Proceedings of the International Conference on Fisheries and Aquaculture, Vol. 11, Issue. 1, 2024, pp.16-35

ISSN 2386-1282 online
DOI: https://doi.org/10.17501/23861282.2024.11102

THE IMPACT OF PROBIOTICS ON THE GROWTH AND SOIL AND WATER QUALITY OF FRESHWATER FISH, *Pangasius hypophthalmus* AND *Piaractus brachypomus*

Bazar KK and Nagajyothi P*

Dept. of Fishery Science & Aquaculture, S.V.U. College of Sciences, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

Abstract: In aquatic farming, probiotic products are seen as an alternative to the use of antibiotics. In freshwater fishes can benefit from probiotic organisms in a number of ways, including increased growth, reduced pathogen colonization, improved nutrient digestion, improved soil and water quality, increased stress tolerance, improved feed utilization, and an increased survival rate. can benefit from probiotic organisms in a number of ways, including increased growth, reduced pathogen colonization, improved nutrient digestion, improved soil and water quality, increased stress tolerance, improved feed utilization, and an increased survival rate. Present study techniques to include the probiotic strains in the fish feed pellets to development of fish biochemical and digestive system and organic waste buildup degrades the quality of the soil and water, which inhibits the growth of freshwater fish in earthen ponds. In order to prevent this issue, a 90-day supplementation experimentation was carried out to ascertain the impact of commercial probiotics "Super-PS" on the maintenance of beneficial soil and water quality parameters as well as the development of *Pangasius hypophthalmus* and *Piaractus brachypomus* fish cultured in freshwater earthen ponds. There are numerous ways that probiotic bacteria can enhance the quality of soil and water. *Rhodococcus* and *Rhodobacter* species are combined in "Super-PS." During the culture period, a freshwater pond was treated with water probiotic "Super PS" for 15 lit/ha from 1 to 30 days, 30 lit/ha from 31 to 60 days, 40 lit/ha from 61 to 90 days, and 91 days, depending on the pond condition, stocking density, and culture days, until harvest. The factors responsible for the improved water quality and significant growth up to harvest fresh water fishes under the influence of soil and water probiotics was analysed and together with the digestive enzymes, biochemical composition and histologically confirmed.

Keywords: *Rhodococcus*, *Rhodobacter*, Fresh water fishes, growth & Soil & water quality

Introduction

The International Conference on Fisheries and Aquaculture 2024 will serve as a platform for innovative research, continuous professional development and information exchange in the field of aquaculture and sustainable fisheries in support of the blue revolution. It is essential in forming frameworks that effectively address upcoming difficulties and Fisheries and Aquaculture are significant factors in creating a future that is more prosperous, inclusive and ecologically sustainable. For the benefit of global and expanding consumers, policymakers, managers, scientists, fishermen, farmers, traders and civil society activists, this conference provides knowledgeable support to scientific and technical insights on challenges, opportunities and innovations shaping the present and future of the sector. Millions of people, particularly in developing countries, depend on the fishing industry for food, nutrition, employment opportunities and revenue. As such, it is an essential component of the global economy. Fish are an essential source of animal protein and contribute to the stability of regional aquatic

*Corresponding Author's Email: *pemminetinagajyothi9@gmail.com

environments. Through cultural practices, they are also helpful in raising the financial status of marginalized communities.

Probiotics, essentially live bacteria, benefit the host by promoting the harmonious functioning of the intestinal microbiota and enhancing the well-being of the animals (Fuller R et al, 1989). Probiotics assessments have been made for a variety of gram positive (*Bacillus, Lactococcus, Micrococcus, Rhodococcus, Enterococcus, Streptcoccus, etc.)* and gram negative (*Aeromonas, Alteromonas, Rhodobacter, Photorhodobacterium, Pseudomonas* and *Vibrio etc*.) bacterias and microalgae yeast mixtures (Gatesoupe FJ et al, 1999). In particular, the probiotics 'Super PS' contain *Rhodococcus* and *Rhodobacter* Probiotics play a central role in transforming soil and water quality and promoting aquatic health. Probiotics for fish compete with pathogens in the host gut and limit their access to food and colonization. They also secrete antibiotics and other antibacterial metabolites that help reduce the pathogen population, which helps protect the host organisms from various infectious diseases, so they can become good growth promoters for aquatic animals. The probiotics played a major role in maintaining water quality parameters and control bacterial load. The probiotic used ponds when compared to the control pond. The isolated probiotic bacteria was identified by morphology and biochemical characterization(Sunitha, K., and P. V. Krishna. 2016, Verschuere L et, al., 2000; Harikrishnan R, 2010).

The freshwater proteinate fishes *Pangasius hypophthalmus*, also known as striped catfish and *Piaractus brachypomus*, also known as pacu, they are omnivorous fishes. The human body's potentized low-fat diet omega-3 fatty acids found in both fish are important for heart health, brain function and immune system function and repair of tissues in the body. The vitamin-D *P.hypophthalmus* fish helps the body absorb calcium, which is important for bone health.

The objectives of the present study are to examine the growth parameters of *Pangasius hypopthalmus* and *Piaractus brachypomus* freshwater fishes, as well as their biochemical makeup, digestive enzymes, soil and water quality metrics, and histological analysis.

Methodology

Origin and accumulation:

In four rectangular freshwater earthen ponds each pond size 0.4 hectares in Kovur Mandal, Nellore District, Andhra Pradesh, India. Fish research earthen ponds, fingerlings of *Pangasius hypophthalmus* and *Piaractus brachypomus* (Figs.1, 2) were obtained from a nearby hatchery and brought to the laboratory to undergo the study.

Fig 1: Piaractus brachypomus

Fig 2: Pangasius hypophthalmus

The *Pangsius hypophthalmus* was formerly long but is now flattened and scale-free, according to Storer, D. et al. (2024). comparatively small head, wide mouth with front incisors, palatal and mandibular bones, rather large eyes and one shorter set of barbels than the other. *Piaractus brachypomus* possesses a compressed body form, six dorsal-fin rays that branch, gill rakers that normally mature and black or dark grey fins. The mandible has a slight downward projection. The belly is serrated both before and after the pelvic fins. On the ventral side of the body, the pre-operculum and operculum are vermilion, reaching from the anterior tip of the lower jaw to the anus. (Ribeiro F. M. et al., 2016).

Experimental Probiotics

The probiotics utilized in this study were commercially available. They are called "Super PS," and they are made and marketed by CPF (India) Private Limited,. They are designed for use in soil and water. At a concentration of 10^9 CFU/mL, the predominant species present are *Rhodococcus* and *Rhodobacter*. A cutting-edge biotechnology product called "Super PS" has populations of helpful bacteria in it. Together, these bacteria can biodegrade organic contaminants, minimize hydrogen sulfidelower and toxic gas levels.

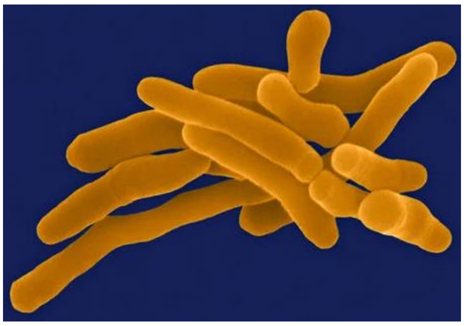


Fig 3: Rhodococcus

Fig 4: Rhodobacter

Fig 5: Probiotics “Super PS”

Experimental Design and Feeding Method

The four earthen pond treatments in this study were "Super PS" probiotics distributed throughout the pond preparation and combined with pond water and instud feed (E1, E2) and probiotic-free in the control ponds (C1, C2), for each pond collected six replicates for experiment. *P.hypophthalmus* fingerlings of uniform size weighing 5-7 g were used for the study. The control and experimental ponds (C1, E1) were both 0.4 ha in size, with a stocking density of 2.5 pieces/m2 total 10,000 fingerlings and *P.brachypomus* fingerlings of uniform size weighing 5-7 g were used in the study. With a stocking density of 1.1 fish/m2 and 4,500 fingerlings, the control and experimental (C2, E2) ponds were each 0.4 hectares in size. The samples were taken on the last day of the 90^{th} day experiment. Fish fingerlings are fed commercial fish feed, which is available as floating pellets in the local market, until they reach adult maturity (Vinod, S.A. et al., 2021). During the culture period, the pond water was administered weekly at 15 l/ha for the first 30 days, then every 31 days at 30 l/ha and finally every 61 days at 40 l/ha, while the probiotic "Super PS" was added to the feed of the experimental ponds (E1, E2) twice a week at a rate of 10 ml/kg. The control ponds (C1, C2) did not contain any probiotics. A feed with 28 crude protein, 3 fat, 7 fiber, 20 ash and 11 moisture of 1 was used in the control and experimental ponds.

Ponds Preparation and Stocking of Fingerlings

To maintain farm biosecurity, the experimental and control earthen ponds were emptied and allowed to dry for fifteen days before stocking. Wahab et al. (2002) state that drying aids in pond sterilization and pH balance maintenance. The calcium oxide (CaO) application rate was 50 kg/acre when using the dusting method of liming. The water inputs and outputs were filtered as a biosecurity measure to prevent unwanted fish and predators from entering the ponds. Every pond was filled to a depth of 1.3 to 1.7 meters. Each pond was initially treated with 200 kg/acre of cow manure to increase productivity, subsequently inorganic fertilizers were applied to the ponds: 2.5 kg/acre of triple superphosphate and 5 kg/acre of urea. Simultaneously, the control and experimental ponds (C1, C2, E1 and E2) were subjected to similar treatments. To further use the organic matter to establish the beneficial bacterial population, 20 liters/acre of "Super PS" probiotic were sprayed on the bottom of the experimental ponds (E1, E2) after the sediment was removed.

The estimated bacterial load of the fish gut was determined, the *Pangasius hypophthalmus* and *Piaractus brachypomus* fingerlings quality and freshness were verified. Selected two fingerlings (ABW, 6.0 g) were placed in the Control and Experimental (C1, E1 and C2, E2) ponds in this study monoculture (Datta, Surjya et al., 2017). The fingerlings were suitably acclimated to the water in the Experimental and Control ponds before being stocked (Nur Mohammad et. al, 2021).

Fig. 6: Culture Pond

Analysis Of Growth Performance

To determine the weight gain Sambhu and Jayaprakas (2001), Survival percentage (G. Biswas et al., 2011), Feed conversion ratio (Mustafa and Ridzwan, 2000), *Pangasius hypophthalmus* and *Piaractus brachypomus* fish 6 replicates were collected from each pond (C1, E1 and C2, E2) on the 90th day.

Weight gain (WG %) = final body weight – initial body weight ÷ initial weight

Weight gain = final body weight (g) – initial body weight (g).

Feed conversion ratio (FCR) = feed intake (dry weight) (g) ÷ body weight gain (wet weight) (g).

Survival rate (%): (total number of surviving fish ÷ total number of stocked fish) x 100

Determination of Biochemical Parameters

Following the 90th day of the culture period, biochemical analyses (C1, C2, E1 and E2) were conducted out on the muscle of *Pangasius hypophthalmus* and *Piaractus brachypomus* fishes to determine the amounts of moisture, ash, total protein, lipid and carbohydrate. Fish lipids (Folch J, et al., 1957), carbohydrates (Dubois et al., 1956), protein content (Lowry et al., 1951), moisture and ash content (AOAC 2000) and amino acid content (Ishida et al., 1981) were all assessed.

Activities of Digestive Enzymes

By monitoring the intestinal metabolizing enzyme activity in *Pangasius hypophthalmus* (C1, E1) and *Piaractus brachypomus* (C2, E2) fish, the effects of the control and experimental diets were investigated. On day 90th of the experiment, aseptic samples of the fish's gut were collected in order to analyze the intestinal enzymes' activity, including lipase, amylase and protease respectively. The stomach was homogenized in ice-cold distilled water and the supernatant was used as a source of crude enzymes after the tissue was centrifuged for 20 minutes at 4 oC and 10,000 rpm. The method of Furne et al. (2005) was utilized to evaluate the lipase activity, while the casein hydrolysis method was utilized to determine the total protease activity. Triacylglycerol was broken down into free f atty acids. The amylase activity was calculated using Bernfeld P. 1955, technique for starch hydrolysed.

Parameters of Soil And Water Quality

The pH of the soil was measured electrochemically using a C1, C2, E1 and E2 ponds. The electrical conductivity measured with an pH and EC meter. The wet oxidation method created by Walkley and Black was used to determine the samples' organic carbon content. (1983, Ghosh et al).

Fish development is dependent on the qualities of the water. Weekly measurements of the water quality were monitored. Temperature, pH, dissolved oxygen (DO), ammonia and nitrite were between factors. Water samples were taken from the ponds and tested for dissolved oxygen, total ammonia (APHA 1989), nitrite (Boyd 1984), pH (Corning pH meter) and other parameters. A portable mercury thermometer was used to measure the temperature.

Statistical Analysis

The statistical analysis was conducted using SPSS version 23. A mean ± SD is displayed for the data. The data were analyzed using one-way analysis of variance or ANOVA. A ($P<0.001$) was considered statistically significant when the significant means were compared using Duncan's multiple range test (DMRT).

Results and Discussion

Growth Prameters of Pangasius hypophthalmus and Piaractus brachypomus

Pangasius hypophthalmus and *Piaractus brachypomus* growth parameters The total body weight variations between fed on the control (C1, C2) and experimental (E1, E2) diets for 90 days are shown in Table 1. It is evident from the results that fingerlings' total body length rises significantly ($P<0.001$) in both control and experimental ponds as raising period increases up to 90 days. In comparison to fingerlings fed the control diet (C1, C2), the amount of this rise is greater in those fed probiotic experimental diets (E1, E2).

The fish were raised from the fingerling stage to the adult stage following stocking. In comparison to the control diet fish, the experimental diet fish showed superior growth performance after 90 days. *Piaractus brachypomus* fish (C2, E2) acquired 473.60g and 583.65g, while *Pangasisu hypopthalmus* fish (C1, E1) gained 362.58g and 498.50g, respectively. The current increase in the experimental probiotic diet (E1, E2) is significant when compared to the (C1, C2) diet and the results indicate that the E1, E2 probiotic diet is more successful at boosting weight than the Control C1, C2 diet.

P.hypophthalmus and *P.brachypomus* fed different control (C1, C2) and experimental (E1, E2) diets over the course of a 90th day culture period had different feed conversion ratios (FCRs). The findings unequivocally demonstrate that the fingerlings' feed conversion ratio changes during the experimental diet groups' rearing time ($P<0.001$), with the fingerlings fed the probiotic diet (E1, E2) demonstrating improved growth performance and a decrease in FCR compared to those fed the control diet (C1, C2).

Table 1: Weight growth (g) in rearing days of culture periods 1 to 90 days Pangasius hypophthamus and Piaractus brachypomus fed with control (C1, C2) and experimental probiotic (E1, E2) diets.

Growth parameters	*P.hypophthalmus* Diet (C1, E1)		*P.brachypomus* Diet (C2, E2)	
Pond – DOC (1-90)	C1 - 90 days	E1- 90 days	C2 - 90 days	E2 - 90 days
Initial weight (g)	5.84 ± 0.25	5.72 ± 0.19	5.42 ± 0.50	5.28 ± 0.41
Final weight (g)	362.58 ± 3.86	498.50 ± 2.94	473.60 ± 3.74	583.65 ± 2.80
Weight gain (%)	356.74 ± 1.58	492.78 ± 1.64	468.18 ± 1.33	578.37 ± 1.27
FCR %	1.809 ± 1.62	1.53 ± 0.018	1.908 ± 0.024	1.58 ± 0.023
Survival rate %	80.38 ± 4.36	96.9 ± 3.42	85.33 ± 5.62	97.77 ± 5.69

After 90 days, the probiotic experimental diet (E1, E2) and control diet (C1, C2) were compared for percentage survival rate (%). The survival rate of *P.hypophthalmus* and *P.brachypomus* in the control ponds (C1, C2) dropped over the culture period when the ponds fed with probiotic feed (E1, E2) were compared to the ponds. Table 1 presents the survival rate (%) at day 90th using statistical analysis. These findings show that compared to the control ponds (C1 and C2), the probiotic-fed ponds (E1 and E2) had a greater survival rate.

Biochemical Composition Of Pangasius hypophthalmus And Piaractus brachypomus Fishes

The biochemical properties of *P.hypophthalmus* and *P.brachypomus* control (C1, C2) and experimental (E1, E2) muscle tissues are shown in Table 2. In the current study, the muscle tissue protein values of the experimental probiotic diet (E1, E2) were significantly higher than those of the Control diet (C1, C2). For 90th day, the protein levels in the experimental and control groups were assessed. During the 90th day culture period, the protein values of *P.hypophthalmus* C1, E1 (25.73±2.41 to 38.08±1.23) and *P.brachypomus* C2, E2 (31.52±2.68 to 42.12±3.45) were significantly ($P<0.001$) higher in the experimental probiotic treatment group than in the control group.

The tissue lipid content of *P.hypophthalmus* in the control (C1,C2) and experimental probiotic supplementation (E1) groups was assessed utilizing after cultured 90th day. The lipid content of the experimental probiotic-treated group was significantly higher than that of the control group. On 90 days, there was a considerable rise in the lipid content from C1, E1 (21.28±3.26 to 36.94±1.87) and C2, E2 (26.22±2.23 to 33.58±1.86).

The carbohydrate content of the *P.hypophthalmus* and *P.brachypomus* control (C1,C2) and experimental probiotic-treated (E1,E2) groups was assessed. Compared to the control group, the experimental probiotic-treated group's carbohydrate concentration progressively increased from day one to 90th day. The results are C1, E1 (27.24±3.07 to 45.36±2.21) and C2, E2 (40.52±2.61 to 48.75±2.18) recorded on 90th day, the experimental group's carbohydrate intake was significantly higher than that of the control diet.

Table 2: Biochemical parameters of the Pangasius hypophthalmus and Piaractus brachypomus in control (C1,C2) and experimental probiotic treated (E1,E2) ponds

Biochemical Parameters	*P.hypophthalmus* Diet (C1, E1)		*P.brachypomus* Diet (C2, E2)	
Pond – DOC (1-90)	C1 – 90th day	E1- 90th day	C2 - 90th day	E2 - 90th day
Protein (mg/g)	25.73 ± 2.41	38.08 ± 1.23	31.52 ± 2.68	42.12 ± 3.45
Lipids (mg/g)	21.28 ± 3.26	36.94 ± 1.87	26.22 ± 2.23	33.58 ± 1.86
Carbohydrates (mg/g)	27.24 ± 3.07	45.36 ± 2.21	40.52 ± 2.61	48.75 ± 2.18
Moisture (%)	77.48 ± 2.92	79.12 ± 3.22	74.89 ± 3.24	76.32 ± 3.47
Ash (%)	2.81 ± 0.28	3.92 ± 0.84	3.34 ± 0.38	4.25 ± 0.21
Amino acid (mg/g)	35.82 ± 2.56	46.74 ± 2.65	63.62 ± 1.97	78.44 ± 1.89

Following the 90th day of the culture period, the percentages of moisture and ash in the fish tissues of the control (C1,C2) and experimental (E1,E2) diet groups were measured. On day 90, there was a little increase in the percentage of moisture in experimental groups C1, E1 (77.48±2.92 to 79.12±3.22) and C2, E2 (74.89 ± 3.24 to 76.32 ± 3.47). And with time, the amount of ash increased. It increased from (C1, E1) 2.81 ± 0.28 to 3.92 ± 0.84 and (C2, E2) 3.34 ± 0.38 to 4.25 ± 0.21 on day 90, according to Table 2.

The amino acid composition of *P.hypophthalmus* and *P.brachypomus* fishes was also assessed on day 90 following the initiation of a control (C1, C2) and an experimental (E1,E2) diet. The experimental group's amino acid content was (C1,E1) 35.82 ± 2.56 to 46.74 ± 2.65) and (C2,E2) 63.62 ± 1.97 to 78.44 ± 1.89 significantly higher than that of the control group.

Digestive Enzymes Of Pangasius hypophthalmus And Piaractus brachypomus

The intestinal digestive enzymes of *P.hypophthalmus* and *P.brachypomus* were examined on days 1 through 90 following feeding with a control (C1, C2) and an experimental (E1, E2) probiotic diet. Protease, lipase and amylase activity were all considerably higher in the experimental group that received probiotic treatment than in the control group (Table 3). Probiotic dietary supplements have been studied for their effects on the growth, health, feed utilization, disease resistance, and digestive enzyme activities of freshwater fishes, specifically *P.hypophthalmus* and *P.brachypomus*, from fingerling to adult stages. Probiotics have been utilized more frequently to reduce stress and infections using a variety of strategies, including enhancing digestion by providing exoenzymes and stimulating the growth of advantageous microflora in the digestive system. Probiotics are utilized in finfish and shellfish aquaculture as an alternative to antibiotics (Chauhan and Singh, 2019), to improve feed efficiency (Ahmed et al., 2014), to enhance growth (Martinez Cruz P et al., 2012) and to improve fish safety during cultivation (Dawood et al., 2019).

Table 3: Pangasius hypophthalmus and Piaractus brachypomus digestive enzymes in the experimental probiotic-treated (E1, E2) and control (C1, C2) diets.

Digestive Enzymes	*P.hypophthalmus* Diet (C1, E1)		*P.brachypomus* Diet (C2, E2)	
Pond – DOC (1-90)	C1 - 90 days	E1- 90 days	C2 - 90 days	E2 - 90 days
Protease (U/mg protein/min)	97.82±3.11	121.37±3.74	16.88±1.07	19.21±1.37
Lipase (µmole ρ-nitro phenol/mg protein/min)	1.31±0.18	1.61±0.11	2.17±0.02	2.92±0.06
Amylase (µ mole maltose/ mg protein/min)	53.46±2.58	65.43±2.14	5.23±0.81	7.34±0.46

Soil and Water quality parameters

Samples of soil were collected from the C1, C2 and E1, E2 cultivation ponds. Soil samples from the cultivation ponds (C1, C2 and E1, E2) were taken in order to determine the factors related to soil quality. At different times during the experiment's culturing period, samples were gathered. After measurement, it was discovered that the samples were range indicated. Parameters of the soil are one of the most important elements of a successful fish farming operation. In comparison to the control ponds, the probiotic-treated pond's pH, electrical conductivity and organic carbon levels were maintained at optimal levels. Experimental (E1, E2) ponds treated with commercial probiotics "Super PS" for both soil and water demonstrated observable optimum level parameters maintained in sediment.

Table 4: Soil quality parameters of C1, C2 and E1, E2 ponds

Soil parameters ranges	*P.hypophthalmus* Culture ponds (C1, E1)		*P.brachypomus* Culture ponds (C1, E1)	
Pond – DOC (1-90)	C1	E1	C2	E2
pH	6.4±0.65	7.1±0.40	6.5±0.44	7.0±0.80
Electrical Conductivity (ds/m)	5.1±0.058	5.8±0.848	4.5±0.126	5.9 ±0.437
Organic Carbon (%)	1.12±0.08	1.28±0.09	1.13±0.064	1.14±0.085

Temperature, dissolved oxygen, ammonia, nitrite, pH, experimental significant *P.hypophthalmus* and *P.brachypomus* ponds were studied water quality during the culture period in the current study. The ranges and mean ± standard deviation (n = 3) of these measurements are displayed in Table 5 for ponds treated with probiotics (E1, E2) and ponds not treated with probiotics (C1, C2). Every water quality indicator was within permissible bounds for the growing of freshwater fish. Because the bacteria play a variety of roles, the current study found that the water quality measurements exhibited positive improvements.

Probiotics were helpful keeping right pH (Sambasivam, S. et al., 2003). The ponds treated with the experimental diet maintained their optimal levels of dissolved oxygen. The impact of probiotics on the mineralization of organic compounds could be the cause of this. As per Stumm & Morgan et al. (1996), there was a difference in the distribution of two water parameters in the pond water, namely nitrate (NO2) and ammonia (NH3). These differences might have come from interactions between biology and chemistry or from both working together. Table 5 illustrates that the experimentally treated ponds E1, E2 had lower levels of ammonia and nitrite than the control-treated ponds C1, C2.

Table 5: Water quality parameters of C1, C2 and E1, E2 ponds

Water quality Parameter	*P.hypophthalmus* Culture ponds (C1, E1)		*P.brachypomus* Culture ponds (C1, E1)	
Pond – DOC (1-90)	C1	E1	C2	E2
Temperature (C)	30.44 ± 1.10	32.80 ± 1.05	30.40 ± 1.90	32.10 ± 0.36
pH	8.80 ± 0.95	7.60 ± 1.05	8.40 ± 0.96	7.60 ± 1.08
Dissolved oxygen (mg/L)	4.0 ± 1.35	7.5 ± 1.20	4.5 ± 1.9	8.5 ± 1.22
Ammonia (mg/L)	0.31 ± 0.03	0.00 ± 0.01	0.30 ± 0.06	0.00 ± 0.01
Nitrite (mg/L)	1.18 ± 0.03	0.00 ± 0.01	1.82 ± 0.05	0.00 ± 0.02

* Data are Mean values ± S.D (n=3)

* Values in the same row with the same superscripts are not significantly different (P<0.001) (DMRT).

Historical Research

The research study investigated the histological changes in the gills, liver and muscle of the probiotic-treated groups of *Pangasius hypophthalmus* and *Piaractus brachypomus* for the culture period following the 90th day of collection, as well as the experimental (E1, E2) and control (C1, C2) groups.

Gill histology examination of 90th day control (C1, C2) and experimental (E1, E2) diets

Histological investigations have demonstrated that the conventional architecture of the gill filaments, comprising primary and secondary lamellae with bilaterally distributed mucous cells, represents the (10X and 40X) normal architecture of the gills.

Gill histology study of Pangasius hypophthalmus 90th day control (C1) and experimental (E1) diets

Fig 7 : Control (C1) gills (90th day)

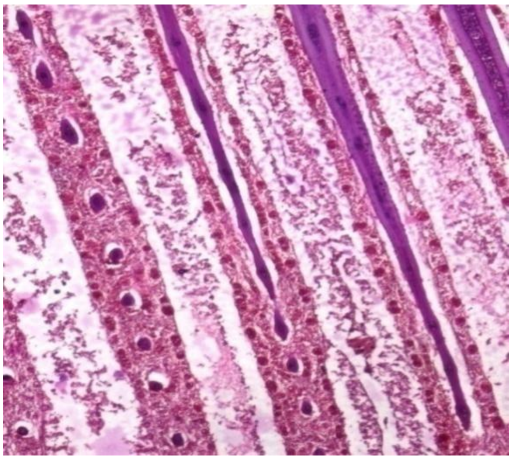


Fig 8: Control (C1) gills (90th day) 40X

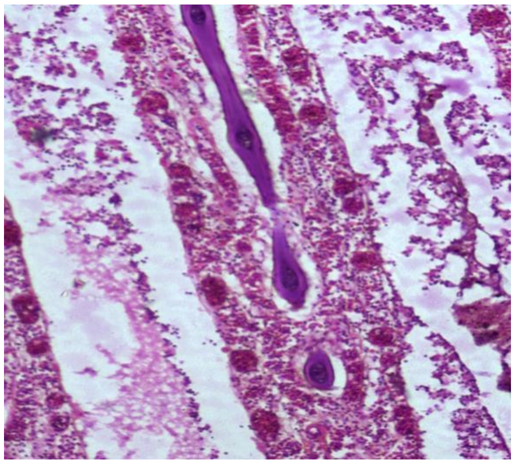


Fig 9: Experimental (E1) gills (90th day) 10X

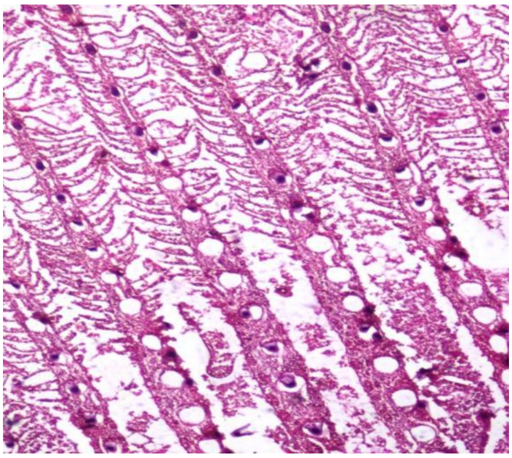
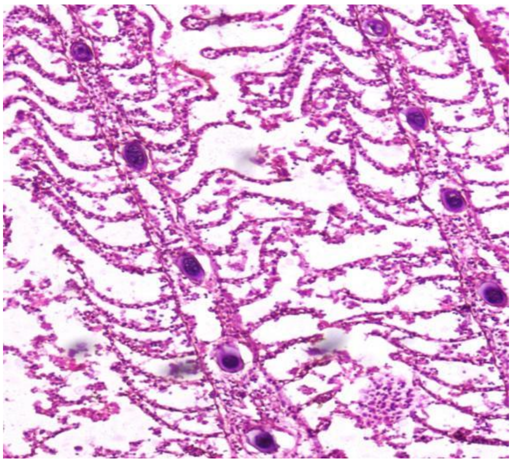


Fig 10: Experimental (E1) gills (90th day) 40X



The gill histology of the experimental (E1) *P. hypophthalmus* fish (Fig. 6 to 9) revealed that both the major and secondary gill lamellae were intact when compared to control pond (C1). Capillaries were divided by mucous cells and the secondary lamellar surface was covered with simple squamous epithelial cells. Each primary gill lamellae's anatomy was similar to that of a flat leaf. The structure was composed of two rows of secondary lamellae with a central supporting axis. They were situated between the interbranch on the septum, laterally. The consistent interlamellar gaps and highly vascularized cell layer covering the secondary lamellae were present on both sides. Control diet (C1) slides simultaneously showed a basement membrane with pillar cells enclosing blood holes beneath it, along with a relatively small and sparse population of mucous cells on main lamellae and a large concentration of them on the epithelial gill rackers (Okomoda, V. T., et al. 2019).

Gills histology study of Piaractus brachypomus 90th day control (C2) and experimental (E2) diet

Fig 11: Control (C2) gills (90th day) 10X

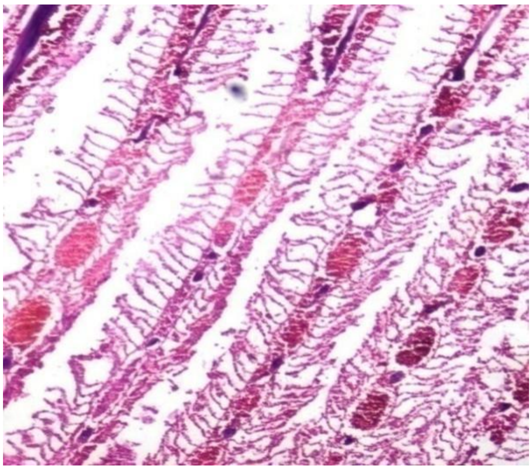


Fig 12: Control (C2) gills (90th day) 40X

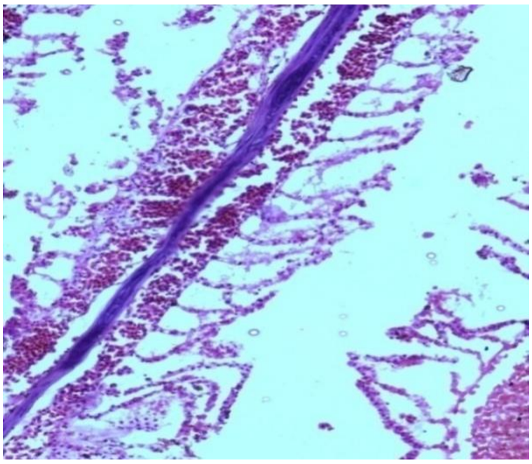


Fig 13: Experimental (E2) gills (90th day) 10X

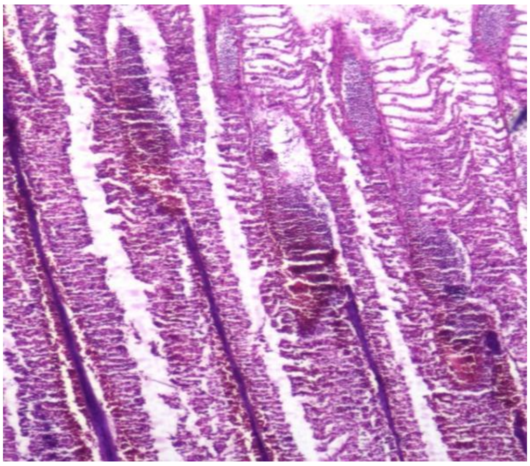
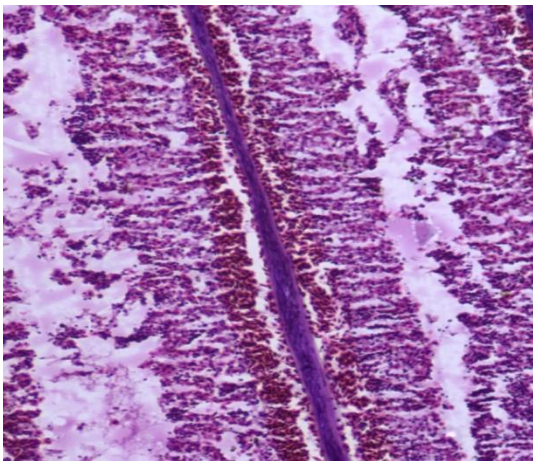


Fig 14: Experimental (E2) gills (90th day) 40X



Piaractus brachypomus in good health was revealed by the histology of the gills in the probiotic-treated (E2) pond. The gill structure had four pairs of gill lamellae and the primary lamellae and bone structure supported the gills on both sides. A single layer of pillar cells divided each of the many blood capillary channels evident when the secondary lamellae were sectioned vertically. The basement membrane was thicker than the laminar epithelium, enclosing blood voids behind the pillar cells (Fig. 10 to 13). The primary lamellae had far fewer and lowernumbers of mucous cells than the epithelial gill rackers, which had a greater number of them. In final analysis, (Ramirez-Duarte, et, al., 2008) the *Piaractus* *brachypomus* fish showed successful culture performance according to the *Rhodococcus* and *Rhodobacter* probiotics.

Liver histology examination of 90th day control (C1, C2) and experimental (E1, E2) diets

The liver is the largest extramural (outside the gastrointestinal tract) organ. Fish livers fulfil tasks comparable to those of mammals. In addition to the processing of proteins, carbohydrates, lipids and vitamins, it is also responsible for the production of bile, detoxification and the maintenance of metabolic balance (Harder, 1975).

Liver Histology study of Pangasius hypophthalmus 90th day control (C1) and experimental (E1) diet

Fig 15: Control (C1) Liver (90th day) 10X

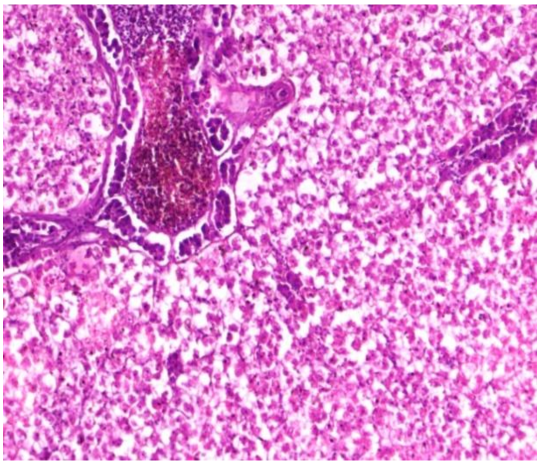


Fig 16: Control (C1) Liver (90th day) 40X

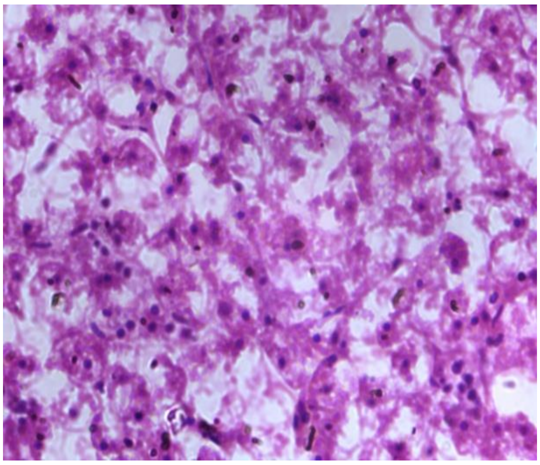


Fig 17. Experimental (E1) Liver (90th day) 10X

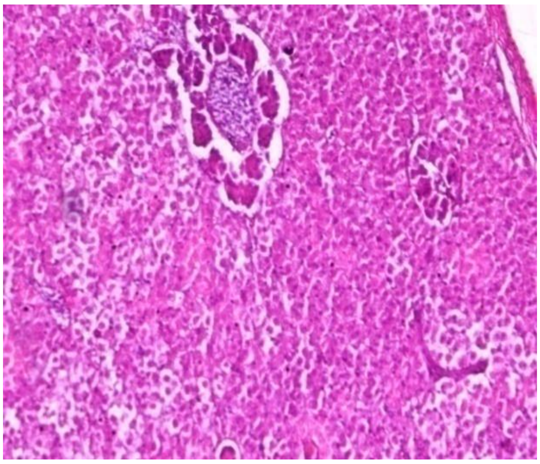
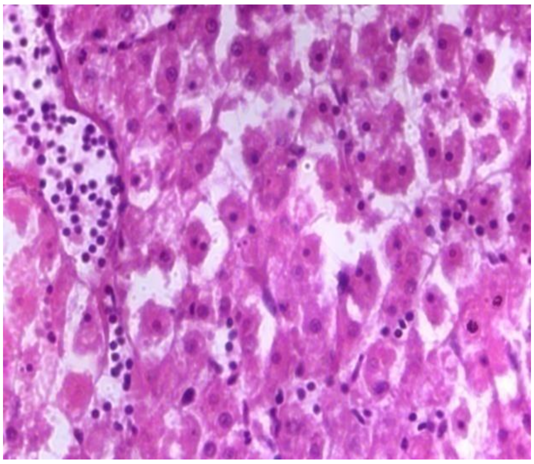


Fig 18. Experimental (E1) Liver (90th day) 40X



Fish reared without probiotics show different characteristics than *P.hypophthalmus* fish reared with probiotics. Probiotics are able to alter the growth performance of *P.hypophthalmus*. It was shown that the amount of neutrophils decreased, the proportion of polymorphonuclear elements increased and the number of lymphocytes increased against the background of probiotics (Fig. 14 to 17). The probiotic bacteria *Rhodococcus* and *Rhodobacter* do not belong to the human pathogens. They are GRAS

(Generally Regarded As Safe) approved. Under the influence of the probiotic microbiota, there was a redistribution of leukocyte cells with functionally dissimilar forms, some of which took on the role of pathogen defence.

Liver histology study of Piaractus brachypomus 90th day control (C2) and experimental (E2) diet

Fig 19: Control (C2) Liver (90th day) 10X

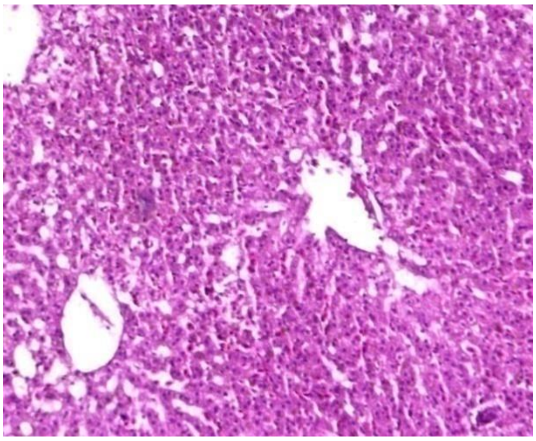


Fig 20: Control (C2) Liver (90th day) 40X

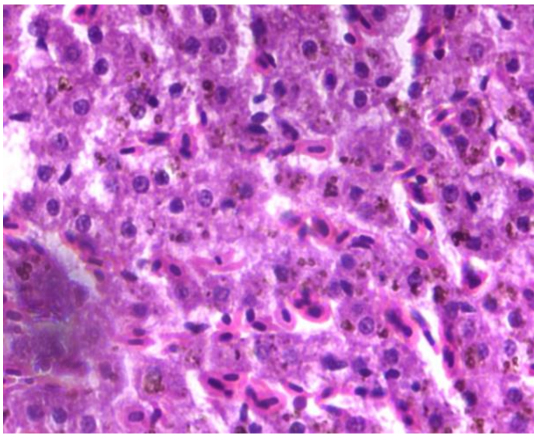


Fig 21: Experimental (E2) Liver (90th day) 10X

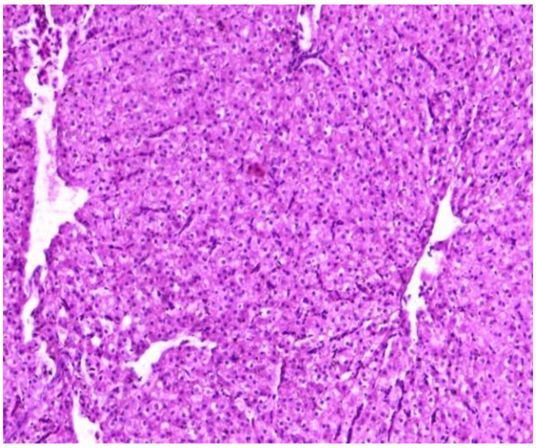
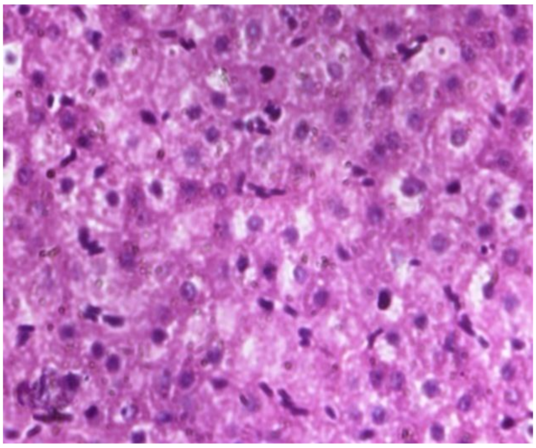


Fig 22: Experimental (E2) Liver (90th day) 40X



Fish liver oil Omega-3 fatty acids are needed daily for humans to perform at their best and taking supplements promotes joint health and protects against various degenerative diseases (Luna 1968). Fish raised with probiotics have a different growth performance *Piaractus brachypomus* than fish produced without probiotics. It was shown that the amount of neutrophils decreased, the proportion of polymorphonuclear elements increased and the number of lymphocytes increased against the background of probiotics (Fig. 18 to 21). Under the influence of the probiotic microbiota, there was a redistribution of leukocyte cells with functionally dissimilar forms, some of which took on the role of pathogen defence.

Muscle histology examination of 90th day control (C1, C2) and experimental (E1, E2) diets

In typical fish the muscle layer consisting of skeletal muscles is called the lateral muscle layer (Rescan, Pierre-Yves 2008). In the muscles of both the experimental group that received the water and soil probiotic 'Super PS' and the control group, the longitudinal muscle fibers are bundled in bundles called myotomes and further separated into myosepta by connective tissue. A nerve normally enters a muscle laterally and divides as it enters the connective tissue. No muscle abnormalities were not observed in fry fed the experimental probiotics diet (E1) for one to 90 days. Feeding P. hypophthalmus with the probiotic 'Super PS' resulted in improved growth of muscle bundles and fibers compared to feeding the control diet (Fig. 22 to 25).

Muscle histology study of Pangasius hypophthalmus 90th day control (C1) and experimental (E1) diet

Fig 23: Control (C1) Muscle (90th day) 10X

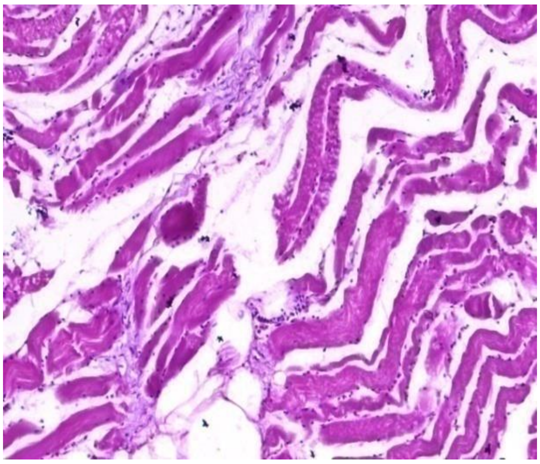


Fig 24: Control (C1) Muscle (90th day) 40X

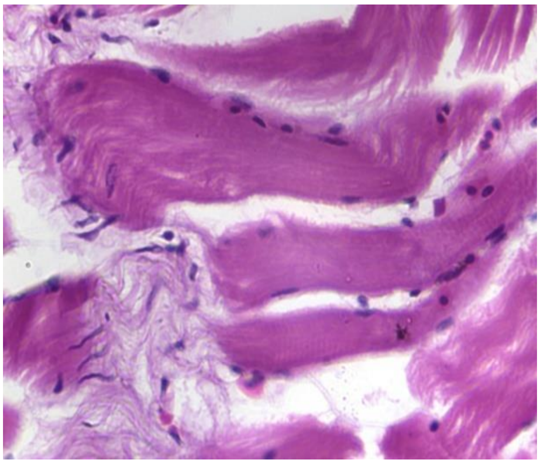


Fig 25: Experimental (E1) Muscle (90th day) 10X

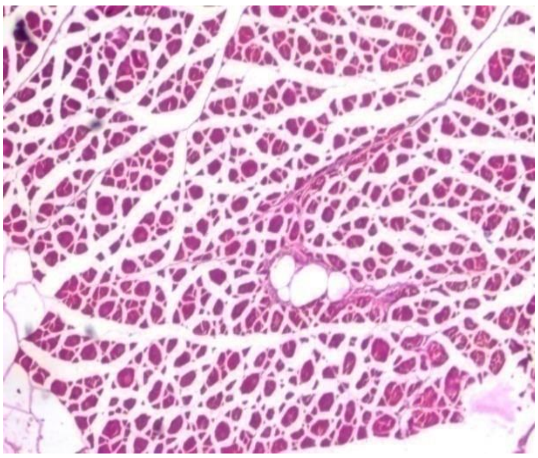
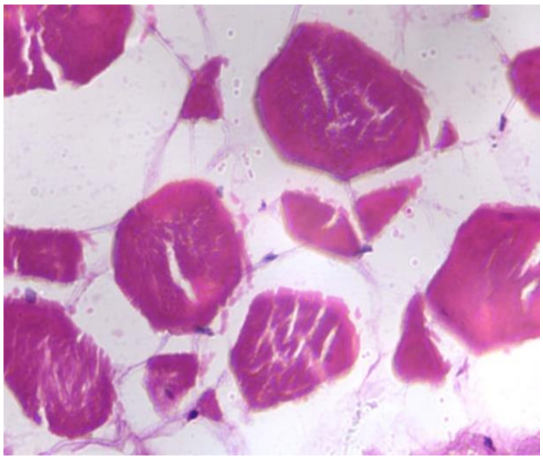


Fig 26: Experimental (E1) Muscle (90th day) 40X



The aquatic ecosystem is a distributed system. Feed additives such as probiotics and growth stimulants can be added to aqua diets to enhance the *P.brachypomus* fish's immune system and growth performance. Benefits depend on the acting mechanism and colonization capacity (Hashem, Aliaa MA et al., 2023). Out of all the protein sources, crude protein which accounts for 28% of fish meal may be replaced by microbiological origin. Treated *P.brachypomus* probiotics "Super PS" On day 90th of the experiment, an individual histological segment of the skeletal muscle was collected and then displayed on various plates (Fig. 26 to 29).

Muscle histology study of Piaractus brachypomus 90th day control (C2) and experimental (E2) diet

Fig 27: Control (C2) Muscle (90th day) 10X

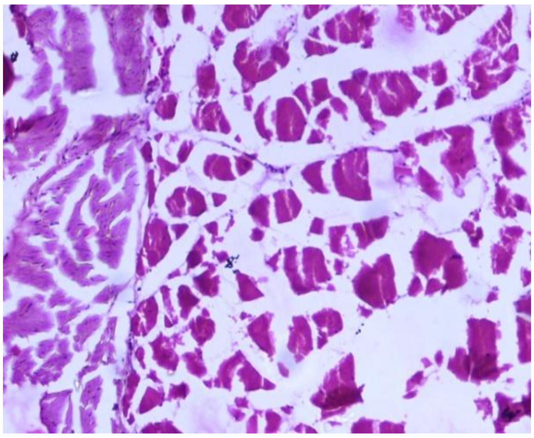


Fig 28: Control (C2) Muscle (90th day) 40X

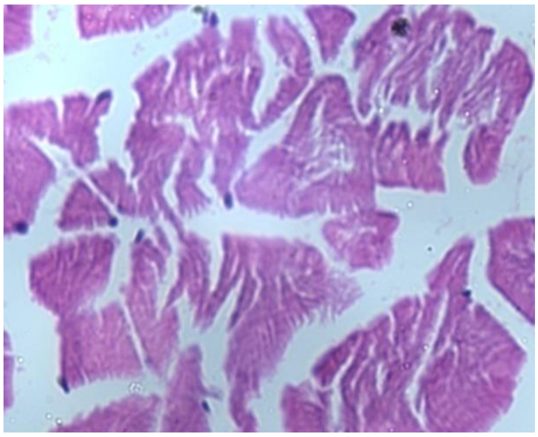
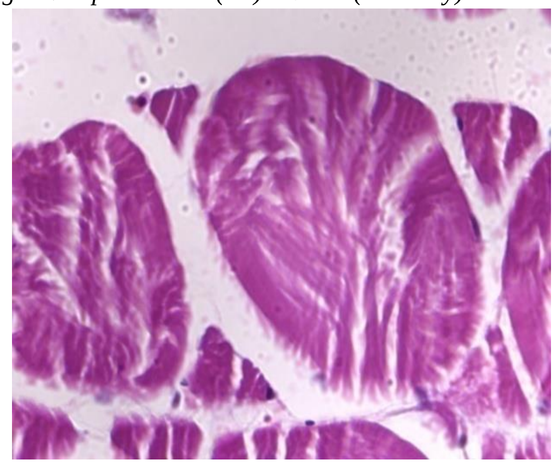


Fig 29: Experimental (E2) Muscle (90th day) 10X

Fig 30: Experimental (E2) Muscle (90th day) 40X



Fish that received probiotics displayed striated skeletal muscle fibers and a discernible heterotrophy with greater cytoplasmic size (Fig. 22 to 29). The muscle fibers of the experimental and control fishes are organized into bundles termed myotomes and these are divided by the connective tissue known as myosepta. Better growth was demonstrated by increased cytoplasmic size and cell proliferation. The study concludes that the commercial probiotics "Super PS" enhanced the quality of the soil and water, leading to increased growth and health preservation.

Discussion

In the current investigation, probiotics called "Super PS" that contain *Rhodococcus* and *Rhodobacter* were added to feed, soil and water. Probiotic use maintained an aquaculture-friendly environment, reduced dangerous microbes and enhanced the health of the pond bottom. Common gram-negative bacteria that can be found in freshwater, saltwater and marine environments are called *Rhodobacter*. It is an important key component in the bioconversion process, which uses biological systems to transform inexpensive starting materials into more valuable molecules (Murthy L. N. et al., 2015; Engelhart-Straub, S. et al., 2022).

The feed supplementation with 'Super PS' probiotics *Rhodococcus* and *Rhodobacter*, improved growth and reduced feed consumption in the current study. In the current study, fish fed diets treated with probiotics showed the best growth performance (Table 1). This could be due to improved performance of biochemical parameters (Table 2). The results of this study indicate that fish fed with probiotics showed the best growth performance, which was also confirmed histologically. This could be due to the fact that the host body receives more digestive enzymes and has better access to nutrients (Table 3).

P.hypophthalmus and *P.brachypomus* produced enough protein and a higher proportion of unsaturated fatty acids and essential amino acids when they were grown in freshwater. *P.hypophthalmus* and *P.brachypomus* require high-quality feed and it's critical to balance the nutritional requirements of proteins and lipids for the fish because the former have higher requirements for proteins than the latter and fish have lower lipid requirements than terrestrial animals (Miller, 2003). Therefore, feed formulations must closely manage the amount of proteins and fats in fish diets to ensure that fish diets are developed that complete their nutritional needs at reasonable prices (Thoman et al., 1999).

The study finds that the clean soil and water ponds (Table 4 and 5) have the maximum fish yield. Pond management, as well as soil and water quality, are highly significant, according to the study's findings. A better management approach and additional feed provided greater nutritional support for increased fish output. The soil and water quality of slightly and heavily contaminated ponds can also be enhanced by using "Super PS," which contains *Rhodococcus* and *Rhodobacter*. Appropriate management can result in increased production and survival rates as well as satisfied the requirement for the supply of biochemical parameters.

Probiotics were used to assess the fishes' growth, survival rate, and nutritional status. In addition to artificial diet, the fish were given nutrients such as calcium, starch, and sardine oil. Protein has the highest value on the biochemical evaluation, followed by other biochemical components such as lipids, amino acids, and carbs. Additionally, the intestinal microbiome was analyzed. When the guts of fish fed probiotics were analyzed, beneficial bacteria were found and harmful bacteria were excluded. The data produced by the current study may help commercial aquaculture incorporate the bacteria as a supplement in fish feed formulations to increase the degree of colonization in fish guts (Munirasu, et, al., 2017).

According to the study, the fish yield is highest in the clean soil and water ponds. As per the study's conclusions, pond management holds great significance along with soil and water quality (Tables 4 and 5). Probiotics treted feed and improved management techniques offered stronger nutritional support for higher fish production. Using "Super PS," which contains *Rhodococcus* and *Rhodobacter*, can also improve the soil and water quality of ponds that are marginally or severely contaminated. Increased production and survival rates as well as meeting the need for the supply of biochemical parameters can be achieved through appropriate management.

Compared to antibiotics, probiotic bacteria offer a healthier, sustainable, and eco-friendly way to lessen the mortality rate of aquaculture fish during illness outbreaks. Probiotics have a number of advantages, such as enhanced immunity, illness resistance, and growth. To provide the best probiotic efficacy in farmed fish, it is crucial to determine the appropriate dosage, the administration period, the fish stage at administration, the administration technique, and the probiotic viability throughout production and storage. Maintain biosecurity measures at the same time (Melo, Javier & Ruiz-Pardo et, al., 2020).

Conclusion

We conclude that the commercial probiotic "Super-PS" consisting mainly of *Rhodococcus* and *Rhodobacter* species is a significant catalyst for improving growth with profitable feed conversion, reducing pathogens and maintaining a friendly breeding environment in fish farms. To increase the level of aquatic productivity in International, the current study could be widely implemented for farmers, which would mean higher productivity for farmers. In the freshwater proteniated fishes *Pangasius hypophthalmus* and *Piaractus brachypomus*, the introduction of *Rhodococcus* and *Rhodobacter* probiotics as feed additives led to a remarkable improvement in the best growth performance, the development of biochemical nutrients, the active support of the digestive system in the production of a healthy body, which was histologically proven and the experimental ponds treated with probiotics achieved optimal soil and water quality parameters. The knowledge gained from ICFA-2024, the benefits of probiotics in the future aquaculture sector will bring sustainable benefits to the economic and blue transfermation of the aquaculture and fisheries industry.

References

Fuller, R. (1989) Probiotics in Man and Animals. Journal of Applied Bacteriology, 66, 365-378. http://dx.doi.org/10.1111/j.1365-2672.1989.tb05105.x

Gatesoupe FJ. The use of probiotics in aquaculture. *Aquaculture.* 1999; 180(1-2):147–165.

Verschuere L, Rombau G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. 2000; 64:655-671.

Harikrishnan R, Balasundaram C, Heo MS. Lactobacillus sakei BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper. Epinephelus bruneus. Fish Shellfish Immunol. 2010; 29:1037-1043.

Sunitha, K., and P. V. Krishna. "Efficacy of probiotics in water quality and bacterial biochemical characterization of fish ponds." International Journal of Current Microbiology and Applied Sciences 5.9 (2016): 30-37.

Storer, David Humphreys. “A History of the Fishes of Massachusetts.” Memoirs of the American Academy of Arts and Sciences, vol. 8, no. 2, 1863, pp. 389–434. JSTOR, https://doi.org/10.2307/25057980. Accessed 24 Jan. 2024.

Ribeiro, F. M et al,.(2016). Alimentação e nutrição de pirapitinga (piaractus brachypomums) e tambaqui (colossoma macropomum): revisão. Pubvet, 10(12), 873-882. https://doi.org/10.22256/pubvet.v10n12.873-882.

Wahab, M.A., M.M. Rahman and A. Milstein, 2002. The effect of common carp, Cyprinus carpio (L.) and mrigal, Cirrhinus mrigala (Hamilton) as bottom feeders in major Indian carp polycultures. Aquacult. Res., 33: 547–556.

Nur Mohammad, Mohd. Mainul Haque, Impact of Water Quality on Fish Production in Several Ponds of Dinajpur Municipality Area, Bangladesh, (IJESIR) International Journal of Science and Innovative Research: 2724-3338: 2021, 02(04), id:0100042IJESIR.

G. Biswas, A.R. Thirunavukkarasu, J.K. Sundaray, M. Kailasam, Culture of Asian seabass *Lates calcarifer* (Bloch) in brackishwater tide-fed ponds: growth and condition factor based on length and weight under two feeding systems, Indian J. Fish., 58 (2011), pp. 53-57.

Mustafa, S. And Ridzwan, A.R. Sustainable marine aquaculture recent developments with special reference to South East Asia. Kotakinbalu, Malasia., 2000.

Sambhu and Jayaprakas (2001),Livol (IHF-IOOO),a new herbal growth promoter in white prawn, Penaeus indicus (Crustacea), Indian Journal of Marine Sciences, Vol. 30, March 2001, pp. 38-43.

Folch j, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957 May; 226(1):497-509. PMID: 13428781.

Dubois, M., Gillies, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem., 28: 350-356.

Lowry, O.H., Rosebrough, N.J., Fan, A.C., and Randall, R.J. (1951) J. Biol. Chem.193, 265–275.

AOAC (2000). Official Methods of Analysis, 17th Ed. Association of Official Analytical Chemists, Gaithersburg, USA. Broihier K (1999). The Phyto-chemical Renaissance. Food Processing. 44:46-48.

Ishida, Y., Fujita, T. and Asai, K. 1981. New detection and separation method for amino acids by high-performance liquid chromatography. Journal of Chromatography. 204, 143-148.

Furne, M.C. Hidalgo, A. Lopez, M. Garcia-Gallego, A.E. Morales, A. Domenzain, J. Domezain, A. Sanz Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss* a comparative study Aquaculture, 250 (2005), pp. 391-398.

Bernfeld P. 1955. Amylases, α and β. Methods in Enzymology 1 : 149 – 158.

Jackson, M.L. (1958) Soil Chemical Analysis. Prentice-Hall Inc., Englewood Cliffs, NJ, p 498.

Ghosh, A.B., Bajaj, J.C., Hasan, R. and Singh, D. (1983) Soil and Water Testing Methods: A Laboratory Manual. IARI, New Delhi, 31-36.

American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater (twenty-first ed.), American Public Health Association, Washington, D.C. (2005), p. 46.

Boyd, C. E., 1979. Water Quality in Warmwater Fish Ponds, Agriculture Experiment Station, Auburn, Alabama, pp 359.

Chauhan A, Singh R (2018) Probiotics and their applications in aquaculture. In: Sharma D, Saharan BS (eds) Microbial cell factories, 1edn. Taylor & Francis, New York, pp 321–338.

Ahmed, G. U., Aktar, H., Chakma, S., Hasan, N. A., & Shamsuddin, M. (2015). Comparative study on growth of supermale tilapia and monosex tilapia in earthen mini pond. *Research in Agriculture Livestock and Fisheries*, *2*(1), 169–175. https://doi.org/10.3329/ralf.v2i1.23055.

Martinez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC. Use of probiotics in aquaculture. ISRN Microbiol. 2012 Oct 16;2012:916845. doi: 10.5402/2012/916845.

Dawood, Mahmoud & Magouz, Fawzy & Salem, Mahmoud & Abdel-Daim, Hanaa. (2019). Modulation of digestive enzyme activity, blood health, oxidative responses and growth-related gene expression in GIFT by heat-killed Lactobacillus plantarum (L-137). Aquaculture. 505. 10.1016/j.aquaculture.2019.02.053.

Sambasivam, S.; Chandran, R.; Khan, S.A. Role of probiotics on the environment of shrimp pond. Journal of environmental biology 2003, 24, 103-106.

Stumm, W. & J. J. Morgan, 1996. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3 rd. ed. J. Wiley & Sons, Inc. New York, USA.

Okomoda, V. T., et al. 2019, "Water quality tolerance and gill morphohistology of pure and reciprocal crosses of Pangasianodon hypophthalmus and Clarias gariepinus." *Journal of King Saud University-Science* 31.4 (2019): 713-723.

Ramirez-Duarte, et, al., 2008 "Acute toxicity and histopathological alterations of Roundup® herbicide on" cachama blanca"(Piaractus brachypomus)." *Pesquisa Veterinaria Brasileira* 28 (2008): 547-554.

Harder, G., & Narasimhan, M. S. (1975). On the cohomology groups of moduli spaces of vector bundles on curves. *Mathematische Annalen*, *212*(3), 215-248.

Luna, L.G. (1968) Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd Edition, McGraw-Hill, New York.

Rescan, Pierre-Yves. "New insights into skeletal muscle development and growth in teleost fishes." *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 310.7 (2008): 541-548.

Hashem, Aliaa MA, et, al., 2023."Production Optimization and Characterization of Antioxidant Protein Hydrolysate From Piaractus brachypomus Fish Meat By Probiotic Bacillus Strain Isolated From Chicken Gizzard." Egyptian Journal of Aquatic Biology & Fisheries. ISSN 1110 –6131; Vol. 27(5): 987 – 1010.

L. N. Murthy, B. Madhusudana rao, et,al, Nutritional composition, product development, shelf-life evaluation and quality assessment of pacu Piaractus brachypomus (Cuvier, 1818), ReseachGate, 2015, Indian J. Fish., 62 (1): 101-109.

Engelhart-Straub, S., Cavelius, P., Hölzl, F., Haack, M., Awad, D., Brueck, T., & Mehlmer, N. (2022). Effects of Light on Growth and Metabolism of Rhodococcus erythropolis. *Microorganisms*, *10*(8), 1680.

Thoman ES, Davis DA, Arnold CR. Evaluation of grow out diets varying protein and energy levels for red drum (Sciaenops ocellatus). Aquaculture. 1999; 176:343-353.

Munirasu, Subramani, Vankatachalam Ramasubramanian, and Palanisamy Arunkumar. "Effect of Probiotics diet on growth and biochemical performance of freshwater fish Labeo rohita fingerlings." Journal of Entomology and Zoology Studies 5.3 (2017): 1374-1379.

Melo, Javier & Ruiz-Pardo, Ruth & Hume, Michael & Sidjabat, Hanna & Villamil, Luisa. (2020). Probiotics for cultured freshwater fish. Microbiology Australia. 41. 10.1071/MA20026.