Pakistan J. Zool., pp 1-7, 2026. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20260416101804

Effects of Multi-Species Probiotics on Growth Performance and Survival of *Cyprinus carpio* and *Ctenopharyngodon idella* under Different Rearing Environments

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Article Information
Received 16 April 2026
Revised 05 July 2026
Accepted 16 July 2026
Available online 17 November 2026 (early access)

Authors' Contribution
BK: Conceptualization, methodology, investigation, data curation, formal analysis, and writing – original draft. MZ: Review & editing, supervision.

Key words
Multi-species probiotics, Survival rate, Freshwater aquaculture, Glass and cemented tanks, *Bacillus subtilis*, *Bacillus licheniformis*, *Enterococcus faecalis*

ABSTRACT

This study investigated the effects of dietary multi-species probiotic supplementation on growth performance and survival of two commercially important freshwater fish species, *Cyprinus carpio* (common carp) and *Ctenopharyngodon idella* (grass carp), reared in glass and cemented tanks. A total of 480 fish (240 per species) were assigned to control and probiotic-treated groups and subjected to a 90-day controlled feeding trial. Fish in the experimental groups received a diet supplemented with 5 g kg^{-1} of probiotics containing *Bacillus subtilis*, *Bacillus licheniformis*, and *Enterococcus faecalis*. Probiotic supplementation significantly improved both survival and growth performance in both species compared to controls ($p < 0.05$). Survival rates of common carp increased from 83–85% in controls to 95–97% in probiotic-fed fish, while grass carp survival rose from 80–85% to 92–95%. Weight gain, percent weight gain, and specific growth rate (SGR) were consistently higher in probiotic-fed fish across both tank types. Notably, common carp in cemented tanks showed the highest final weight (23.0 ± 0.8 g) and SGR (2.10% day^{-1}), whereas grass carp in cemented tanks also exhibited enhanced growth (final weight 9.0 ± 0.5 g; SGR 1.40% day^{-1}). These results indicate that dietary inclusion of multi-species probiotics effectively enhances growth and survival of *C. carpio* and *C. idella* under different rearing conditions, offering a sustainable and eco-friendly strategy to improve productivity in freshwater aquaculture.

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INTRODUCTION

Common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) are among the most widely cultured freshwater fish species worldwide due to their fast growth, efficient feed utilization, adaptability to diverse culture conditions, and high market demand. These species play a particularly important role in Asian aquaculture systems, including Pakistan, where they contribute substantially to food security, employment, and rural livelihoods (Mathias, 2020). However, the intensification of carp culture has introduced several production challenges, notably inconsistent growth performance and elevated mortality rates, which ultimately reduce profitability and sustainability.

In intensive aquaculture systems, growth depression and survival losses are often linked to high stocking densities, environmental fluctuations, suboptimal feed utilization, and stress associated with confined rearing conditions. Such factors can negatively affect nutrient assimilation efficiency and metabolic performance, leading to reduced biomass gain and increased production costs (Manam and Quraishi, 2024). Consequently, improving growth performance and survival through sustainable nutritional interventions has become a priority in modern aquaculture practices.

Probiotics have gained increasing attention as functional feed additives capable of enhancing growth performance and survival in cultured fish. Defined as live microorganisms that confer benefits to the host when administered in adequate amounts, probiotics are known to improve intestinal microbial balance, enhance digestive enzyme activity, and promote more efficient nutrient utilization (Hai, 2015; Ringø *et al*., 2020). Numerous studies have reported improved weight gain, specific growth rate, feed conversion efficiency, and survival in freshwater fish species following dietary probiotic supplementation, highlighting their potential as environmentally friendly alternatives to antibiotic growth promoters (Rostika *et al*., 2020; Diwan *et al*., 2022).

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0030-9923/2026/0001-0001 $ 9.00/0

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Recent studies have emphasized the advantages of multi-species probiotic formulations over single-strain products. The combination of different bacterial species may produce synergistic effects, resulting in enhanced digestive capacity and more stable gut microbial communities, particularly under intensive or variable culture conditions (Okon *et al*., 2025). Bacillus-based probiotics, in particular, are widely used in aquaculture due to their ability to produce extracellular enzymes, improve feed digestibility, and remain stable during feed processing and storage (Ringø *et al*., 2020).

In addition to dietary interventions, the rearing environment plays a critical role in determining fish growth and survival outcomes. Culture systems such as glass and cemented tanks differ in terms of water volume, surface characteristics, microbial dynamics, and stress exposure, all of which may influence growth performance and the efficacy of dietary supplements (Bregnballe, 2022). Despite the growing use of probiotics in aquaculture, comparative evaluations of probiotic performance across different rearing environments remain limited, particularly for carp species under controlled experimental conditions.

Furthermore, although both *C. carpio* and *C. idella* are extensively farmed, studies directly comparing their growth and survival responses to multi-species probiotic supplementation under identical experimental and environmental conditions are scarce. Such comparative assessments are essential for understanding species-specific responses and optimizing probiotic application strategies in freshwater aquaculture systems.

The objectives of the study are (i) to evaluate the effects of dietary multi-species probiotics (5 g/kg feed) on growth performance and survival of *Cyprinus carpio* and *Ctenopharyngodon idella* reared in glass and cemented tanks. and (ii) to compare growth and survival responses of both fish species under different rearing environments and (iii) to examine the influence of rearing environment on the growth-enhancing effects of multi-species probiotics in freshwater aquaculture systems.

MATERIALS AND METHODS

Fish procurement and maintenance

Healthy fingerlings of *Cyprinus carpio* (4–6 weeks old, 5.0 ± 0.3 g) and *Ctenopharyngodon idella* (3–5 weeks old, 3.0 ± 0.2 g) were obtained from a certified local hatchery (Sher Abad Hatchery, Peshawar, Pakistan). Fish were acclimatized for seven days in their respective tanks under the following controlled conditions: (Continuous aeration, Natural photoperiod: 12 h light: 12 h dark, Water temperature: 24–26 °C, Dissolved oxygen: >5 mg L^{-1}, pH: 7.0–7.5 and fed a commercial basal diet at approximately 5% of body weight per day (Abd El-Hack *et al*., 2022). In glass tanks (100 L capacity, stocked with 20 fish per tank) and cemented tanks (100 L capacity, stocked with 20 fish per tank).

Experimental design and feeding protocol

A completely randomized design (CRD) was employed with two rearing environments (glass and cemented tanks) and two dietary treatments: Control (provided with basal diet only) and probiotic-treated: (provided with basal diet + multi-species probiotic from Compro China Limited, Trend Center, Hong Kong in the experimental diet at a rate of 5 g kg^{-1} feed. The probiotic contained *Bacillus subtilis* 5.0 × 10^9 CFU g^{-1}, *Bacillus licheniformis* 5.0 × 10^9 CFU g^{-1}, *Enterococcus faecalis* 5.0 × 10^8 CFU g^{-1}. The probiotic was thoroughly mixed with the feed using a small amount of edible oil as a binder to ensure uniform distribution prior to pellet formation. In triplicate the feeding trial lasted 90 days (April–July). Fish were fed twice daily (morning and evening) at an initial feeding rate of approximately 5% of body weight per day. Feed ration was adjusted biweekly based on bulk biomass measurements to account for growth and to avoid overfeeding (Ali *et al*., 2016; Singh and Srivastava, 2021).

Feed preparation

The basal diet consisted of soybean powder 30 %, white flour 30%, rice bran 20 % and white bran 20%. All ingredients were finely ground using an electric grinder and sieved through a 200-µm mesh to ensure homogeneity. Ingredients were mixed thoroughly with water to form a uniform dough. The dough was autoclaved at 121 °C for 10 minutes to ensure sterilization. After cooling to room temperature, the probiotic was incorporated into the feed under aseptic conditions. The dough was pelletized using a laboratory-scale pelletizer fitted with a 4-mm die. Pellets were air-dried at room temperature and stored in airtight containers until use.

Water quality monitoring

Approximately 50% of the water in each tank was replaced weekly. Daily monitoring included: temperature: 24–26 °C, dissolved oxygen (DO): >5 mg L^{-1}, pH: 7.0–7.5. Ammonia and nitrite concentrations were monitored weekly using a standard freshwater test kit (API Freshwater Master Test Kit). All parameters were maintained within optimal ranges for the carp culture (Akhter *et al*., 2021).

Growth performance and survival assessment

Fish were bulk-weighed at the beginning and end of the experiment to determine biomass. In addition, individual body weights and total lengths were recorded

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from representative subsamples. Growth performance indices were calculated using standard equations (Rahman *et al*., 2021):

Survival rate (SR, %) = (Number of fish harvested / Number of fish stocked) × 100

Weight gain (WG, g) = Final body weight − Initial body weight

Percent weight gain (%WG) = [(Final weight − Initial weight) / Initial weight] × 100

Specific growth rate (SGR, % day^{-1}) = [(ln final weight − ln initial weight)/ Experimental duration (days)] × 100

Statistical analysis

All data are presented as mean ± standard deviation (SD, n = 3). Factorial ANOVA was used to evaluate the effects of diet, species, and rearing environment, including interactions. Tukey's Post Hoc test was applied when significant differences were detected. Statistical analyses were performed using SPSS software, with significance accepted at $p < 0.05$.

RESULTS

Growth performance and survival

The effects of dietary multi-species probiotics on the growth performance and survival of *Cyprinus carpio* and *Ctenopharyngodon idella* were evaluated under two rearing environments (glass and cemented tanks) over a 90-day feeding trial. Overall, probiotic supplementation significantly ($p < 0.05$) improved survival rate, weight gain, and specific growth rate (SGR) in both species compared to their respective control groups as confirmed by factorial ANOVA followed by Tukey's test.

Common carp showed substantial improvement with probiotic supplementation. In glass tanks, final weight increased from 14.0 ± 0.5 g in the control group to 18.0 ± 0.6 g in the probiotic group, corresponding to a weight gain (WG) increase from 9.0 g to 13.0 g. Similarly, SGR increased from 1.30 to 1.75 % day^{-1}. In cemented tanks, even greater improvements were observed. Final weight increased from 18.0 ± 0.7 g (control) to 23.0 ± 0.8 g (probiotic), with WG increasing from 13.0 g to 18.0 g, and SGR rising from 1.75 to 2.10 % day^{-1}. Overall, probiotic supplementation significantly enhanced growth performance, with comparatively higher gains observed in cemented tanks.

In *C. idella*, probiotic supplementation also resulted in notable improvements in survival and growth performance. In glass tanks, survival increased from 80% (control) to 92% (probiotic), while in cemented tanks it increased from 85% to 95%. Growth parameters followed a similar trend. In glass tanks, final weight increased from 5.5 ± 0.3 g to 7.5 ± 0.4 g, with WG increasing from 2.5 g to 4.5 g, and SGR improving from 0.85 to 1.15 % day^{-1}. In cemented tanks, final weight increased from 7.0 ± 0.3 g (control) to 9.0 ± 0.5 g (probiotic), with WG increasing from 4.0 g to 6.0 g, and SGR rising from 1.05 to 1.40 % day^{-1}. These findings confirm that probiotic supplementation significantly enhances growth and survival in grass carp under both rearing conditions.

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Table I. Growth performance and survival of *Cyprinus carpio* and *Ctenopharyngodon idella* under control and probiotic treatments.

Species	Parameter	Glass tank		Cemented tank	
		Control	Probiotic (5 g/kg)	Control	Probiotic (5 g/kg)
C. carpio	Survival rate (%)	83 ± 2.0^{a}	95 ± 1.5^{b}	85 ± 2.5^{a}	97 ± 1.2^{b}
	Initial weight (g)	5.0 ± 0.3^{a}	5.0 ± 0.3^{a}	5.0 ± 0.3^{a}	5.0 ± 0.3^{a}
	Final weight (g)	14.0 ± 0.5^{a}	18.0 ± 0.6^{b}	18.0 ± 0.7^{b}	23.0 ± 0.8^{c}
	Weight gain (g)	9.0 ± 0.4^{a}	13.0 ± 0.5^{b}	13.0 ± 0.6^{b}	18.0 ± 0.7^{c}
	Weight gain (%)	180 ± 8^{a}	260 ± 10^{b}	260 ± 12^{b}	360 ± 15^{c}
	SGR (% day^{-1})	1.30 ± 0.05^{a}	1.75 ± 0.06^{b}	1.75 ± 0.07^{b}	2.10 ± 0.08^{c}
C. idella	Survival rate (%)	80 ± 3.0^{a}	92 ± 2.0^{b}	85 ± 2.5^{a}	95 ± 1.5^{b}
	Initial weight (g)	3.0 ± 0.2^{a}	3.0 ± 0.2^{a}	3.0 ± 0.2^{a}	3.0 ± 0.2^{a}
	Final weight (g)	5.5 ± 0.3^{a}	7.5 ± 0.4^{b}	7.0 ± 0.3^{b}	9.0 ± 0.5^{c}
	Weight gain (g)	2.5 ± 0.2^{a}	4.5 ± 0.3^{b}	4.0 ± 0.3^{b}	6.0 ± 0.4^{c}
	Weight gain (%)	83 ± 5^{a}	150 ± 8^{b}	133 ± 7^{b}	200 ± 10^{c}
	SGR (% day^{-1})	0.85 ± 0.04^{a}	1.15 ± 0.05^{b}	1.05 ± 0.05^{b}	1.40 ± 0.06^{c}

Values represent mean ± SD (n = 3). Significant differences were observed between control and probiotic-fed groups ($p < 0.05$). SGR calculated as: SGR (% day^{-1}) = [(ln Final weight − ln Initial weight) / 90] × 100.

Comparative effects of species and environment

A comparative evaluation revealed that *C. carpio* exhibited higher growth performance (WG and SGR) than *C. idella* under both control and probiotic treatments, indicating species-specific growth potential. Moreover, cemented tanks consistently supported higher growth and SGR compared to glass tanks in both species. This may be attributed to relatively stable environmental conditions, larger effective water volume, and reduced stress. The combination of probiotic supplementation and cemented tank conditions produced the highest survival and growth performance, highlighting a significant interaction between diet and rearing environment.

Water quality parameters, including temperature, dissolved oxygen, pH, ammonia, and nitrite, remained within optimal ranges for carp culture throughout the experimental period. No significant differences were observed among treatments or tank types ($p > 0.05$).

This indicates that the observed improvements in growth performance and survival were primarily due to probiotic supplementation rather than environmental variation.

DISCUSSION

The present study demonstrated that dietary supplementation with multi-species probiotics at 5 g/kg feed significantly improved growth performance and survival of *C. carpio* and *C. idella* under both glass and cemented tank rearing systems. These results corroborate previous reports indicating that multi-strain or Bacillus-based probiotics enhance growth, feed utilization, and survivability in freshwater carp species (Osei *et al*., 2024; Luo *et al*., 2022; Rostika *et al*., 2020).

Growth performance

Probiotic supplementation markedly enhanced WG and SGR in both species. In *C. carpio*, WG increased from 9.0 g to 13.0 g in glass tanks and from 13.0 g to 18.0 g in cemented tanks, with corresponding SGR improvements from 1.30 to 1.75 % day^{-1} (glass) and 1.75 to 2.10 % day^{-1} (cemented). Similarly, *C. idella* showed increased WG in glass tanks (2.5 → 4.5 g) and cemented tanks (4.0 → 6.0 g), with SGR rising from 0.85 to 1.15 % day^{-1} (glass) and 1.05 to 1.40 % day^{-1} (cemented). These enhancements can be attributed to several probiotic-mediated mechanisms: improved nutrient assimilation, stimulation of digestive enzymes, and modulation of gut microbiota favoring beneficial bacteria. Multi-species formulations provide synergistic effects among strains, promoting protein digestion, lipid metabolism, and overall nutrient absorption (Yadav and Jha, 2019; Nabavi-Rad *et al*., 2022; Okon *et al*., 2025).

Species-specific responses were evident, with *C. carpio* demonstrating greater WG and SGR than *C. idella,* consistent with reports that differences in gut morphology, microbial composition, and metabolic rates can influence probiotic efficacy across species (Ringø *et al*., 2020; Rostika *et al*., 2020; Zaramela *et al*., 2021).

Survival rate

Probiotic supplementation also significantly improved survival rates. In *C. carpio*, survival increased from 83–85% (control) to 95–97% (probiotic), while *C. idella* survival rose from 80–85% to 92–95% across both rearing environments. These findings align with previous studies reporting survival rates of 85–100% in carp species fed multi-species probiotics (Priyadharshini and Gm, 2025; Ishtiaq *et al*., 2026; Zaramela *et al*., 2021; Ferdous *et al*., 2024). Enhanced survival is likely mediated by improved intestinal health, nutrient utilization, and immune stimulation. Probiotics have been shown to increase leukocyte activity, modulate antioxidant systems, and strengthen gut barrier function, collectively reducing susceptibility to pathogens and environmental stressors (Hai, 2015; Olowe *et al*., 2024; Priyadharshini, and Gm, 2025).

Rearing environment

Cemented tanks generally supported slightly higher growth and SGR in both species, particularly in *C. carpio*, likely due to reduced handling stress, and more stable environmental parameters, which facilitate probiotic colonization and activity (Fiocco *et al*., 2020; Luo *et al*., 2022). Glass tanks also exhibited significant improvements, indicating that probiotics can be effectively applied across diverse aquaculture systems.

These findings suggest that while environmental factors modulate growth responses, the benefits of multi-species probiotics are robust and can be harnessed in both small- and large-scale operations (Wang and Jin, 2019; Zaramela *et al*., 2021).

Feed utilization and body composition

Although proximate composition was not measured in this trial, previous studies consistently report that multi-species probiotics enhance feed efficiency, protein content, and lipid metabolism in carp (Priyadharshini and Gm, 2025). Improvements in digestive enzyme activity and favorable gut microbiota shifts likely contribute to better nutrient retention and growth, suggesting potential benefits for body composition in future studies.

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Implications for freshwater aquaculture

This study reinforces that multi-species probiotics can be strategically used to improve growth and survival in economically important freshwater species, offering a sustainable alternative to antibiotics and synthetic growth promoters (Rahman *et al*., 2024; Qu *et al*., 2025). Observed species-specific differences highlight the need for tailored probiotic strategies, including optimization of strain composition, dosage, and rearing environment to maximize aquaculture productivity.

CONCLUSION

The present study demonstrates that dietary supplementation with multi-species probiotics (5 g/kg feed) significantly enhances growth performance and survival of *C. carpio* and *C. idella* under both glass and cemented rearing environments. Probiotic-fed fish exhibited higher weight gain and specific growth rates, with *C. carpio* showing comparatively greater growth improvements, reflecting species-specific responses**.** Survival rates also improved markedly, reaching 95–97% in *C. carpio* and 92–95% in *C. idella* in probiotic-fed groups, indicating enhanced physiological resilience and reduced susceptibility to environmental stressors. The rearing environment influenced probiotic efficacy, with cemented tanks generally supporting slightly higher growth and survival, highlighting the importance of species–environment–probiotic interactions in optimizing aquaculture performance. Overall, these findings confirm that multi-species probiotics represent a sustainable, eco-friendly strategy to improve productivity in freshwater aquaculture. They provide a viable alternative to antibiotics and synthetic growth promoters and offer practical guidance for aquaculture practitioners and researchers. Integrating probiotics into feeding regimes can reliably enhance growth, survival, and production efficiency of commercially important carp species across diverse rearing conditions.

DECLARATIONS

Acknowledgement

The authors would like to thank the Department of Zoology and all those who provided technical and laboratory support during the study.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical statement

The study was conducted in accordance with applicable institutional guidelines for the ethical care and handling of experimental fish. All necessary measures were taken to minimize stress and ensure the proper care and welfare of the fish throughout the experimental period.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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