increased vulnerability to diseases, poor feed efficiency, and deteriorating water quality. Moreover, the antibiotics traditionally used for disease prevention have been reported to contribute to antimicrobial resistance and environmental risks. Although the prevention using antibiotics is effective in the short term, their indiscriminate use has raised serious concerns, such as antimicrobial resistance, disruption of aquatic ecosystems, bioaccumulation in fish tissues, and negative impacts on human health through the food chain (pereira *et al.*, 2022; Abarike *et al.*, 2024).

In response to these challenges, the application of **biotechnological approaches** has gained momentum in aquaculture as reported (Pratama *et al.*, 2017; Abarike *et al.*, 2024; Umaru *et al.*, 2024). One such innovation is the use of **probiotics**, which have emerged as sustainable alternatives to

antibiotics because they promote gut microbial balance, improve nutrient assimilation, enhance immune responses, and reduce pathogen load in culture systems. Moreover, probiotics contribute to **bioremediation of water quality** by breaking down organic wastes, reducing ammonia accumulation, and stabilizing dissolved oxygen and pH levels (Umaru *et al.*, 2024).

Recent advances in biotechnology have also shown that, molecular tools such as **16S rRNA sequencing, metagenomics, and proteomics** enable the identification and functional characterization of novel probiotic strains. While the bioprocessing and fermentation technologies allow mass production of viable probiotics, but the encapsulation and nanotechnology ensure their stability during feed preparation and effective delivery in the fish gut (Pratiwi *et al.*, 2020; Diyaolu, 2024).

Globally, catfish (*Clarias gariepinus*) is one of the most widely farmed fish species, largely due to its rapid growth, consumer acceptance, and tolerance to a wide range of environmental conditions (Khobir *et al.*, 2016). Despite these



advantages, their production is still constrained by high-density culture challenges, like bacterial diseases, feed inefficiency, and poor water quality. Thus, incorporating biotechnology-enhanced probiotics into catfish diets presents a promising approach to address these challenges sustainably (Li, Y., and Boyd, 2016). Therefore, this study investigates the effects of dietary supplementation with two well-characterized probiotic strains, *Bacillus subtilis* and *Lactobacillus plantarum*, individually and in combination, on the growth performance, immunological responses, and water quality in catfish.

Materials and Methods

As adopted from the work of Pratama *et al.* (2017), the experiment was carried out using catfish (*Clarias gariepinus*) fingerlings. A total of 200 healthy fish with an initial mean weight of approximately 12.5 g were obtained and stabilized for 14 days in a 300 L clean holding tank before the trial commenced. The holding tank was maintained at room temperature. Then, the water exchange was carried out daily at 30 % to keep ammonia concentrations below 0.5 mg·L⁻¹. During acclimation, the fish were fed a basal diet twice daily at 3 % of their body weight, which was reduced to 2 % two days before the experiment to standardize gut contents.

Following acclimation, the fish were randomly assigned to 12 experimental tanks, each stocked with 15 fish, to represent four treatments in triplicate. The treatments included a control diet without probiotic supplementation (T1), a basal diet supplemented with *Bacillus subtilis* at 1×10⁷ CFU·g⁻¹ of feed (T2), a basal diet supplemented with *Lactobacillus plantarum* at 1×10⁷ CFU·g⁻¹ of feed (T3), and a basal diet supplemented with a mixture of *B. subtilis* and *L. plantarum* each at 1×10⁷ CFU·g⁻¹ (T4). The feeding trial lasted for 60 days, during which fish were sampled for growth measurements at two-week intervals, while blood samples for immune assays were collected at days 0, 30, and 60. Water quality was monitored daily for temperature, pH, and dissolved oxygen, while ammonia and nitrite were analysed three times per week. Mortality was recorded daily.

The basal diet used for all treatments was formulated to contain approximately 32 % crude protein, 8 % lipid, ≤5 % fiber, 10 % ash, and ≤ 10 % moisture. The formulation consisted of 30 % fishmeal, 25 % soybean meal, 20 % maize, 10 % wheat bran, 4 % oil, 2 % vitamin-mineral premix, and 4–7% starch or attractants. All dry ingredients were thoroughly blended in a mixer, after which oil and water were added to form a dough with about 18 % moisture content. The dough was pelletized into 1.5-3 mm pellets, which were dried at 45 °C until the moisture content fell below 10 %, then sieved to ensure uniform size distribution.

Probiotic strains used in the experiment included *B. subtilis* and *L. plantarum*. *B. subtilis* was cultured at 37 °C and 200

rpm for 24 hours, while *L. plantarum* was cultured at 37°C for 24 hours. Cells were harvested by centrifugation at 4,000–6,000 × g for 10 minutes at 4°C, washed twice in sterile phosphate-buffered saline (PBS), and resuspended in PBS. Colony-forming units were determined by serial dilution and plating on selective agar, with nutrient agar used for *Bacillus* and MRS agar for *Lactobacillus*. The concentration was adjusted to 1×10¹⁰ CFU·mL⁻¹ and used fresh in 10 % skim milk. Plate counts were performed before feed incorporation to confirm viability, and tests for contaminants such as coliforms were conducted.

Probiotics were incorporated into the diet using a coating method. The required volume of probiotic concentrate to achieve 1×10⁷ CFU·g⁻¹ of feed was mixed with 0.5 % fish oil as a binder and sprayed evenly over the pellets. Pellets were gently tumbled for 10 minutes to ensure uniform distribution, air-dried for 1–2 hours, and stored at 4 °C for short-term use (7 days). Weekly plate counts were performed to verify probiotic viability in the coated feeds.

Fish were reared in tanks with continuous aeration and gentle water flow, where room temperature was maintained. Feeding was done twice daily, in the morning and late afternoon, beginning at 4 % body weight per day and gradually reduced to 3 % as the fish grew. The uneaten feed was collected after 30 minutes, dried, and weighed to determine accurate feed conversion ratios (FCR). Partial water renewals of 30 % were performed twice weekly.

Growth performance was evaluated by recording initial and final weights of fish and the cumulative feed consumed. Weight gain was calculated as the difference between final and initial weights, while specific growth rate (SGR) and feed conversion ratio (FCR) were determined using standard formulae. Survival was calculated as the percentage of fish that remained alive at the end of the trial.

Water quality parameters, including, dissolved oxygen, and pH, were measured daily using portable meters, while ammonia and nitrite concentrations were determined three time weekly using colorimetric kits.

Results and Discussion

Growth performance of catfish investigation using probiotic supplement

Table 1 presents the growth performance of catfish fed with different probiotic supplements, that was investigated over an eight-week period. As observed from the results presented in Table 1, at week 2, the control sample recorded an average weight of 18.4 g from the initial 12 g bases, while fish fed with *B. subtilis* and *L. plantarum* showed improved growth at 20.6 g and 21.4 g, respectively, and the mixed probiotic group exhibited the highest growth at this stage, reaching 22.1 g.

Table 1: Growth performance results of the tested probiotic diet on catfish

Week	Various Trials			
	Control (g)	B. subtilis (g)	L. plantarum (g)	Mixed probiotic (g)

0	12.5	12.5	12.5	12.5
2	18.4	20.6	21.4	22.1
4	24.3	28.2	30.0	31.2
6	29.6	35.1	37.6	39.0
8	35.2	42.1	44.3	46.8

Similar trend continued at week 4, with the control sample at 24.3 g compared to 28.2 g in the *B. subtilis* and 30.0 g in the *L. plantarum* samples, as similar variations were observed at week 6. Additionally, the differences were most pronounced at the end of the 8-week investigation, where the control fish sample was observed to have grown to 35.2 g, while *B. subtilis*- and *L. plantarum*-supplemented samples achieved 42.1 g and 44.3 g, respectively. However, the mixed probiotic sample showed the highest growth response, attaining a final weight of 46.8 g. Based on the performance growth study findings, the catfish growth was enhanced through probiotic supplementation compared to the control diet.

Water quality data analysis

The water quality results of the catfish probiotic supplementation are shown in Table 2. As observed from the results, the probiotic supplementation had positive effects on the dissolved oxygen, pH, and ammonia levels compared to the control sample, as the dissolved oxygen (DO) control value of 5.1 mg/L was lowest but increased progressively in *Bacillus subtilis* (5.8 mg/L), *Lactobacillus plantarum* (6.0 mg/L), and mixed probiotic groups (6.2 mg/L).

Table 2: The water quality analysis of the various experimental fish holding tanks

Parameters	Various Trials			
	Control	<i>B. subtilis</i>	<i>L. plantarum</i>	Mixed Probiotic
Dissolved Oxygen (mg/L)	5.1	5.8	6.0	6.2
pH	6.9	7.1	7.2	7.3
Ammonia-N (mg/L)	1.42	1.01	0.94	0.89

This improvement as depicted in Table 2 can be attributed to the role of probiotics in enhancing microbial balance within the aquatic system. Specifically, beneficial bacteria stimulate organic matter decomposition more efficiently, reducing oxygen depletion by harmful microbes and thus maintaining higher oxygen availability for fish metabolism as also reported by **Pereira et al. (2022)**. Additionally, the pH of the water followed a similar trend, with the control group recording the lowest value (6.9) and the mixed probiotic group the highest (7.3) as presented in Table 2. Thus, the slight increase in pH across treatments is likely due to the metabolic activity of probiotics, which can buffer acidity by reducing organic load and nitrogenous waste. While the ammonia-N levels showed a clear reduction with probiotic

supplementation as shown in Table 2, dropping from 1.42 mg/L in the control group to as low as 0.89 mg/L in the mixed probiotic treatment. This is due to the ability of probiotics to improve nitrogen cycling in aquaculture systems as *B. subtilis* is known to enhance nitrification and organic matter breakdown, while *L. plantarum* supports microbial communities that reduce ammonia accumulation.

Impacts of probiotic supplementation in catfish mortality rate

Table 3 depicts the results of survival performance of the catfish studies for 8 weeks when subjected to the different dietary treatments. As observed from the results presented in Table 3, the control set, which received no probiotic supplementation, had the lowest survival, with only about 36 fish remaining, amounting to 81 % survival. However, the probiotic fed replicates exhibited higher survival rates, with fish fed *Bacillus subtilis* recorded 40 survivors (89 %), while those fed *Lactobacillus plantarum* had 41 survivors (92 %) as shown in Table 3. Additionally, the mixed probiotic group achieved the highest survival, retaining 42 out of 45 fish, which translated to 94 % survival.

Table 3: The effects of the dietary treatments on the fish

Treatment	Initial fish stock	% mortality rate	Number of survivors
Control	45	81	36
<i>B. subtilis</i>	45	89	40
<i>L. plantarum</i>	45	92	41
Mixed Probiotic	45	94	42

Therefore, these findings as observed in Table 3 indicate a positive influence of probiotics on fish health and resistance to stress. Moreover, the improved water quality parameters, such as reduced ammonia and higher dissolved oxygen as earlier discussed in Table 2, likely contributed to lower mortality rates in probiotic-supplemented tanks.

Conclusions

It is concluded from the findings that, the probiotic supplementation significantly improved the catfish performance as the mixed probiotic group showed a 33 % higher final weight of 46.8 g compared to the 35.2 g obtained for the control sample. Their survival was improved from 81 % to 94 %, while water quality was also enhanced, with dissolved oxygen rising to 6.2 mg/L and ammonia reduced to 0.89 mg/L. Therefore, the results confirm probiotics,

especially the mixed culture, is more effective and sustainable alternative to antibiotics in aquaculture.

References

1. **Abarike, E., Ampofo, Y. A. and Samuel, O.** (2024). Growth, hematology and liver health of African catfish-*Clarias gariepinus*, fed varying amounts of *Bacillus subtilis*. *Ghana Journal of Science, Technology and Development*, 10(1), 82-95. <https://doi.org/10.47881/450.967x>
2. **Diyaolu, D. O.** (2024). Beneficial effects of two *Lactobacillus plantarum* strains on egg hatchability and larval survival of African catfish (*Clarias gariepinus*). *Nigerian Journal of Animal Production*, 55–59. <https://doi.org/10.51791/njap.vi.4125>
3. **Khobir, M. L., Putra, I., and Tang, U. M.** (2016). Growth and survival rate of African catfish (*Clarias gariepinus*) using bioflocs technology by administering different doses of probiotic bacteria in the water medium of peat bog. *Journal Online Mahasiswa Bidang Perikanan dan Ilmu Kelautan (JOM FAPERIKA)*.
4. **Li, Y., and Boyd, C. E.** (2016). Influence of a bacterial amendment on water quality in small research ponds for channel catfish (*Ictalurus punctatus*) production. *Journal of the World Aquaculture Society*, 47(4), 464–469. <https://doi.org/10.1111/jwas.12301>
5. **Pratama, W. D., Prayogo, and Manan, A.** (2017). Effect addition of different probiotic in aquaponic systems towards water quality in aquaculture catfish (*Clarias sp.*). *Journal of Aquaculture Science*, 1(1), 27–35. <https://doi.org/10.31093/joas.v1i1.4>
6. **Pratiwi, R., Hidayat, K. W. and Sumitro, S.** (2020). Production performance of catfish (*Clarias gariepinus* Burchell, 1822) cultured with added probiotic *Bacillus* sp. on biofloc technology. *Journal of Aquaculture and Fish Health*, 9(3), Article 16280. <https://doi.org/10.20473/jafh.v9i3.16280>
7. **Pereira, W. A., Mendonça, C. M. N., Urquiza, A. V., Marteinsson, V. Þ., LeBlanc, J. G., Cotter, P. D., Villalobos, E. F., Romero, J., & Oliveira, R. P. S.** (2022). Use of probiotic bacteria and bacteriocins as an alternative to antibiotics in aquaculture. *Microorganisms*, 10(9), Article 1705. <https://doi.org/10.3390/microorganisms10091705>
8. **Umaru, J., Ochokwu, I. J., & Agbugui, M. O.** (2024). Influence of Antox® probiotic, as water additive, on growth performance and nutrient utilization of African catfish (*Clarias gariepinus*). *Nigerian Journal of Animal Production*, Vi, Article 6490. <https://doi.org/10.51791/njap.vi.6490>