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Effect of probiotic (*Lactobacillus acidophilus*) and prebiotic (*Spirulina platensis*) Dietary supplementation on the growth performance and Biochemical composition of *Labeo rohita* fingerlings

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Abstract

This study aimed to evaluate the growth performance of *Labeo rohita* fingerlings in response to dietary supplementation with (*Lactobacillus acidophilus)* and (*Spirulina platensis)*. Experimental diets were formulated by incorporating *L.acidophilus* and *S. platensis* at three different concentration levels: 2%, 4%, and 6%. These diets were administered to (*Labeo rohita)* fingerlings over a 75th day period. The fish were maintained under continuous natural aeration, with water exchange conducted once daily for 14 days, maintaining water parameters at pH 7.5, temperature between 30-33°C, dissolved oxygen levels at 6.5 mg/L, and salinity at 0.5 ppt. During the acclimatization phase, fingerlings were fed a Control Diet. Results indicated that the Survival Rate (SR), Specific Growth Rate (SGR), Food Conversion Ratio (FCR), and Weight Gain (WG) were significantly higher ($P < 0.05$) in the group of *Labeo rohita* fingerlings fed with a 6% supplementation of *L. acidophilus* and a similar 6% supplementation of *S. platensis* (210 individuals). Furthermore, the levels of total carbohydrates, proteins, lipids, and triglycerides were also significantly higher ($P < 0.05$) in the 6% *L. acidophilus*-supplemented diet compared to the 6% *S. platensis* supplemented diet in Rohu fingerlings. Among all the experimental groups, the group fed with a diet that replaced 6% of fish meal with *L. acidophilus* exhibited the most significant performance improvement, followed by the group fed with a 6% *S. platensis* supplemented diet. These results suggest that partially replacing fish meal with *L. acidophilus* is advantageous for the culture of Rohu fingerlings, with similar benefits observed for a 6% supplementation of *S. platensis* in the diet.

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Introduction

The most important of the three principal carp species that are used in carp polyculture systems in India is rohu (*Labeo rohita*). (FAO, 2009). This graceful Indo-Gangetic riverine species is endemic to the rivers of northern and central India, as well as Pakistan, Bangladesh, and Myanmar. It has been introduced successfully into practically all riverine systems in India, including the freshwater parts of Andaman, where its population has grown. This species has also been introduced into Sri Lanka, the former Soviet Union, Japan, China, the Philippines, Malaysia, Nepal, and numerous African nations. This carp's traditional cultivation extends back centuries and has been performed in tiny ponds in eastern Indian regions.

The production of *Labeo rohita* fingerlings plays a pivotal role in sustaining and enhancing the aquaculture industry in India, meeting the increasing demand for fish protein and generating employment opportunities in rural areas. As per the Food and Agriculture Organization (FAO, 2020). India is one of the world's leading producers of fish, and Rohu ranks among the top species cultured in the country. With its excellent taste and high nutritional value, Rohu has earned a prominent place in Indian cuisine, making it a preferred choice among consumers. Now a days, Probiotics have been employed as dietary supplement to enhance fish growth and improve resistance against disease at every stage (Faramarzi *et al.*, 2011; Allameh *et al.*, 2017).

The dietary use of certain probiotics, like *Lactobacillus* spp., continues to exhibit promising outcomes in the growth and survival of both freshwater and marine fish species. Numerous studies over the years have supported this notion. For instance, research conducted by Taoka *et al.*, (2006), Al - Dohail *et al.*, (2009), Wang *et al.*, (2011), Lara-Flores and Olvera-Novoa 2013, Rodrigues *et al.*, (2014), Hoseinifar *et al.*, (2016), Akter *et al.*, (2019), and Dawood *et al.*, (2019) have all contributed to our understanding of the positive effects of probiotics on fish.

One particular strain, *Lactobacillus acidophilus*, has exhibited various beneficial characteristics in relation to fish health. It has been found to adhere to the mucosal lining of fish intestines, as demonstrated by Liu *et al.*, (2013). Additionally, it showcases the ability to withstand a wide range of pH levels, a trait highlighted by Gebara *et al.*, (2013). Furthermore, *L.acidophilus* has been linked to an increase in enzyme activity within the fish's gastrointestinal tract, as noted by Akter *et al.*, (2019). Moreover, it has been shown to produce antimicrobial compounds, as elucidated by Todorov *et al.*, (2011). Lastly, this probiotic strain has the capacity to modulate the immune response in fish, as supported by Villamil *et al.*, (2014). These collective attributes establish *L.acidophilus* as a valuable probiotic for fish, as previously demonstrated in the case of *C. estor*, where its use resulted in positive effects on the growth and survival of fish larvae, as reported by Martínez-Ángeles *et al.*, (2022). These findings continue to underscore the potential benefits of probiotics in the aquaculture industry and their role in enhancing the health and performance of fish species.

Spirulina platensis continues to be a widely utilized nutritional supplement in many countries for both human and animal consumption, it is renowned for its nutritional prowess, being recognized as a potent source of proteins, carbohydrates, polyunsaturated fatty acids, sterols, minerals, and vitamins, as demonstrated in the study by Sarma *et al.*, (2010). Additionally, research conducted by Amin *et al.,* (2006) has highlighted *S.platensis* remarkable ability to provide protection against the harmful effects of heavy metal toxicity. This versatile and nutrient-rich microorganism remains a valuable asset in promoting health and combatting environmental challenges in today's world.

S.platensis is widely recognized as one of the most abundant natural sources of essential nutrients such as proteins, vitamins, minerals, essential amino acids, fatty acids, and antioxidant pigments, including carotenoids Radhakrishnan *et al.*, (2014). The unique composition of *Spirulina* protein stands out due to its

balanced blend of amino acids, including methionine and tryptophan, which closely resembles that of casein, a protein found in dairy products. Notably, *Spirulina* protein holds great promise as a cost-effective alternative to animal-derived feeds in aquaculture Habib *et al.*, (2008). Its potential to serve as either a partial supplement or a complete replacement for protein in aqua-feeds not only offers economic advantages but also aligns with sustainable practices in the aquaculture industry. This remarkable versatility and nutritional richness make *S.platensis* a valuable resource for improving the quality and sustainability of aquaculture operations.
This study investigates the impact of dietary probiotic and prebiotic supplementation on growth profiles such as survival rate (SR), specific growth rate (SGR), food conversion ratio (FCR), and weight gain (WG). and biochemicals (carbohydrate, protein, lipid, and triglyceride).

Materials and methods

Collection and acclimation

Labeo rohita fingerlings, weighing 5±0.4 grams and measuring 5.25±0.2 centimeters in length, were collected from the Surya Fish Farm in Kallidaikurichi, (Latitude 8° 40' 47'' N and Longitude 77° 28' 35'' E), Ambasamudram, Tirunelveli District, Tamil Nadu, India. These fingerlings were captured using a 12-meter cast net and transported to the CARE (Center for Aquaculture and Research Extension) laboratory at St. Xavier's College (Autonomous) (Latitude 77.73.88°E and Longitude 8.7180°N), Palayamkottai, Tamil Nadu, India, in oxygenated polythene bags (with a density of 100-130 kg/m³ and thickness of 0.1-0.15 millimeters).

In the CARE laboratory, the fingerlings were placed in a cement tank measuring nine feet and five feet (9' × 5'). They were acclimated to tap water with continuous aeration for fourteen days before the commencement of the experiment. The ingredients of the control feed consisted of (Anchovy) Fish meal (25%), Soya bean meal (25%), Groundnut oil cake (GNOC) (25%), Rice bran (12%), Tapioca flour (5%), Sunflower oil (3 ml), Egg albumen (4 ml), and Multivitamin tablets (Minerals and Vitamins) (1 gm). Control feed Rohu fingerlings were used as a reference for comparing growth rates and biochemical performance against those fed with experimental diets containing *Lactobacillus acidophilus* and *Spirulina platensis*.

Experimental Procedure and Feeding Regimen

Labeo rohita fingerlings of uniform size (5±0.4 grams in weight and 5.25±0.25 centimeters in length) were meticulously selected for the feeding experiments. Various feeds at different concentrations were used, including *Spirulina platensis* at different concentrations: T_1S (2%), T_2S (4%), and T_3S (6%), and the probiotic bacterium *Lactobacillus acidphilus* at different concentrations: T_1L (2%), T_2L (4%), and T_3L (6%). TO (0%) represented the control group, which received no supplementary feed. Each treatment was conducted in triplicate. Ten fishes were introduced into each plastic trough, and they were fed with an amount equivalent to 3% of their body weight twice a day (10AM and 5PM).

The water in the aquarium tank was siphoned off every day to remove and store the remnants of the feed (unconsumed) and fecal matter in separate containers for sun drying. The experiment spanned 75 days, during which the effects of different bacterial probiotic feeds *Lactobacillus acidophilus* and algal feed *Spirulina platensis* on the growth of *Labeo rohita* fingerlings were observed. After the feeding experiments, growth parameters such as Weight Gain (WG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR), and Survival Rate (SR) were recorded.

After sufficient acclimation, 210 numbers of healthy Rohu fishes weighing 5±0.4 grams each were chosen and divided into four groups for the experiment. In this experiment, the first group of fishes was fed the same diet without experimental ingredients, referred to as the control diet (0%). The next groups, T_1L (2%) of *L. acidophilus*, T_2L (4%), and T_3L (6%), gradually increased the concentration of L. acidophilus in their diet. Simultaneously, *Spirulina platensis* was replaced with fish meal. The ingredients included

Fish meal (Anchovy) (25%), Soya bean meal (25%), Groundnut oil cake (GNOC) (25%), Rice bran (12%), Tapioca flour (5%), Sunflower oil (3%) (Gold winner), Egg albumen (4%), Supradyn Multivitamin tablets (Vitamins (0.5%) and Minerals (0.5%)). *S.platensis* was included in the fish meal. Fish meal was replaced with the probiotic bacterium *L.acidophilus* at different levels, T_1L (2%), T_2L (4%), and T_3L (6%), while *S.platensis* was included at different levels, T_1S (2%), T_2S (4%), and T_2S (6%). The control diet, To (0%), was not supplemented with bacterial feeds. The experiment was conducted for 75th days, from December 1st to February 15th. For fish feed formulation, a trace amount of multivitamin (1g) was used, specifically Supradyn, which is a vitamin and mineral mixture.

Formulation and Preparation of Experimental Diet

The basal ingredients, such as processed fish meal (Anchovy) and sun-dried soya bean mesh, groundnut oil cake, and wheat bran, were ground separately and filtered through a 60-mesh sieve. The sieved feed ingredients were blended with a manual blender, and the blends were used for the preparation of experimental diets. Two groups were created: one with control diet (no microalgae or probiotics) and the other with three experimental diets containing *Lactobacillus acidophilus* (2%, 4%, 6%) as a replacement for fishmeal. The blends were steam-cooked for 15 minutes at 95-100°C and allowed to cool at room temperature. The steam-cooked blends were mixed with the replacement material, the probiotic bacterium *Lactobacillus acidophilus*, with each coated tablet containing not less than 120 million spores in the form of SPORALAC-DS. After the experimental period (75 days), three test species (fishes) from each trough (triplicate) were removed and starved for 24 hours before being sacrificed. The whole alimentary tract was dissected out in ice-cold fish ringer solution and washed thoroughly externally. The tissue was then rinsed with cold distilled water, split open, and washed thoroughly in fish groups at the respective concentrations. The multivitamin tablets (Supradyn), sunflower oil (Gold winner), and binder (Egg albumen and Tapioca flour) were added to the blender and manually blended. Boiled water was added to prepare a dough form. The dough was pelletized with a manual pelletizer fixed with a 3mm die, and the pellets were collected in aluminum trays. Then, the feeds were dried in a thermostatic oven until the moisture content was less than 10%. The dried pellets were physically examined for visual appearance, uniformity, color, and fragrance. The selected ingredients and feed proximate composition are shown as mentioned above in the paragraph.

Growth parameter formulas

1. Weight gain = Final weight - Initial Weight (Chaudhary *et al.*, 2007)
2. Specific growth rate (SGR %) = Ln $W_{t-}w_0$ / t × 100 (Krishnaveni *et al.*, 2013)

Where, Ln=Natural logarithm

Wt. = Final Wet wt. of fish (mg)

W_0 = Initial wet wt. of fish (mg)

t = Experimental duration (days)

Natural log of final Weight-Natural log of initial weight / Experimental duration × 100

3. Food Conversion ratio (FCR) = Food Consumed (mg) / Wet weight gain (mg)
4. Survival rate (%) (SR) = Total number of animal harvested / Total number stocked.

Biochemical study

Carbohydrate estimation was carried out using the Anthrone method as described by Carroll *et al.* (1956). Protein estimation was performed using the Lowry method as outlined by Lowry *et al.* (1951). Lipid estimation was conducted using the Bragdon method (1951).and the estimation of Triglyceride-Cholesterol was accomplished following the procedure by Werner *et al.* (1981).

Statistical analysis

Calculated values were expressed as mean ± SD for each group two statistically significant differences between mean values were determined by OW Analysis of Variance (ANOVA) followed by the Turkey's test for multiple comparisons. Difference is significant at the 0.05 level. The results were statistically analysed by SPSS software version 22.

Results and discussion

Results

The enhanced growth of aquatic fauna fed with bacterial and algal probiotic diets may be attributed to improved digestive functions, including enhanced enzymatic activity and vitamin synthesis, leading to improved digestibility and weight gain. Fish from each of the triplicate aquariums were sampled at 15-day intervals, and the total feed administered to each aquarium increased progressively. Recorded values for feces and unconsumed feeds were collected. As the sampling period concluded, both experimental groups receiving probiotic feeds (6% of body weight) exhibited a decrease in recorded faeces and unconsumed feed values compared to the control group.

Table 1. Ingredients of experimental diets for 100 gms.

S. No.	Ingredients	CONTROL T_0 (0%)	DIET 1 T_1 (2%)	DIET 2 T_2 (4%)	DIET 3 T_3 (6%)
1.	*Lactobacillus acidophilus* / *Spirulina platensis*	0	2	4	6
2.	Fish meal (Anchovy)	25	23	21	19
3.	Soya bean meal	25	25	25	25
4.	Ground nut oil cake (GNOC)	25	25	25	25
5.	Rice bran	12	12	12	12
6.	Tapioca flower	5	5	5	5
7.	Sun flower oil	3	3	3	3
8.	Egg albumen	4	4	4	4
9	Minerals & Vitamins	1	1	1	1
	Total	100	100	100	100

The results of the growth and biochemical parameters of rohu fingerlings under different feeding regimes are presented in Table 2. The growth parameters of rohu with varying feed concentrations indicated that a 6% probiotic *Lactobacillus* diet resulted in a Weight Gain = 37.50±0.58 grams, FCR = 35±0.04, SGR = 1.14±0.03, and 100% Survival Rate. On the other hand, a 6% prebiotic *Spirulina* diet exhibited the highest growth rate with an WG=20.83±0.2gm, FCR = 68.4±0.090, SGR = 0.80±0.79, and Survival Rate of 100%.

Regarding the composition of muscle, carbohydrate, protein, lipid, and triglycerides, the 6% Probiotic *Lactobacillus* diet showed carbohydrate levels of 64.20±0.05, protein levels of 2.93±0.00, lipid levels of 62.00±0.00, and triglycerides at 198.50±0.00.

In contrast, the 6% probiotic *Spirulina* diet exhibited higher values for carbohydrate (69.00±0.00) µg/mg/day, protein (3.07±0.00) µg/mg/day, lipid (69.00 ± 0.00) µg/mg/day, and triglycerides (150.43±0.12) µg/mg/day.

The control feed-fed fishes showed a weight gain of 16.66±0.05 gm, Food Conversion Ratio (FCR) = 56±0.05, Specific Growth Rate (SGR) = 0.672±0.006, and a survival rate of 100%. Additionally, their muscle composition included carbohydrate levels = 60.20±0.05 µg/mg/day, protein levels = 2.82±0.00 µg/mg/day, lipid levels of 51.00±0.00 µg/mg/day, and triglycerides = 198.50±0.00 µg/mg/day.

The growth and biochemical parameters of freshwater fish *Labeo rohita* fingerlings under different feeding regimes at 75 days varied significantly. The inclusion of bacterial probiotics (2%, 4%, and 6%) resulted in higher carbohydrate, protein, lipid, and triglyceride levels compared to the control group.

The 6% bacterial probiotic feed exhibited the highest weight gain, specific growth rate (SGR), and survival rate. Similarly, *Spirulina* supplementation (2%, 4%, and 6%) led to increased carbohydrate, protein, lipid, and triglyceride levels, with the 6% *Spirulina* feed showing the highest growth performance and survival rate.

Table 2. Impact of *Lactobacillus* and *Spirulina* on *Labeo rohita* fingerling growth and Biochemistry in 75th day with varied feeding.

Types of feed	Carbohydrate µg/mg/day	Protein µg/mg/day	Lipid µg/mg/day	Triglyceride µg/mg/day	Weight Gain (gm)	Food Conversion Ratio (FCR)	Specific Growth Rate (SGR)	Survival Rate (%)
Control	60.20±0.05	2.82±0.00	51.00±0.00	127.50±0.00	16.66±0.05	56±0.05	0.672±0.006	100±0.00
lactobacillus (2%)	62.36±0.08	2.85±0.00	57.00±0.00	173.50±0.00	25.16±0.152	46±0.07	0.91±0.04	100±0.00
lactobacillus (4%)	63.23±0.08	2.90±0.00	59.00±0.00	192.50±0.00	33.4±0.57	37±0.40	1.8±0.2	100±0.00
lactobacillus (6%)	64.20±0.05	2.93±0.00	62.00±0.00	198.50±0.00	37.50±0.58	35±0.04	1.14±0.03	100±0.00
Spirulina (2%)	62.50±0.11	2.83±0.00	62.00±1.93	128.76±0.12	16.60±0.16	75.3±0.08	0.67±0.2	100±0.00
Spirulina (4%)	63.20±0.05	2.85±0.003	63.00±0.00	129.50±0.00	18.33±0.17	74.6±0.12	0.73±0.74	100±0.00
Spirulina (6%)	69.00±0.00	3.07±0.00	69.00±0.00	150.43±0.12	20.83±0.2	68.4±0.090	0.80±0.79	100±0.00

The table provides a summary of the types of feed used and their respective biochemical parameters and growth characteristics. For the control group, the carbohydrate content was 60.20±0.05 µg/mg/day, protein content was 2.82±0.00 µg/mg/day, lipid content was 51.00±0.00 µg/mg/day, and triglyceride content was 127.50±0.00 µg/mg/day. The weight gain was 16.66±0.05 gm, with a food conversion ratio (FCR) = 56±0.05, a specific growth rate (SGR) = 0.672±0.006, and survival rate = 100±0.00%.

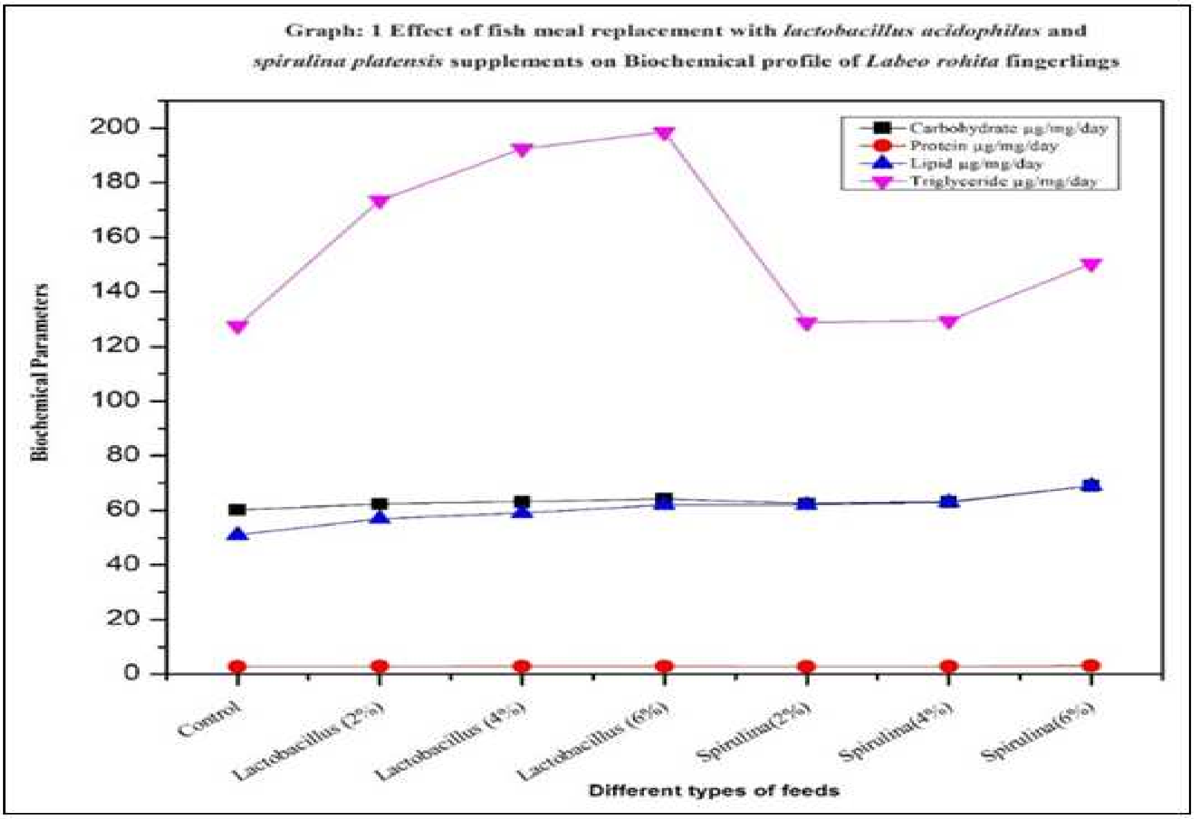

Fig. 1. Shows the different concentrations of *Lactobacillus* and *Spirulina* feed and shows a biochemical parameter.

In the group fed with bacterial probiotics (6%), the carbohydrate content was 64.20±0.05 µg/mg/day, protein content was 2.93±0.00 µg/mg/day, lipid content was 62.00±0.00 µg/mg/day, and triglyceride content was 198.50±0.00 µg/mg/day. The weight gain was 37.50±0.58 gm, with an FCR of 35±0.04, an SGR of 1.14±0.03, and a survival rate of 100±0.00%.

In the *Spirulina*-fed group (6%), the carbohydrate content was 69.00±0.00 µg/mg/day, protein content was 3.07±0.00 µg/mg/day, lipid content was 69.00±0.00 µg/mg/day, and triglyceride content was 150.43±0.12 µg/mg/day. The weight gain was 20.83±0.2 gm, with an FCR = 68.4±0.090, an SGR = 0.80±0.79, and a survival rate = 100±0.00%.

Discussion

The use of *Lactobacillus*, a prominent group of probiotic bacteria, is common in animal nutrition to enhance growth performance, survival rate, feeding efficiency, and to mitigate intestinal ailments and anti-nutritional factors in feed items. Suzer *et al.*, (2008). *Lactobacillus* also contributes to maintaining a healthy balance of beneficial microbes in the gastrointestinal tract, promoting overall animal health. Fish meal is highly regarded as a superior dietary protein source due to its palatability and provision of an optimal balance of essential amino acids, essential fatty acids, and digestible energy (Tacon, 1993). Research by Sharma *et al.* (2018) suggests that probiotic feed containing *Lactobacillus* can improve dry matter intake, daily feed conversion, and digestibility compared to control groups. Dietary protein is of paramount importance in fish feeding for weight gain (Yambo & Zirong, 2006). Adequate dietary protein supply is essential for rapid growth, and the effects of *Spirulina* on body protein and lipid contents are associated with their synthesis and accumulation rate in muscle (Nandeesha *et al.*, 1998; Nandeesha *et al.*, 2001).

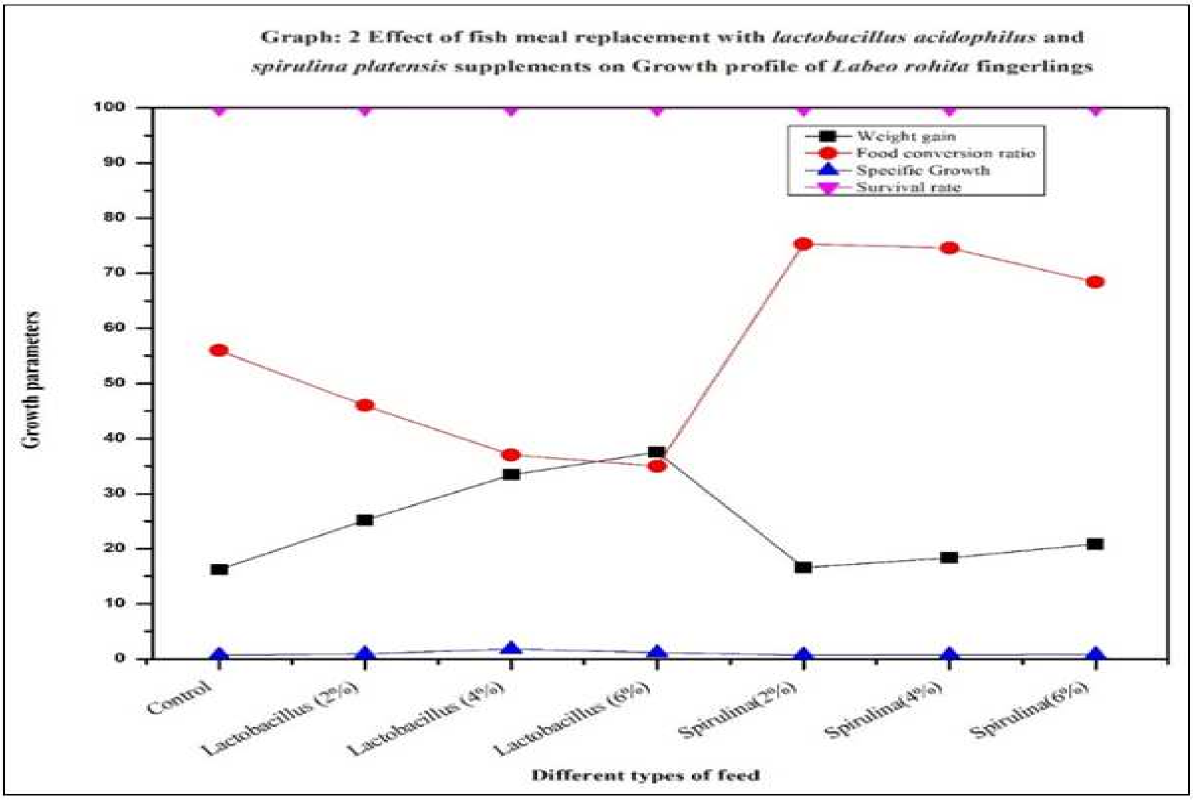

Fig. 2. Shows the different concentrations of *Lactobacillus* and *Spirulina* feed and shows a Growth parameter.

The study showed that *Spirulina* could replace fish meal as the sole protein source for *Labeo rohita*, resulting in significantly higher growth compared to diets using fish meal as the primary protein source, with minimal effect on the growth of catla (*Catla catla*). These results highlight that the response to *Spirulina* in fishes is likely species-specific. Man *et al.* (2020), it was found that adding a low concentration (5%) of *S. platensis*, a type of blue-green algae, to the diet of Sabah giant grouper *E. lanceolatus* can lead to improved growth performance. The growth response to *Spirulina* is also influenced by the composition of experimental diets in which *Spirulina* is incorporated (Takeuchi *et al.*, 2002), as well as the growth rate of the organism (Abdel Tawwab and Ahmad, 2009).

Both fish meal and soya bean meal were used as dietary protein sources, similar to the findings of Tongsiri *et al.* (2010), where the best performance was achieved by replacing 5% of fish meal with Spirulina. Fish meal was the sole protein source for *Spirulina* supplementation.

Overall, the results indicate that the concentration of both probiotics and *Spirulina* at 6% exhibited the highest growth rate in *Labeo rohita*. *Spirulina*-incorporated diets demonstrated better SGR and FCR compared to the probiotic diet, while the control diet had the highest FCR (1.93±0.05).

The survival rate was higher in groups receiving probiotics, possibly due to the production of vitamin B complex by intestinal probiotic bacteria. This study aligns with previous research indicating that probiotic diets enhance survival and growth performance in fish (Ghosh *et al.*, 2007; Del Carmen *et al.*, 2015; Subramani Munirasua, 2017).

Aman *et al.* (2023) propose a potential strategy for enhancing the growth of *Labeo rohita* under controlled aquaculture conditions. They advocate for a combination of plant-based feeds and the use of probiotics. the optimizing survival rates and promoting enhanced growth while achieving a higher fish yield. This approach holds the promise of economizing aquaculture production and contributing to the sustainability of the industry. Incorporating probiotics in a polyculture system was found to significantly boost the growth, digestive enzyme functions, and immune system of *L. rohita*, as demonstrated in a study by Ullah *et al.,* (2020).

Conclusion

The present study demonstrates that supplementary feeds significantly improved the growth performance of Indian Major carp Rohu fingerlings. This improvement can be attributed to enhanced health status and physiological responses. The growth performance indices (SGR, FCR, and Survival Rate) and biochemical parameters (carbohydrate, protein, lipids, and triglycerides) were notably higher in the fish fed with Probiotic feed at all dietary inclusion percentage levels. Additionally, this approach enhanced the efficiency of nutrient utilization and reduced input costs. Importantly, no mortality was observed among the fishes fed with probiotic bacteria *L.acidophilus* and prebiotic *S.platensis* under various feeding regimes in this study.

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Conflict of the Interest

The authors declare that they have no conflict of interest.

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