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Comparative Evaluation of Probiotic Treatments for Growth Performance and Water Quality in Molly Fish (*Poecilia sphenops*), with Emphasis on *Lactobacillus pentosus* B25

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ABSTRACT

Probiotics have gained increasing attention in aquaculture as potential dietary supplements for improving fish growth and supporting sustainable production. The present study investigated the growth-promoting potential of different probiotic preparations in molly fish (Poecilia sphenops), with particular emphasis on Lactobacillus pentosus B25 isolated from a marine sample. The effects of three probiotic treatments, Darolac, Viabact, and L. pentosus B25, were compared with a commercial-feed control over a 15-day experimental period. Growth performance was evaluated by recording changes in body weight and length, while qualitative observations of fish activity and colour variation were documented. Water-quality parameters, including temperature, pH, dissolved oxygen, total ammonia nitrogen, nitrite, and nitrate, were also monitored. The recorded results showed weight gains of 0.328 g (19.16%) in the control group, 0.418 g (24.22%) in the Darolac group, and 0.531 g (27.73%) in the Viabact group. The L. pentosus B25 treatment was reported to produce a weight gain of 0.560 g (29.17%), representing the highest reported growth among the treatments. Dissolved oxygen was slightly higher, while nitrogenous waste parameters were comparatively lower in the probiotic-treated groups than in the control. Qualitative observations also indicated greater activity and colour variation in the probiotic-treated fish. These findings suggest that probiotic supplementation, particularly with L. pentosus B25, may have potential for promoting growth in P. sphenops under the conditions investigated. However, further studies with adequate biological replication, longer experimental periods, and standardized probiotic doses are required to validate these preliminary findings and establish the effectiveness of B25 for sustainable aquaculture applications.

Keywords: *Probiotics; Lactobacillus pentosus B25; Poecilia sphenops; Aquaculture; Fish growth performance; Probiotic feed supplementation; Water quality; Sustainable aquaculture.*

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1. Introduction

Aquaculture plays an important role in food production and the ornamental fish trade. Maintaining fish health, promoting growth, and ensuring suitable culture conditions are important objectives in fish farming. However, fish growth can be affected by several factors, including feed composition, water quality, microbial communities, and disease pressure. Consequently, researchers have investigated nutritional and biological approaches that may improve fish performance and support sustainable aquaculture practices [1]. Probiotics have received considerable attention as potential supplements in aquaculture. They are generally defined as live microorganisms that confer a health benefit on the host when administered in adequate amounts. In aquatic animals, probiotic microorganisms may influence host–microbe interactions and contribute to maintaining a favourable microbial environment. Their application as biological control agents in aquaculture has been widely discussed in the scientific literature [2].

Probiotic microorganisms may affect fish through different mechanisms, including competition with potentially harmful microorganisms, modulation of immune responses, and changes in digestive processes. However, the effects of probiotics depend on the microbial strain, host species, administration method, dose, and environmental conditions. Therefore, probiotic treatments should be evaluated experimentally rather than assumed to produce identical results across different aquaculture systems [3]. The scientific use of the term *probiotic* also requires careful consideration. The International Scientific Association for Probiotics and Prebiotics has emphasised that probiotic claims should be supported by appropriate evidence of a health benefit. This principle is important when evaluating commercial preparations or individual bacterial isolates because the presence of microorganisms alone does not establish their effectiveness as probiotics [4].

Several studies have investigated probiotic applications in aquaculture, including their potential effects on growth, feed utilisation, and microbial balance. However, outcomes can differ among fish species and probiotic preparations. The identity of the microorganism, its viability, the concentration administered, and the conditions under which it is used are therefore important considerations when interpreting experimental results [5]. The relationship between probiotics and fish immunity has also received scientific attention. Certain microorganisms may influence immune-related responses, but these effects are not necessarily uniform across bacterial strains or fish species. Consequently, growth and behavioural observations should be interpreted alongside appropriate experimental measurements rather than treated as direct proof of improved immunity or health [6].

Research on probiotic and prebiotic applications in aquaculture has highlighted the potential value of these approaches while also identifying the need for further evidence. In particular, probiotic performance must be assessed under defined conditions, with sufficient replication and clear reporting of the microorganisms and experimental procedures used [7]. Lactic acid bacteria have been investigated as potential probiotic organisms in finfish. Nevertheless, the biological properties of these bacteria are strain-dependent, and identification at the genus or species level alone may not establish probiotic efficacy. Accurate identification and appropriate evaluation are therefore important when investigating an individual isolate for possible aquaculture applications [8].

Experimental studies have explored the use of microbial supplements to promote fish growth. For example, Lara-Flores and colleagues investigated bacterial and yeast supplements in Nile tilapia (*Oreochromis niloticus*), providing a basis for examining the relationship between microbial supplementation and fish growth performance [9]. Such studies demonstrate the relevance of evaluating probiotic treatments using measurable outcomes, including changes in body weight and length. Reviews of probiotic use in aquaculture have also described potential applications in different aquatic production systems. However, the effectiveness of a particular treatment cannot be assumed to transfer directly from one fish species or culture environment to another. Comparative studies are therefore useful for investigating the responses associated with different commercial formulations and individual bacterial isolates [10].

Probiotics have additionally been investigated as potential tools for disease management in aquaculture. Their effects depend on the microorganism and the conditions of application, and their use should be supported by experimental evidence. Studies comparing different treatments can help identify promising candidates for further investigation, but observed differences should be interpreted in light of the experimental design and the limitations of the available data [11]. Water quality is another important factor in fish growth experiments. Parameters such as dissolved oxygen, temperature, pH, and nitrogenous waste concentrations can influence the physiological condition of fish and the interpretation of growth responses. Appropriate monitoring and management of water quality are therefore essential when comparing fish maintained under different feeding or supplementation regimes [12].

Ammonia is particularly important in aquatic systems because its toxicity is influenced by environmental conditions. The equilibrium between ionised ammonium and un-ionised ammonia varies with pH and temperature, affecting the potential toxicity of ammonia to aquatic organisms. Monitoring ammonia alongside other water quality parameters can therefore help contextualise observations made during fish growth experiments [13]. Adequate nutrition is also essential for interpreting fish growth. Fish require suitable levels and balances of nutrients to support maintenance, tissue development, and growth. Consequently, growth comparisons should consider the composition and quantity of the basal diet, feeding rate, and experimental conditions, as these factors may influence the observed response to a probiotic treatment [14]. Despite the growing body of literature on probiotics in aquaculture, further investigation is needed to understand how different preparations and bacterial isolates perform under specific experimental conditions. In particular, comparative studies that record fish growth and relevant water quality parameters can provide preliminary information for selecting treatments for more extensive evaluation.

The present study investigated the growth-promoting potential of three probiotic treatments, Darolac, Viabact, and *Lactobacillus pentosus* B25, in comparison with a commercial-feed control in fish identified as *Poecilia sphenops*. Over a 15-day experimental period, the study recorded body weight and length changes, qualitative observations of swimming activity and external colour variation, and selected water quality parameters.

The objective was to compare the recorded responses among the four experimental groups and identify treatments that may warrant further investigation. The findings were interpreted as preliminary observations, with consideration given to the completeness of the experimental records, replication, and the limitations of the available measurements.

2. Materials and Methods

2.1. Collection and Selection of Experimental Fish

Three mollies representing golden, black, and platinum colour varieties were obtained from a fish market in Malad, Mumbai, Maharashtra, India. The fish were recorded as belonging to the genus *Poecilia*, with *Poecilia sphenops* designated as the species in the original experimental records. We used borewell water collected from Borivali East, Mumbai, Maharashtra, to rear the experimental fish. Initial body weight and body length were recorded before the commencement of the feeding experiment to establish baseline measurements for assessing growth performance. The initial body weight and length of the golden variety were 1.712 g and 4.3 cm, respectively. The black variety weighed 1.726 g and measured 4.8 cm, whereas the platinum variety weighed 1.915 g and measured 4.8 cm. Consistent monitoring of rearing conditions is important in aquaculture experiments because environmental factors can influence fish health and growth [12].

2.2. Collection of Feed Ingredients and Probiotic Preparations

Soybean and soya chunks were procured from local markets in Mumbai, Maharashtra, for preparing the basal experimental feed. Approximately 50 g of soybean and 20 g of soya chunks were obtained for the study. Commercial fish feed (Optimum) was used as the control diet. Two commercial probiotic preparations, Darolac and Viabact, were obtained from a local pharmacy in Mumbai. In addition, the bacterial isolate *Lactobacillus pentosus* B25, obtained from a marine sample, was included as a candidate probiotic treatment.

The experimental design specified four dietary treatments: a control receiving commercial fish feed, a Darolac-associated treatment, a Viabact-associated treatment, and a treatment associated with *L. pentosus* B25. Probiotics have been investigated in aquaculture for their potential to support fish growth, digestive processes, and host health [1].

2.3. Description of Probiotic Preparations

2.3.1. Darolac

Darolac was used as the first commercial probiotic preparation. According to the product information recorded in the original study, it contained *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces boulardii*. The reported microbial concentration was approximately 1.25 billion microbial cells per gram. Darolac was included in the experimental feeding protocol to investigate the potential influence of this commercial probiotic preparation on fish growth. Probiotic formulations containing beneficial microorganisms have been investigated for their possible contributions to fish health and aquaculture performance [3].

2.3.2. Viabact

Viabact was used as the second commercial probiotic preparation. The original experimental records described its composition as including *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, and lactic acid bacilli. The original product description also mentioned *Lactobacillus sporogenes*. Viabact was included as a separate dietary treatment to compare its potential influence with that of the control diet, Darolac, and *L. pentosus* B25. The reported composition reflects the original experimental records and should be verified against the product label before publication. Different probiotic microorganisms may have different effects depending on the host species, microbial strain, and rearing conditions [5].

2.3.3. *Lactobacillus pentosus* B25

The bacterial isolate *Lactobacillus pentosus* B25, obtained from a marine sample, was included as a candidate probiotic strain. Its concentration was recorded as 1.5 billion microbial cells, although the corresponding unit and measurement basis were not specified in the available experimental records. The B25 treatment was included to investigate the potential influence of this bacterial isolate on fish growth during the feeding experiment. Evaluating individual bacterial strains is important because probiotic effects may be strain-specific and cannot automatically be generalized to all microorganisms belonging to the same group [8].

2.4. Preparation of Experimental Feed

Soybean and soya chunks were ground separately into fine powders and mixed in a 1:1 ratio to prepare the basal feed mixture. For the documented preparation procedure, 1 g of the soybean–soya mixture was placed in a 50 mL beaker, and 2 mL of distilled water was added to form a paste. The mixture was blended thoroughly to obtain a uniform consistency, passed through a sieve, and sun-dried before feeding. Commercial fish feed (Optimum) was used for the control group, while Darolac, Viabact, and *L. pentosus* B25 were associated with their respective experimental dietary treatments. The quantities of soybean and soya chunks procured for the study were approximately 50 g and 20 g, respectively; however, the documented feed-preparation procedure specified a 1:1 ratio for the portion used to prepare the basal mixture.

The original records did not specify the exact quantity of each probiotic added to the feed or the final viable-cell concentration per gram of prepared feed. Therefore, these values have not been estimated or assigned.

2.5. Feeding Protocol and Experimental Duration

The feeding experiment was conducted over a period of 15 days. The fish were fed twice daily at a reported feeding rate of 4% of body weight per day. The daily feed quantity was calculated using the body weight of the fish and the prescribed feeding percentage.

Daily feed quantity (g) = Body weight (g) × 4/100

The four dietary conditions comprised a control group receiving commercial fish feed (Optimum), a Darolac-associated treatment, a Viabact-associated treatment, and a treatment associated with *L. pentosus* B25. Body weight and body length were scheduled for measurement at regular intervals throughout the experimental period. The documented feeding rate was 4% of body weight per day; however, the original records did not clearly indicate whether the feed quantity was recalculated after each growth measurement or remained based on the initial body weight. Consequently, no additional feeding adjustment procedure has been assumed.

2.6. Water-Quality Monitoring

Borewell water collected from Borivali East, Mumbai, Maharashtra, was used for rearing the experimental fish. Water temperature was monitored using a thermometer, and pH was assessed during the experimental period. Temperature was recorded in the morning and evening to monitor daily variations. The original Materials and Methods records referred to a pH meter, whereas the original Results section mentioned indicator strips. The precise pH measurement method should therefore be confirmed from the laboratory records before submission.

The water-quality parameters documented during the experiment included temperature, pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite, and nitrate. These parameters were monitored across the control, Darolac, Viabact, and *L. pentosus* B25 treatment conditions. Water-quality monitoring is an important component of aquaculture research because changes in temperature, pH, dissolved oxygen, and nitrogenous compounds can influence fish physiology and rearing conditions [12]. The relationship between ammonia toxicity, pH, and temperature is particularly important when assessing water suitability for fish [13]. However, the available records did not consistently identify the instruments, reagents, or analytical procedures used for measuring dissolved oxygen, ammonia, nitrite, and nitrate. Accordingly, specific analytical methods have not been attributed to these measurements.

2.7. Measurement of Fish Growth Parameters

Fish growth was evaluated by measuring body weight and body length during the 15-day feeding experiment. Measurements were scheduled at three-day intervals on days 0, 3, 6, 9, 12, and 15. Body weight was recorded in grams (g) using a weighing balance, while body length was measured in centimetres (cm) using a measuring scale. The initial measurements served as baseline values, and the measurements recorded at the end of the experimental period were used to calculate growth. Body weight and body length are commonly used indicators in fish nutrition studies for evaluating changes in growth during experimental feeding [14].

2.7.1. Absolute Weight Gain

Absolute weight gain was calculated by subtracting the initial body weight from the final body weight. This parameter represents the net change in body weight during the experimental period and was expressed in grams.

Weight gain (g) = Final body weight (g) – Initial body weight (g)

2.7.2. Percentage Weight Gain

Percentage weight gain was calculated to express the change in body weight relative to the initial body weight. The value was expressed as a percentage using the following equation:

Percentage weight gain (%) = [(Final body weight – Initial body weight) / Initial body weight] × 100

2.7.3. Length Gain

Length gain was calculated by subtracting the initial body length from the final body length. The value was expressed in centimetres.

Length gain (cm) = Final body length (cm) – Initial body length (cm)

These calculations were used to quantify changes in body weight and body length between the beginning and the end of the feeding experiment. The resulting growth parameters were intended to facilitate comparisons among the dietary treatments. Calculations were based on recorded measurements, and missing measurements were not estimated.

2.8. Qualitative Observation Procedure

In addition to quantitative growth measurements, qualitative observations of fish movement and external colour were scheduled on days 1, 5, 8, 11, and 15 of the experimental period. The observations focused on visible swimming activity, movement, general responsiveness, and external colour variation. These characteristics were documented descriptively to monitor observable changes during the experiment. The qualitative observation schedule was distinct from the three-day interval schedule used for body-weight and body-length measurements. Since the original experimental records did not specify a standardized numerical scoring system for movement or colour, these characteristics were treated as qualitative observations rather than quantitative growth parameters.

2.9. Statistical Analysis

The original study records identified Minitab version 21 as the statistical software used for analysis and one-way analysis of variance (ANOVA) as the method for comparing treatment-related growth measurements. One-way ANOVA is generally used to evaluate whether the means of a numerical response variable differ among multiple groups. In the present study, the intended comparison involved the control, Darolac, Viabact, and *L. pentosus* B25 dietary treatments. However, the available records do not fully specify the number of independent fish allocated to each treatment, the replication structure, or the exact dataset used for the reported analysis. These details are necessary to establish the appropriate experimental unit and assess the validity of the statistical comparison.

Therefore, the statistical procedure is described according to the original records, without introducing unverified sample sizes or making unsupported claims about statistical significance.

3. Results and Discussion

3.1. Initial Characteristics of Experimental Fish

Three molly fish, identified as *Poecilia sphenops*, were obtained from a fish market in Malad, Mumbai, Maharashtra, India. The fish represented golden, black, and platinum colour varieties. Their initial body weight and length were recorded before the 15-day feeding experiment.

Table 1: Initial characteristics of experimental fish

Fish Identification	Initial Body Weight (g)	Initial Body Length (cm)	Colour Variety
Fish 1	1.712	4.3	Golden
Fish 2	1.726	4.8	Black
Fish 3	1.915	4.8	Platinum

Initial body weights ranged from 1.712 to 1.915 g, while body lengths ranged from 4.3 to 4.8 cm. These measurements provided baseline information for evaluating growth during the experimental period. The experiment included four dietary treatments: commercial fish feed (control), Darolac, Viabact, and *Lactobacillus pentosus* B25.

3.2. Water-Quality Analysis

Water temperature, pH, dissolved oxygen, and nitrogenous waste concentrations were recorded to assess the rearing conditions during the experiment.

3.2.1. Temperature and pH

Table 2: Temperature and pH in the experimental fish pots

Treatment	Morning Temperature (°C)	Evening Temperature (°C)	pH
Control	26.0–26.5	27.0–27.5	7.7–7.8
Darolac	26.0–26.5	27.0–27.5	7.6–7.8
Viabact	26.0–26.5	27.0–27.5	7.6–7.8
L. pentosus B25	26.0–26.5	27.0–27.5	7.6–7.8

The morning temperature ranged from 26.0 to 26.5°C, while the evening temperature ranged from 27.0 to 27.5°C. The pH ranged from 7.6 to 7.8 across the experimental treatments. The recorded temperature and pH ranges were broadly comparable among the treatments, providing similar environmental conditions for fish rearing.

3.2.2. Dissolved Oxygen

Table 3: Dissolved oxygen concentrations in different treatments

Treatment	Dissolved Oxygen (mg/L)
Control	6.2
Darolac	6.4
Viabact	6.5
L. pentosus B25	6.6

Dissolved oxygen concentrations ranged from 6.2 to 6.6 mg/L. The highest recorded concentration was observed in the B25 treatment, followed by Viabact, Darolac, and the control. Dissolved oxygen is essential for fish respiration and normal metabolic activity. The recorded values indicated slight differences among the treatment groups.

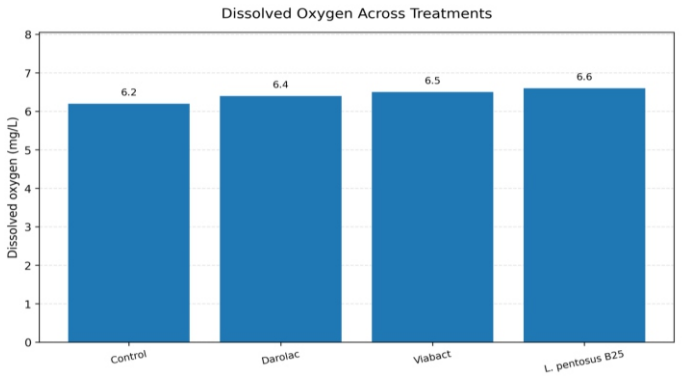


Figure 1. Comparison of dissolved oxygen concentrations (mg/L) in the water of molly fish (Poecilia sphenops) across different probiotic treatments during the experimental period.

3.2.3. Nitrogenous Waste Concentrations

Table 4: Nitrogenous waste concentrations in different treatments

Treatment	Total Ammonia Nitrogen (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
Control	0.20	0.05	5.0
Darolac	0.18	0.04	4.8
Viabact	0.16	0.04	4.5
L. pentosus B25	0.15	0.03	4.3

Total ammonia nitrogen ranged from 0.15 to 0.20 mg/L, nitrite ranged from 0.03 to 0.05 mg/L, and nitrate ranged from 4.3 to 5.0 mg/L. The B25 treatment showed the lowest recorded concentrations of total ammonia nitrogen, nitrite, and nitrate. Compared with the control, total ammonia nitrogen was 25% lower, nitrite was 40% lower, and nitrate was 14% lower in the B25 treatment. These findings indicate differences in the recorded water-quality parameters among the experimental treatments.

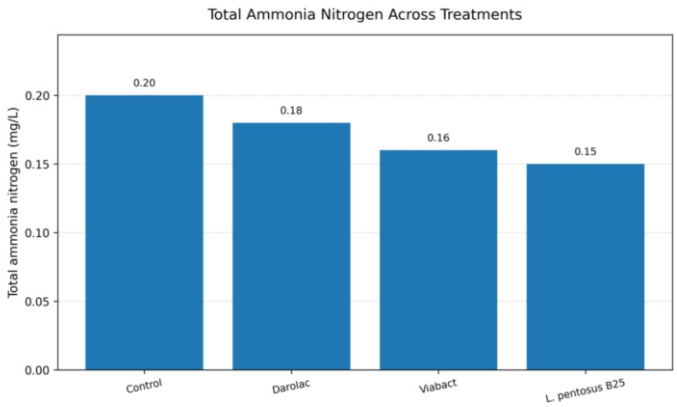


Figure 2. Total ammonia nitrogen concentrations (mg/L) recorded in the rearing water of molly fish (Poecilia sphenops) receiving different probiotic treatments.

3.3. Comparative Analysis of Body Weight

Body-weight gain was used to compare the growth performance of fish receiving different dietary treatments over the 15-day experimental period.

Weight gain was calculated using the following formula:
Weight Gain (g) = Final Weight – Initial Weight
Weight Gain (%) = (Weight Gain / Initial Weight) × 100

Table 5: Body-weight changes during the 15-day experimental period

Treatment	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)
Control	1.712	2.040	0.328	19.16
Darolac	1.726	2.144	0.418	24.22
Viabact	1.915	2.446	0.531	27.73
L. pentosus B25	1.920	2.480	0.560	29.17

The control group showed a weight gain of 0.328 g (19.16%). The Darolac group showed a gain of 0.418 g (24.22%), while the Viabact group gained 0.531 g (27.73%). The B25 treatment showed the highest weight gain of 0.560 g (29.17%). Compared with the control, the reported weight gain was approximately 27.4% higher in the Darolac group, 61.9% higher in the Viabact group, and 70.7% higher in the B25 group. Among the four treatments, B25 showed the highest reported weight gain, followed by Viabact, Darolac, and the control. These results suggest that probiotic supplementation may have potential benefits for fish growth.

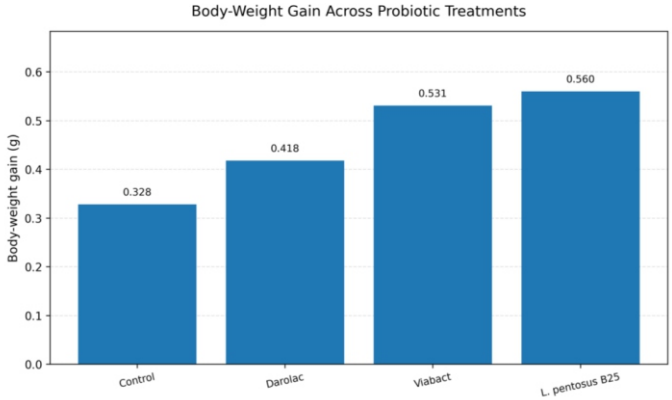


Figure 3. Comparative body-weight gain (g) of molly fish (Poecilia sphenops) fed different probiotic-supplemented diets over the 15-day experimental period.

3.4. Comparative Analysis of Body Length

Body length was evaluated as an additional indicator of fish growth during the experimental period.

Table 6: Body-length changes during the 15-day experimental period

Treatment	Initial Length (cm)	Final Length (cm)	Length Increase (cm)
Control	4.3	4.9	0.6
Darolac	4.8	5.3	0.5
Viabact	4.8	5.5	0.7
L. pentosus B25	4.8	5.6	0.8

The control group showed a length increase of 0.6 cm, while the Darolac group showed an increase of 0.5 cm. The Viabact group showed a length increase of 0.7 cm, whereas the B25 group showed an increase of 0.8 cm. The highest recorded length increase was observed in the B25 treatment, followed by Viabact, the control, and Darolac. These results indicate a potential positive effect of probiotic supplementation on fish growth.

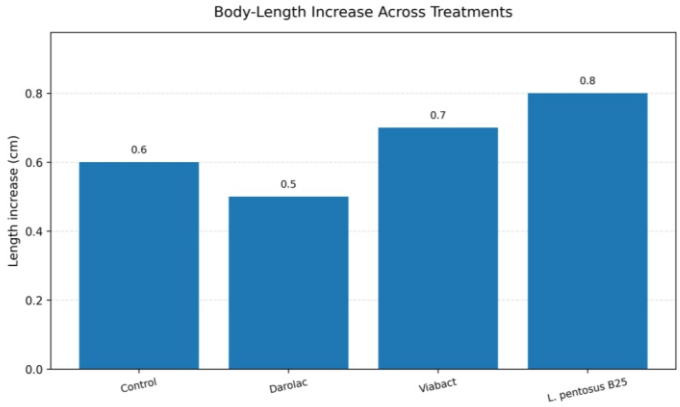


Figure 4. Comparison of body-length increase (cm) in molly fish (Poecilia sphenops) receiving different dietary treatments over the 15-day experimental period.



Figure 5. Photographic documentation of body-length measurements in molly fish (Poecilia sphenops) during the 15-day experimental period under different dietary treatments.

3.5. Qualitative Observations of Fish Movement and Colour

Fish movement and external colour were observed during the 15-day experimental period.

Table 7: Observations of fish movement and colour

Observation Day	Control	Darolac	Viabact	*L. pentosus* B25
Day 1	Baseline condition	Baseline condition	Baseline condition	Baseline condition
Day 5	No noticeable change	Improved movement and colour variation	Improved movement and colour variation	Improved movement and colour variation
Day 8	No noticeable change	Improved movement	Improved movement	Improved movement
Day 11	No noticeable change	Improved movement and colour variation	Greater movement and colour variation	Greater movement and colour variation
Day 15	Comparatively unchanged	Improved movement and colour variation	Greatest observed movement and colour variation	Greatest observed movement and colour variation

The observations indicated differences in movement and colour variation among the treatments. By Day 15, the Viabact and B25 groups showed the greatest qualitative changes in movement and colour, while the control group remained comparatively unchanged. These observations were consistent with the overall pattern of higher reported growth in the probiotic-treated groups.

3.6. Statistical Analysis

The reported one-way analysis of variance (ANOVA) yielded an F-value of 5.05 and a p-value of 0.026. A p-value below 0.05 generally indicates a statistically significant difference when the analysis is appropriate for the experimental design and its assumptions are satisfied. The reported values suggest differences among the treatment groups. However, the statistical result should be interpreted in conjunction with the underlying experimental data and design.

3.7. Overall Interpretation

The results showed differences in body-weight gain, body-length increase, water-quality parameters, and qualitative observations among the dietary treatments. The B25 treatment showed the highest reported weight gain and length increase. It also showed the lowest recorded concentrations of total ammonia nitrogen, nitrite, and nitrate, along with the highest dissolved oxygen concentration. Viabact also showed higher reported weight gain than Darolac and the control. The qualitative observations indicated greater movement and colour variation in the Viabact and B25 groups towards the end of the experimental period. Overall, the findings suggest that probiotic-supplemented diets may have potential benefits for fish growth and rearing conditions.

4. Conclusion

The present study investigated the growth-promoting potential of three probiotic preparations, Darolac, Viabact, and *Lactobacillus pentosus* B25, in molly fish (*Poecilia sphenops*) over a 15-day experimental period. The results indicated differences in growth performance, water-quality parameters, and behavioural observations among the dietary treatments. B25 showed the highest reported weight gain of 0.560 g (29.17%), followed by Viabact at 0.531 g (27.73%) and Darolac at 0.418 g (24.22%), compared with 0.328 g (19.16%) in the control group. Viabact also showed the greatest recorded increase in body length among the treatments with available measurements.

Water-quality analysis revealed lower concentrations of total ammonia nitrogen, nitrite, and nitrate in the probiotic-treated groups, with B25 showing the lowest recorded values and the highest dissolved oxygen concentration. Qualitative observations also indicated greater movement and colour variation in the Viabact and B25 groups towards the end of the experiment. These findings suggest that probiotic supplementation may contribute to improved fish growth performance and support suitable rearing conditions, although the observed differences require further validation.

Overall, *L. pentosus* B25 emerged as a promising candidate for further investigation as a dietary probiotic in ornamental aquaculture. However, the short experimental duration and limitations in the available growth and replication data restrict definitive conclusions regarding its effectiveness. Further studies involving adequately replicated groups, verified probiotic concentrations, longer feeding trials, and appropriate statistical analyses are necessary to confirm these findings and establish the practical application of probiotics in sustainable fish culture.

5. Declarations

5.1. Funding

This research was conducted without any external financial support from government agencies, private organisations, commercial institutions, or other funding bodies. The study was carried out using the resources available to the researcher. No specific grant was received for conducting the research, preparing the manuscript, or publishing the findings.

5.2. Conflict of Interest

The author declares that there are no financial, personal, professional, or commercial conflicts of interest that could have influenced the design of the study, experimental procedures,

interpretation of the findings, or preparation of the manuscript. The selection and evaluation of the probiotic treatments were undertaken for academic and scientific purposes.

5.3. Ethics Statement

The study involved the maintenance and observation of molly fish (*Poecilia sphenops*) under aquarium conditions during a 15-day experimental period. The fish remained alive at the end of the experimental period.

5.4. Data Availability Statement

The data associated with this study include the recorded fish growth measurements, water-quality parameters, and qualitative observations collected during the experimental period. Data availability will be determined in accordance with the researcher's records and institutional requirements. Supporting data may be provided by the corresponding author upon reasonable request, where available.

5.5. Use of Artificial Intelligence

An artificial intelligence (AI)-assisted tool was used exclusively to support language editing, grammatical correction, and improvement of the clarity and readability of the manuscript. The tool was not used as a substitute for experimental work or the collection of research measurements. The author is responsible for reviewing the manuscript, verifying the accuracy of the scientific content, and ensuring that the final version complies with the ethical and publication requirements of the target journal.

5.6. Author Responsibility

The author accepts responsibility for the accuracy and integrity of the manuscript, including the presentation of experimental observations, interpretation of the results, and appropriate acknowledgement of relevant scientific literature. The author also acknowledges the importance of transparent reporting, accurate data presentation, and compliance with the publication ethics requirements of the selected journal.

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