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Effect of *Lactobacillus* Probiotic Supplement on Growth and Haematological Performance of Indian Major Carp *Labeo rohita*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The influence of probiotics bacteria *Lactobacillus acidophilus* improves the survival, growth, and Hematological performance of the Indian major carp Rohu (*Labeo rohita*). After acclimation, a 60-day experimental study was conducted with four different types of treatments; namely, T1: Control,

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i.e. Basal feed without Probiotic supplements, T2: Basal feed with *Aeromonas hydrophila*, T3: *Lactobacillus* supplemented probiotic feed without *Aeromonas hydrophila*, and T4: *Lactobacillus* supplemented probiotic feed with *A. hydrophila*. After the experimental study, growth parameters were monitored through Weight gain, Specific growth rates, survival percentage, and haematological parameters including WBC, RBC, haemoglobin (Hb), and haematocrit (Hct) values of probiotics treated group (T3 and T4) were significantly (P<0.05) higher than control and T2 group. The Present study indicated that the positive effects of the *L. acidophilus* probiotic supplement diet improved the survival, growth, and Haematological performance in aquaculture.

Keywords: Probiotic; lactobacillus; Labeo rohita; growth performance.

1. INTRODUCTION

"Aquaculture is one of the emerged and foremost promising sectors in India and it constantly provides high-quality animal protein, raises nutritional levels, and generates income and employment around the country during the last three decades" (Department of Fisheries, 2023). "It has attracted the attention of both the public and private sectors. However, Unwise management practices, disease outbreaks, and bad environmental conditions continually hinder the productivity of aquaculture industries" (FAO 2022). "*Aeromonas hydrophila* is a Gram-negative bacterium widely distributed in aquatic environments and an opportunistic pathogen for fish and humans, which can cause severe hemorrhagic septicemia and skin ulceration in aquatic animals and diarrhea in mammals. It causes motile *Aeromonas* septicemia (MAS), also named bacterial hemorrhagic septicemia, that affects numerous species of freshwater carp and marine fishes and leads to massive mortalities of wild and farmed fish. For control the pathogenic bacteria and disease management, chemotherapies and, antibiotics are commonly used in fish farming industries, on the other hand, the overuse of chemical antibiotics has led to the development of antibiotic-resistant microorganisms, which has been causing havoc in the aquaculture industry in recent times" (Arumugam et al. 2023). "They caused severe problems not only for fish but also for fish consumers and the environment. The problems outlined above and recent restrictions on the use of antibiotics have resulted in aquaculture, natural immunostimulants, and probiotics being considered as an alternative strategy for disease management and better yield. The application of probiotics in aquaculture is such a technology and research as bioremediation and biocontrol agents are increasing with the demand for eco-friendly aquaculture" (Ramesh et al. 2019).

"The use of probiotics is critical for improving the habitat of aquatic animals and increasing their performance while having no negative consequences for consumers. There is a lot of interest in using probiotics in fish as feed additives to improve feed values, nutrient absorption, gut microbial community, production of lactic acid, digestive enzymes and increase the available nutrients to the host" (Yonar 2019; Subasinghe et al. 2023; Romano 2020, and Dahiyart al. 2012). Several probiotics have been used in aquaculture but probiotics from lactic acid bacteria (LAB) are often used to improve growth performance and disease resistance in freshwater fish culture, especially carp. Therefore, the present study was carried out using the feed supplement of probiotic bacteria *L. acidophilus* to evaluate their potential benefits on the survival, growth, and Haematological parameters of Indian major carp *Labeo rohita* against the potential fish pathogen of *A. hydrophila*.

2. MATERIALS AND METHODS

Collection and maintenance of Experimental fish: The present research was conducted at the PG Department of Zoology at Sarah Tucker College, Tirunelveli, Tamilnadu, India. Rohu (*Labeo rohita*) mean body weight of 7 ± 0.5 g and length of 3.5 ± 1.5 cm were purchased from Commercial Fish Farm, Kallidaikurichi, Tirunelveli district, Tamil Nadu, India, and were stocked for acclimation to the laboratory conditions for about 10 days before they were used for experimentation. The dechlorinated river water was used during the acclimatization and experimental period. The water tanks were well-oxygenated with electrical aerators. The water pH and temperature were maintained during the experimental period.

Experimental diet preparation: The experimental diet containing varying amounts of the ingredients used in the formulation of basal

feed were fishmeal, rice bran, groundnut oilcake, vitamin capsules, and mineral premix. In probiotic-supplemented feed, *Lactobacillus acidophilus* 1.5 ×10^9CFU g/ml was added along with the basal diet. Fish were fed experimental diets at a rate of 6% in the first month and 5% in the second month of their body weights, and feeding was done twice daily at 09: 00 AM and 5: 00 PM for 60 days. Every fifteen days, the fish of each group were live-weighed to calculate the amount of feed consumed during the experimental period.

Experimental setup: For experimental setup 4 plastic troughs were used. Each trough was stacked with *L. rohita* fingerlings of 20 numbers (7.0 ± 1. 5g and mean body length 3.5 ± 1.5 cm). In the control trough T1: Basal feed without Probiotic supplements, T2: Basal feed with *Aeromonas hydrophila*, T3: *Lactobacillus* supplemented probiotic feed without *Aeromonas hydrophila*, and T4: *Lactobacillus* supplemented probiotic feed with *A. hydrophila* respectively. The length and weight of 10 fish randomly selected from each trough were measured every 15 days and the experiment was performed for 60 days. After the 45th day of the experiment, the fish were challenged with the bacterial pathogen *A. hydrophila* for 15 days. Blood was collected by the caudal vein puncture and pooled from a random sample of five fish in each experimental tank after anesthetizing them with Eugenol after 60 days. The blood (heparinized 150 in 1 ml) collected from each group was tested for Total Leucocyte counts (WBC), Total Erythrocyte count (RBC), haemoglobin (Hb), and haematocrit (Hct) values.

Growth parameters: Fish of each experimental tank were recorded in length and weight every fifteen days. The fish were weighed and lengthed, to calculate a final weight (FW), weight gain (WG), specific growth rate (SGR) feed conversion rate (FCR), and Survival Rate. Two fish were randomly collected from each tank (n = 10) (Markowiak and Slizewska 2018).

Growth Measurements: The following growth parameters were assessed during the experimental period.

Table 1. Composition of feed ingredients

S. No.	Ingredients	Composition (%) Basal Feed	Composition (%) Probiotic Feed
1.	Soybean meal	15	15
2.	Fish meal	15	15
3.	Tapioca Powder	10	10
4.	Ground nut oil cake	30	30
5.	Rice Bran	20	20
6.	Maize	5.0	5.0
7.	Sunflower oil	3.5	2.0
8.	Vitamin and Mineral Mixture	1.5	1.5
9	*Lactobacillus acidophilus*	-	1.5

a) %Weight Gain in g $= \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} \times 100$

b) Total length gain (TLG) = Final total length (TL)(cm) - Initial total length (TL)(cm)

c) Specific Growth Rate (SGR) $= \frac{Ln\,Wt - LnWo}{t} \times 100$

Were, Wt = Final weight, Wo= Initial weight and t = time duration

d) Food Conversion Ratio (FCR) = $\frac{Weight\ of\ food\ given\ (g)}{Weight\ gain\ of\ fish\ \ (g)}$

e) Survival Rate = $\frac{Nt \times 100}{No}$

Where, Nt = Final number of fish and No = Initial number of fish

2.1 Haematological Examination

Total Erythrocyte count (RBC): Total Erythrocyte count (RBC) was done in an Improved Neubauer counting chamber using RBC diluting fluid (Clark et al. 2015). The following formula is used to calculate the number of RBC per µl of the blood sample:

$$\mathrm{RBC}(10^6/\mathrm{mm}^3) = \frac{\text{Total no. of cell in 5 large squares} \times \text{df} \times \text{depth factors}}{\text{No. of small square counted}} \times 16$$

Total Leucocyte counts (WBC): 20 µl of blood was mixed with 380µl of WBC diluting fluid in a clean glass vial. The number of WBC per µl of the blood sample was calculated using the following formula:

$$\mathrm{WBC}(10^3/\mathrm{mm}^3) = \frac{\text{Total no. of cell in1 large square} \times \text{df} \times \text{cf}}{\text{Volume factor}(0.1)}$$

Haemoglobin (Hb): The blood's haemoglobin level was determined by using 5 ml of Drabkin's working solution combined with 20 µl of blood. The following formula was then used to determine the haemoglobin concentration:

Conc. of Hb in sample = Abs. of sample÷ Abs of Standard × Conc. of standard.

Haematocrit value (Hct): Haematocrit value (Hct), was determined by centrifugation at 2000 rpm for 20 min. A suitable quantity of whole blood mixed with an anticoagulant is centrifuged in a haematocrit tube until all blood cells are packed at the bottom of the tube.

Haematocrit value (Hct) = (Height of RBCs in mm/Height of RBC and plasma) X 100

Statistical Analysis: The data were statistically analyzed by one-way ANOVA using SPSS software version 22.0. Further evaluation was conducted by Duncan's multiple comparisons to compare the differences among groups. The results are shown as the mean values ± standard error of the mean, and the levels of significance among treatment groups were set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Growth performance and feed utilization: The effect of *L. acidophilus* probiotic supplement on the growth performance of Indian major carp *L. rohita* under different treatments and control groups (T1) is presented in Table 2. The main beneficial effects of probiotics in fish farming are improvements in growth performance, haematology, and immunity (Sun et al. 2010 and Kondera et al. 2019). The present study, the final average length of fish ranged from 10.2± 12.8 cm. The lowest mean length of fish was 10.2±0.5 cm in the T2 trough, while the highest length was observed 12.8±1.0 and 12.2±1.5 cm in the T3 and T4 trough respectively. The mean weight of fish ranges from 7.2±1.0 to 26.4±1.0 g. The lowest in 22.2±0.5g and 25.2±1.0g in the T2 and T3 trough respectively. Significantly (P< 0.05) increased length and weight were noticed in the experimental fish group than in the control and pathogen-treated group. It indicated that the selected probiotics have influenced the growth of *L. rohita*. Similar observations have been reported on *L. rohita (*Munirasu & Ramasubramanian 2017). Similarly, Saha et al. (2015*)* tested different concentrations of *S. cerevisiae-supplemented* diets in *M. rosenbergii* and after 90 days of feeding, they observed significant differences in morphometric data (length and weight) with more in control. Also, in agreement with the *Bacillus* probiotic that resulted high in terms of weight gain (g), length gain (cm), SGR (%), Percentage of weight gain, and percentage of length gain growth of *O. niloticus* was found in T1 (0.2% probiotic) diet group (Das et al. 2019).

The specific growth rate (% /day) of fish *L. rohita* ranges from 3.10± 1.5 to 3.82± 1.7 for the experimental and control group. The highest value was 3.82± 1.7 in T4 and 3.10 in T2 tank was recorded. The lowest value of SGR was 3.10 in the T2 tank and 3.45 in the T1 group respectively (Table 2). There are no significant differences (P< 0.05) in the SGR of fish, among control and treatment groups respectively (Table 2). Mahmoud et al. (2021) reported the improved specific growth rate of *Oreochromis niloticus* by using natural and biological feed additives. Mohapatra et al. (2014) reported SGR of *L. rohita,* fed the diet with graded levels of probiotics Rahman et al. (2019) reported that probiotic feed PRO2 fed *O. pabda* fish showed a higher SGR.

The maximum food conversion efficiency was recorded in T4 trough (5.8 ± 1.0) and T3 (Control) 5.2 ± 0.6 in T3 tank. The minimum was recorded in T2 tank (4.4 ± 1.0). Effect of *Lactobacillus* dietary supplement improves growth performance and maximum FCR of European Carp. Ali et al. (2018) reported that FCR were significantly (*P*<0.05) lower in *Mystus cavasius* fishes of T1 group fed with probiotics *Bacillus* sp. compared with the other groups. The range of FCR improved the *Lactobacillus bulgaicus* dieted group on carp. These findings are consistent with the findings of the present study.

The survival of fish recorded in different treatments and control group (T1) are presented in Table 2. The highest average survival rate of 88.2± 1.2% in T4 trough and the lowest average survival rate was observed in T2 (70.6±1.2). Significant differences (P< 0.05) in the survival of fish were observed among both control and T3 treatment groups. This is an agreement with the findings of Munirasu & Ramasubramanian (2017) who reported 96-99% survivability of *L. rohita* by using probiotics mixed feed. Ahmed et al. (2020) reported that the overall survival of Tilapia was 95.76-97.54% by using commercial floated feed with probiotics in cages at Dakatia river.

Haematology is an important factor that could be considered for the fish diet quality assessment. Fazio et al. (2019); Galagarza et al. (2017) and Seibel et al. (2021) reported that the most common blood variables consistently influenced by diet are the haematocrit (Hct) and haemoglobin (Hb) levels. This study evaluates the effect of the probiotic on the blood parameters of the fish *Labeo rohita* using *L. acidophilus*-supplemented probiotic diets are included in Table 3. Overall, the dietary probiotic induced significant changes (P < 0.05) in leucocyte counts, compared with the T2 and Probiotic treated T3 group (6.6±1.2 and 8.1 ±1.0).

There was an increased Total erythrocyte count in fish that received T3 and T2 group (10.2±0.5 and 12.8±1.0) (Table 3). The percentage of haematocrit was significant (P < 0.05) when compared to the *A. hydrophila* treated group T2. However, non-significant differences in the haemoglobin contents of the control and the treatment groups were observed. The haemoglobin content oscillated from the T3 and T4 group values of 12.4±0.2 and 12.2±0.2. Overall high significance of haematological parameters was recorded between T2 and T4 group. These results supported the results of Azarin et al. (2015) and Sharma et al. (2013).

Probiotics have been used in Nile tilapia Elsabagh et al. (2018); Reda and Selim (2015) and Silva et al. (2015) which reported positive effects on haematological parameters. On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis* or supplemented with *Pediococcus acidilactici* Ferguson et al. (2010) observed some variation (but not significant) in Hb and Hct contents among the control and fish-fed enriched diet with probiotics. Fish fed the diet supplemented with probiotics showed the highest values of Hb, RBCs, and WBCs. Mocanu Cretu et al. (2011) Earlier Reports indicated that fish groups fed the diet supplemented with dead *Saccharomyces cerevisiae* yeast and both of live *B. subtilis* and *S. cerevisiae* showed significant (P < 0.05) increase in the Hct level when compared to fish fed the control diet. Firouzbakhsh et al. (2012) Reported that Hb concentration, in rainbow trout (*Oncorhynchus mykiss*) fed different levels of probiotics was significantly (P < 0.05) different from the control.

Table 2. Growth performance of *L. rohita* under different treatments observed during experimental periods

Treatments	Growth parameters						
	Initial length (cm)	Final length(cm)	Initial weight(g)	Final weight(g)	Specific growth rate%	Food Conversion Ratio (FCR)	Survival rate %
T1(Control)	3.5±1.0	11.8±1.5	7.2±1.0	26.4±1.0	3.45± 1.2	4.8 ± 1.2	82.5±1.4
T2	3.6±1.0	10.2±0.5	7.4±1.0	22.2±0.5	3.10± 1.5	4.4 ± 1.0	70.6±1.2
T3	3.6±1.0	12.8±1.0	7.2±1.0	25.2±1.0	3.62± 1.4	5.2 ± 0.6	74.8 ± 1.8
T4	3.5±1.0	12.2±1.5	7.2±1.0	27.4±1.5	3.82± 1.7	5.8 ± 1.0	88.2 ± 1.2

T1: Basal feed without Probiotic supplements
T2: Basal feed with Aeromonas hydrophila
T3: Lactobacillus supplemented probiotic feed without Aeromonas hydrophila
T4: Lactobacillus supplemented probiotic feed with A. hydrophila

Table 3. Haematological parameters of *L. rohita* under different treatments observed during experimental periods

Treatments	Haematological parameters			
	(WBC)	Total Erythrocyte count (RBC)	Haemoglobin (Hb)	Haemocytocrit value (Hct)
T1(Control)	7.8 ±1.0	4.8±0.1	11.6±0.1	36.8±0.8
T2	6.6±1.2	4.0±0.5	11.2±0.4	32.8±0.2
T3	8.0±1.4	4.8±0.6	12.4±0.2	35.2±0.4
T4	8.1 ±1.0	4.6±0.4	12.2±0.2	37.4±0.6

4. CONCLUSION

Based on the obtained data on growth parameters, feed utilization, and haematological parameters the experiment revealed that the *L. acidophilus* probiotic supplement diet has significant efficiencies in improving growth performance in *L. rohita*. This experiment can recommend that probiotics be used in the culture of Rohu as a potential supplement and an eco-friendly tool for healthy aquaculture. However, a more in-depth study is required to elucidate the biochemical and immunological parameters for probiotic supplementation(s) towards sustainable carp culture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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