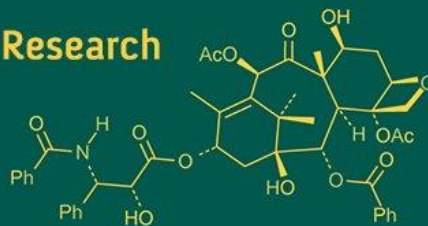
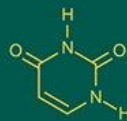
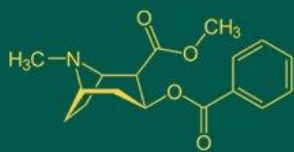


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Effects of fructooligosaccharide on growth performance, survival rate, feed utilization and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*

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Abstract

The present study was conducted to evaluate the effects of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival rate, feed utilization and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*. The experiment was carried out for 60 days at the College of Fisheries Science, Kamdhenu University, Veraval, using a Completely Randomized Design (CRD) with five treatments and four replicates each. Experimental diets were formulated with 0% (control), 0.5%, 1%, 1.5% and 2% FOS inclusion levels. Results revealed that FOS supplementation significantly ($p < 0.05$) enhanced growth and feed utilization parameters compared to the control. The highest performance was observed in the 2% FOS-supplemented group (T4), which recorded the greatest mean weight gain (2.64 ± 0.039 g), specific growth rate ($3.06 \pm 0.006\%$) and lowest feed conversion ratio (2.26 ± 0.017). Protein efficiency ratio and feed conversion efficiency were also markedly improved in the FOS-treated groups. Following the disease challenge with *A. hydrophila*, survival rate increased progressively with FOS inclusion, reaching a maximum of 80% in the 2% FOS group. Throughout the study, water quality parameters remained within optimal ranges for *L. rohita* culture. Overall, the findings demonstrate that dietary inclusion of 2% fructooligosaccharide effectively improves growth performance, feed utilization efficiency and disease resistance in *Labeo rohita*. Thus, FOS can be considered a promising prebiotic additive for sustainable aquaculture, enhancing fish health and productivity while reducing reliance on antibiotics.

Keywords: Fructooligosaccharide, *Labeo rohita*, growth performance, *Aeromonas hydrophila*

1. Introduction

Aquaculture, the cultivation of aquatic organisms such as fish, molluscs, crustaceans, amphibians, reptiles and aquatic plants under controlled or semi-controlled conditions, has become one of the fastest-growing food production sectors globally (Huntingford *et al.*, 2012; Stickney *et al.*, 2022) [15, 30]. It can be described as “underwater farming,” practiced across freshwater, brackishwater, marine and hypersaline environments, each characterized by distinct salinity levels. In India, aquaculture plays a crucial role in food security and economic development, contributing significantly to total fish production. During 2022-23, India produced 17.4 million tonnes of fish, accounting for about 8% of global production, ranking third in total fish production and second in aquaculture output (Sahu & Mohanta, 2024) [20].

Among freshwater species, *Labeo rohita* (Rohu) is one of the most commercially important Indian Major Carps (IMCs), preferred for its taste, rapid growth and high market demand across India, Bangladesh, Pakistan and Myanmar (Talwar & Jhingran, 1991; Kaur & Ram, 2017) [31, 17]. Rohu exhibits omnivorous feeding behavior, consuming plankton in its early life stages and shifting to filamentous algae, detritus and decomposed vegetation as it matures (Chondar, 1999) [7]. Its feeding intensity and growth are influenced by environmental and physiological factors such as temperature, reproductive cycle and water quality (Khan & Jhingran, 1975) [18].

In recent decades, aquaculture intensification has enhanced productivity but also increased susceptibility to stress and disease outbreaks (Pohlenz & Gatlin III, 2014; Magnadottir, 2010) [21, 19]. The extensive use of antibiotics for disease control has led to several adverse effects,

including the development of antibiotic-resistant bacteria, immune suppression and environmental contamination (Cabello, 2006; Reverter *et al.*, 2014) ^[5, 23]. Consequently, there is growing interest in eco-friendly alternatives such as functional feed additives, including prebiotics, to promote health and disease resistance in cultured species (Hoseinifar *et al.*, 2015) ^[13].

Prebiotics are nondigestible dietary ingredients that beneficially affect the host by selectively stimulating the growth or activity of beneficial gut microorganisms (Ringø *et al.*, 2010) ^[24]. Their inclusion in aquafeeds has shown positive effects on growth performance, nutrient utilization, immune response, gut microbiota composition and disease resistance (Guerreiro *et al.*, 2018; Rohani *et al.*, 2021) ^[12, 16]. Common prebiotics used in aquaculture include β -glucans, inulin, mannanoligosaccharides (MOS), galactooligosaccharides (GOS) and fructooligosaccharides (FOS) (Wee *et al.*, 2024).

Among these, FOS naturally found in plants such as onions, garlic, asparagus and bananas—has emerged as a promising prebiotic that enhances fish growth, immunity and resistance to pathogens while reducing reliance on antibiotics (Fuller & Gibson, 1998; Sridevi *et al.*, 2014) ^[10, 29]. FOS are short-chain β -D-fructans synthesized enzymatically via transfructosylation of sucrose using β -fructofuranosidase from *Aspergillus niger* (Roberfroid, 2007) ^[25].

Given its potential benefits, the present study was conducted to evaluate the effects of fructooligosaccharide supplementation on the growth performance, feed utilization and disease resistance against *Aeromonas hydrophila* in *Labeo rohita*.

2. Materials and Methods

2.1 Experimental Setup

The experiment entitled “Effects of Fructooligosaccharide on Growth Performance, Survival Rate, Feed Utilization and Disease Resistance against *Aeromonas hydrophila* in *Labeo rohita*” was conducted at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval. The study was carried out for 60 days, from 19 September 2024 to 17 November 2024, followed by a 7-day disease challenge test against *Aeromonas hydrophila*. The experiment was designed under a Completely Randomized Design (CRD) with five treatment groups, each replicated four times.

2.2 Experimental Tanks

A total of 20 rectangular plastic tanks ($2 \times 1 \times 1$ ft, capacity 55 L) were used for the study. All tanks were thoroughly cleaned and disinfected with 4 ppm potassium permanganate (KMnO_4) solution before use and then filled with 35 L of filtered, disinfected freshwater. Continuous aeration was provided throughout the experimental period.

2.3 Experimental Fish

Healthy *Labeo rohita* fry were procured from a commercial Indian Major Carp hatchery and transported to the experimental facility in oxygenated polythene bags. Fish were acclimated for six days in a 500 L tank under continuous aeration and regular feeding. After acclimation, fish with an average initial body weight of 0.50 ± 0.023 g were randomly distributed among 20 tanks, with 20 fish per tank (total 400 fish).

2.4 Treatment Details

Table 1: Treatment details

Treatment	Amount
T ₀	Diet prepared with 0% prebiotic (Control)
T ₁	Diet prepared with 0.5% prebiotic Fructooligosaccharide
T ₂	Diet prepared with 1% prebiotic Fructooligosaccharide
T ₃	Diet prepared with 1.5% prebiotic Fructooligosaccharide
T ₄	Diet prepared with 2% prebiotic Fructooligosaccharide

2.5 Experimental Diet Preparation

2.5.1 Procurement of Feed Ingredients

The prebiotic Fructooligosaccharide (FOS) was procured from Sharrets Nutritions LLP, Rajasthan. Other feed ingredients such as fish meal, groundnut oil cake (GNOC), wheat flour, tapioca flour, plant oil, vitamins and mineral mix were purchased from the local market in Veraval. Tapioca flour was finely ground using a grinder, while high-quality fish meal and fish oil were obtained from a commercial fish processing unit in Veraval.

2.5.2 Proximate Analysis of Ingredients

The proximate composition of the feed ingredients was analyzed following AOAC, 2019 procedures. Crude protein was estimated by the micro-Kjeldahl method, moisture content by oven drying at 100 °C and ash content using a muffle furnace at 550 °C until white ash was obtained.

Table 2: Proximate composition of feed ingredients (%)

Ingredients	Crude protein (%)	Crude-fat (%)	Ash (%)	Moisture (%)
Fish meal	55.07	11.41	22.62	8.22
Groundnut oil cake	35.15	7.54	5.12	12.15
Wheat flour	10.70	0.72	1.16	6.05
Tapioca flour	3.95	10.26	1.45	5.20

2.5.3 Feed Formulation

Experimental feeds were formulated to contain 30% crude protein using Pearson's square method. Five diets were prepared as per treatment details (Table 3.3).

Table 3: Composition of experimental diets (30% protein)

Ingredients of feed	Diets (30% protein)				
	T ₀	T ₁	T ₂	T ₃	T ₄
FOS	0	0.5	1	1.5	2
Fish meal	30	30	30	30	30
GNOC	40	40	40	40	40
Tapioca flour	14	13.7	13.4	13.2	12.8
Wheat flour	12	11.8	11.6	11.3	11.2
Fish oil	2	2	2	2	2
Vitamin & Mineral	2	2	2	2	2
Total	100	100	100	100	100

2.5.4 Feed Preparation

The weighed ingredients were mixed thoroughly with water to form dough and steam-cooked at 121 °C and 15 psi for 15 minutes. After cooling, vitamins, minerals and FOS (as per treatment) were added and mixed thoroughly. The mixture was pelletized using a mechanical pelletizer, sun-dried to less than 10% moisture and stored in labelled airtight plastic containers.

2.6 Experimental Procedure

2.6.1 Growth and Survival Study

Fish were fed twice daily (09:00 and 17:00 hrs) at 10% of their body weight, adjusted at *ad libitum* based on fortnightly weight measurements. The experiment was conducted for 60 days and fish were weighed every 15 days using an electronic balance.

Mean Weight Gain (MWG) = Final mean weight - Initial mean weight

% Weight Gain = [(Final weight - Initial weight) / Initial weight] × 100

Specific Growth Rate (SGR, %/day) = [(ln Final weight - ln Initial weight) / Duration] × 100

Survival (%) = (Number of fish survived / Number of fish stocked) × 100

2.6.2 Feed Utilization Study

Feed utilization was assessed through Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Feed Efficiency Ratio (FER):

FCR = Feed intake (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

FER = Weight gain (g/day) / Feed intake (g/day)

2.6.3 Disease Resistance Test

2.6.3.1 Preparation of *Aeromonas hydrophila* Stock

Aeromonas hydrophila culture was obtained from the Department of Aquatic Animal Health Management, College of Fisheries Science, Vervaval. The bacteria were cultured in nutrient broth at 37 °C for 24 hours, centrifuged at 3000 rpm for 10 minutes and the pellet resuspended in phosphate-buffered saline (PBS, pH 7.4). The suspension was adjusted to 0.5 McFarland standard (1×10^7 CFU/ml) and serially diluted in PBS (Sahu *et al.*, 2007).

2.6.3.2 Challenge Test

After the feeding trial, ten fish from each treatment were injected intramuscularly with 0.1 ml of *A. hydrophila* suspension (1×10^6 CFU/ml) using a 26-gauge needle. The fish were observed for 7 days for mortality and clinical symptoms. A positive control group received the same bacterial suspension, while the negative control group was injected with 0.1 ml of sterile saline solution.

2.7 Water Quality Management

Water quality parameters such as temperature, pH, dissolved oxygen (DO) and alkalinity were monitored weekly. Temperature was measured using a thermometer, pH with a digital pH meter and DO by Winkler's method. Alkalinity was determined by the titration method and expressed in mg/L. Continuous aeration was maintained, and tanks were siphoned daily to remove waste and replenish with fresh filtered water.

2.8 Statistical Analysis

All data were analyzed statistically using one-way ANOVA under a Completely Randomized Design (CRD) with SPSS software. Differences among means were compared using

Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$).

3. Results and Discussion

3.1 Effects of Dietary Fructooligosaccharide Supplementation on the growth of *Labeo rohita*

The growth performance of *Labeo rohita* was evaluated by measuring mean weight, weight gain, percentage weight gain, specific growth rate (SGR) and total length across different dietary treatments of fructooligosaccharide (FOS) supplementation. The experiment was conducted for 60 days, during which samples were collected every 15 days to assess growth performance.

3.1.1 Mean Weight (g)

The initial average weight of *L. rohita* was 0.50 ± 0.005 g across all treatment groups. After 60 days of feeding, the final mean weights were recorded as 2.66 ± 0.046 g, 2.86 ± 0.043 g, 2.88 ± 0.049 g, 3.06 ± 0.048 g and 3.14 ± 0.048 g in the control group (T0), T1, T2, T3 and T4, respectively. The results revealed a significant difference ($p < 0.05$) among the treatments, with T4 showing the highest final mean weight. Although T4 recorded a higher weight than T3, the difference was not statistically significant. In contrast, the control group exhibited the lowest mean weight.

The consistent increase in mean weight across treatments indicates that dietary supplementation of FOS positively influenced growth in *L. rohita*. These findings align with Poolsawat *et al.* (2020) [22], who reported a significant increase in final body weight of *Oreochromis niloticus* when fed a 2% FOS-supplemented diet. Similarly, El Latif *et al.* (2015) [9] observed improved growth performance in tilapia fed diets containing 2% FOS compared to the control. However, Chitmanat *et al.* (2017) [6] found no significant difference in *Anabas testudineus*, suggesting that variations in species, fish size and culture conditions can influence the growth response to prebiotic supplementation.

3.1.2 Weight Gain (%)

The percentage weight gain of *L. rohita* fry was calculated based on the recorded mean weights. The mean percentage weight gains for treatments T0, T1, T2, T3 and T4 were 423.03 ± 9.84 , 462.60 ± 9.01 , 489.77 ± 2.44 , 512.50 ± 1.89 and 528.11 ± 2.46 , respectively. The results revealed that all FOS-supplemented diets significantly improved weight gain compared to the control ($p < 0.05$), with T4 showing the highest percentage gain. Although T4 recorded higher values than T3, the difference was not statistically significant.

These findings corroborate the results of Akrami *et al.* (2013) [3], who reported higher weight gain percentages in juvenile stellate sturgeon (*Acipenser stellatus*) fed FOS-supplemented diets compared to controls. Likewise, Hoseinifar *et al.* (2014) [14] and Soleimani *et al.* (2012) [28] observed improved growth in common carp (*Cyprinus carpio*) and *Rutilus rutilus*, respectively, with 2% FOS inclusion. The present results therefore confirm that moderate FOS supplementation effectively enhances growth in *L. rohita*.

3.1.3 Mean Weight Gain (g)

Throughout the experimental period, *L. rohita* exhibited consistent improvement in mean weight gain across all FOS-supplemented treatments compared to the control. At the end of 60 days, mean weight gains were recorded as 2.15 ± 0.031 g (T0), 2.35 ± 0.027 g (T1), 2.39 ± 0.040 g (T2),

2.56±0.022 g (T3) and 2.64±0.039 g (T4). Among all treatments, T4 showed the highest mean weight gain, followed closely by T3, while the control group showed the lowest.

Statistical analysis indicated that all treatments differed significantly from the control ($p<0.05$). The trend clearly demonstrates that increasing FOS inclusion up to 2% improved the growth performance of *L. rohita*. Similar growth-promoting effects of FOS have been reported by Poolsawat *et al.* (2020) [22] in tilapia and Soleimani *et al.* (2012) [28] in Caspian roach, where diets supplemented with 2% FOS yielded the highest growth rate. Conversely, Grisdale Helland *et al.* (2008) [11] found no significant effect of FOS supplementation on *Salmo salar*, possibly due to differences in species and gut microbiota composition.

3.1.4 Total Length (cm)

The initial average length of *L. rohita* was 3.58±0.006 cm across all treatments. After 60 days, the mean lengths recorded were 5.80±0.022 cm (T0), 6.27±0.030 cm (T1), 6.33±0.026 cm (T2), 6.50±0.046 cm (T3) and 6.56±0.039 cm (T4). Treatment T4 showed a significantly higher length compared to the control group ($p<0.05$).

These findings are consistent with Sepulveda Quiroz *et al.* (2021), who observed increased total length in *Atractosteus*

tropicus fed diets containing 5-10 g/kg FOS and Lima Paz *et al.* (2019) [8], who reported similar results in *Colossoma macropomum*. The improved length in FOS-supplemented groups suggests that prebiotics enhance nutrient absorption and feed utilization efficiency.

3.1.5 Specific Growth Rate (SGR, %)

The specific growth rate (SGR) was significantly influenced by dietary FOS supplementation. The mean SGR values for treatments T0, T1, T2, T3 and T4 were 2.76±0.032, 2.87±0.027, 2.95±0.007, 3.02±0.005 and 3.06±0.006 %, respectively. Treatment T4 recorded the highest SGR, followed by T3, while the control group showed the lowest. Statistical analysis revealed significant differences ($p<0.05$) between all treatments and the control. The enhanced SGR in the FOS-supplemented groups indicates better growth efficiency and metabolic performance. These observations agree with Ahmed *et al.* (2019) [1], who found the best SGR in *Cyprinus carpio* fed 10 g/kg FOS and Akrami *et al.* (2013) [3], who noted higher SGR at 1% inclusion in *A. stellatus*. Such variations in optimal FOS levels among species may be due to differences in digestive physiology and gut microbial communities.

Table 4: Growth parameters (Mean±SE, n=4) of *Labeo rohita* fed with Fructooligosaccharide-supplemented diets

Treatment	Mean Weight (g)	Mean Weight gain (g)	Percentage Weight gain (%)	Mean Length gain (cm)	Specific Growth Rate (%)
T0	2.66 ^c ±0.046	2.15 ^c ±0.031	423.032 ^d ±9.840	5.80 ^d ±0.022	2.76 ^d ±0.032
T1	2.86 ^b ±0.043	2.35 ^b ±0.027	462.595 ^c ±9.011	6.27 ^c ±0.030	2.87 ^c ±0.027
T2	2.88 ^b ±0.049	2.39 ^b ±0.040	489.774 ^b ±2.448	6.33 ^c ±0.026	2.95 ^b ±0.007
T3	3.06 ^a ±0.048	2.56 ^a ±0.022	512.502 ^a ±1.890	6.5 ^b ±0.046	3.02 ^a ±0.005
T4	3.14 ^a ±0.048	2.64 ^a ±0.039	528.109 ^a ±2.456	6.56 ^a ±0.039	3.06 ^a ±0.006

3.2 Influence of Fructooligosaccharide-Incorporated Diet on Feed Utilization of *Labeo rohita*

Feed utilization parameters such as Feed Conversion Ratio (FCR), Feed Conversion Efficiency (FCE) and Protein

Efficiency Ratio (PER) were analyzed to determine the effect of dietary incorporation of fructooligosaccharide (FOS) on *Labeo rohita*.

Table 5: Feed utilization parameters (Mean±SE, n=4) of *Labeo rohita* fed with Fructooligosaccharide-supplemented diets

Treatment	FCR	FCE	PER
T0 (Control)	2.46 ^a ±0.039	0.40 ^d ±0.006	1.35 ^d ±0.021
T1 (0.5% FOS)	2.39 ^{ab} ±0.027	0.42 ^{cd} ±0.005	1.39 ^{cd} ±0.015
T2 (1% FOS)	2.37 ^{bc} ±0.012	0.42 ^{bc} ±0.002	1.41 ^{bc} ±0.007
T3 (1.5% FOS)	2.32 ^{cd} ±0.010	0.43 ^{ab} ±0.003	1.44 ^{ab} ±0.009
T4 (2% FOS)	2.26 ^d ±0.017	0.44 ^a ±0.003	1.47 ^a ±0.011
S.Em. ±	0.024	0.004	0.014
C.D. at 5%	0.072	0.013	0.043
C.V.%	2.03	2.08	1.99

The results revealed that dietary supplementation of fructooligosaccharide (FOS) significantly improved ($p<0.05$) the feed utilization parameters of *Labeo rohita* compared to the control diet. Among all treatments, the group receiving 2% FOS (T4) exhibited the most favorable feed utilization indices, showing the lowest FCR (2.26±0.017), highest FCE (0.44±0.003) and highest PER (1.47±0.011). These findings indicate that the addition of FOS enhanced feed efficiency and protein utilization in *Labeo rohita*.

The decline in FCR observed in FOS-supplemented treatments suggests that FOS improved the digestibility and utilization of dietary nutrients, enabling fish to gain more weight per unit of feed consumed. This improvement may

be due to the prebiotic properties of FOS, which promote the growth of beneficial intestinal bacteria such as *Lactobacillus* and *Bifidobacterium*. These microbes enhance gut health, enzymatic activity and nutrient absorption, leading to improved feed conversion efficiency. Similar findings were reported by Soleimani *et al.* (2012) [28] and Poolsawat *et al.*, who observed improved FCR in fish fed diets containing fructooligosaccharides.

Feed Conversion Efficiency (FCE) and Protein Efficiency Ratio (PER) also showed significant improvement in FOS-supplemented diets, particularly at the 2% inclusion level. The higher FCE and PER values indicate that *Labeo rohita* efficiently converted feed and dietary protein into body tissue when FOS was incorporated into the diet. El Latif *et*

al. (2015) [9] also reported that Nile tilapia fed with 2% FOS exhibited a significantly better FCR and FCE than the control group, supporting the present findings. Similarly, Ahmed *et al.* (2019) [11] found that common carp fed 1% FOS had improved feed efficiency and growth performance.

The observed enhancement in protein efficiency may be due to improved intestinal morphology and increased nutrient digestibility caused by the fermentation of FOS in the gut, which produces short-chain fatty acids beneficial for metabolism. Zhou *et al.* (2010) [34] reported that Red drum fish fed diets with FOS exhibited a higher PER than those fed other prebiotics. However, other studies such as Ai *et al.* (2011) [2] on *Larimichthys crocea* and Grisdale Helland *et al.* (2008) [11] on *Salmo salar* found no significant improvements in feed utilization at high inclusion levels, suggesting that the effects of FOS can vary with species, inclusion levels and culture conditions.

Overall, the present study concludes that dietary inclusion of 2% fructooligosaccharide optimizes feed utilization efficiency in *Labeo rohita*. The improved FCR, FCE and PER values in FOS-supplemented groups demonstrate that FOS serves as an effective prebiotic feed additive, enhancing nutrient absorption, digestion and protein utilization, which ultimately promotes better growth and performance in *Labeo rohita*.

3.3 Influence of Fructooligosaccharide-Incorporated Diet on Disease Resistance in *Labeo Rohita* Against *Aeromonas hydrophila*

At the end of the 60-day experimental period, a disease challenge test was conducted to evaluate the resistance of *Labeo catla* against *Aeromonas hydrophila*. Ten fish from each treatment group were randomly selected and injected intramuscularly with *A. hydrophila* at a concentration of 1×10^6 cells/ml, ensuring minimal stress during handling. Mortality was observed for seven days post-challenge, and survival percentages were recorded.

Table 7: Temperature (°C), pH, Dissolved Oxygen (ppm) and Alkalinity (mg/L) of *Labeo rohita* in each treatment throughout the experimental period

Treatments	Temperature (°C)		pH		Dissolved Oxygen (ppm)		Alkalinity (mg/L)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
T0	25.00	27.25	7.30	7.70	5.20	7.00	164	195
T1	25.50	27.00	7.40	7.70	5.20	7.00	165	188
T2	25.25	27.20	7.20	7.70	5.20	6.90	165	194
T3	25.00	27.50	7.40	7.70	5.00	6.80	165	198
T4	24.50	27.00	7.40	7.70	4.90	6.80	165	205

5. Conclusion

The results of the experiment revealed that incorporating fructooligosaccharide (FOS) into the diet of Rohu (*Labeo rohita*) enhanced growth performance, survival rate and resistance to *Aeromonas hydrophila* infection compared to the control group. Among all treatments, the diet containing 2% FOS showed the best outcomes, with the highest weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio, protein efficiency ratio and disease resistance, indicating its superior potential to support fish health and development.

In summary, this study confirms that dietary supplementation of *Labeo rohita* with fructooligosaccharide has a beneficial effect on overall performance. Fish fed with 2% FOS demonstrated significant improvements in growth parameters and survival. Additionally, FOS inclusion

Dietary supplementation with fructooligosaccharide (FOS) significantly improved the survival rate of *L. catla* compared to the control group. After 7 days, the control group (T0) exhibited a survival rate of 50%, whereas fish fed with FOS-supplemented diets showed higher survival rates. Treatment T4 (2% FOS) achieved the highest survival rate of 80%, followed by T2 and T3 (both 70%), and T1 (60%). This suggests that incorporating 2% FOS into the diet enhances disease resistance in *L. catla* against *A. hydrophila*, demonstrating better protection compared to the control.

These findings align with the results of Poolsawat *et al.* (2020) [22], who observed that supplementation with FOS improved immune responses and disease resistance in tilapia. Similarly, Zhang *et al.* (2014) [33], El Latif *et al.* (2015) [9] and Lima paz *et al.* (2019) [8] reported that FOS supplementation enhanced the survival of blunt snout bream, Nile tilapia and tambaqui, respectively, following exposure to *A. hydrophila*. Moreover, Soleimani *et al.* (2011) [28] demonstrated that 2% FOS supplementation yielded better survival and growth in Caspian roach than other tested levels. These studies collectively support the role of FOS as a beneficial prebiotic additive for improving disease resistance and overall health in fish.

Table 6: Disease resistance of *Labeo rohita* in each treatment group after 7 days of exposure to *Aeromonas hydrophila*

Treatments	T0	T1	T2	T3	T4
Survival (%)	50	60	70	70	80

4. Physico-Chemical Parameters

Throughout the 60-day experimental period, the water quality parameters of all experimental tanks were carefully monitored on a weekly basis to ensure an optimal environment for the growth and survival of *Labeo rohita*. The parameters measured included water temperature, dissolved oxygen (DO), pH and alkalinity.

enhanced immune response, resulting in increased resistance to disease challenges. Therefore, the use of 2% FOS in Rohu diets can be considered an effective and sustainable approach to promote better growth, survival and health in aquaculture systems while reducing dependence on antibiotics and chemical additives.

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