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Study of Probiotic on Growth Performance of Silver Barb (*Puntius gonionotus*)

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ABSTRACT: A 90-day feeding trial was conducted to evaluate the effects of probiotics on the growth and gut health of Silver barb (*Puntius gonionotus*) yearlings. Four iso-nitrogenous diets (32% crude protein) with different probiotic levels were tested: T1 (control), T2 (4% *Lactobacillus sporogenes*), T3 (4% *Saccharomyces cerevisiae*), and T4 (2% each of *L. sporogenes* and *S. cerevisiae*). Fish (initial weight: 63.42 ± 1.13 g to 64.42 ± 0.66 g) were reared in 300-liter aerated tanks, with 12 fish per replicate in triplicate groups. They were fed at 3% of body weight twice daily. At the end of the trial, body weight gain was highest in T4 (73.07 ± 1.72%) compared to T1 (30.55 ± 3.40%), T2 (40.64 ± 1.83%), and T3 (55.91 ± 3.29%). The T4 group also showed the highest final weight (110.31 ± 1.58 g), body weight gain (46.58 ± 1.30 g), specific growth rate (0.61 ± 0.01%/day), and protein efficiency ratio (1.35 ± 0.04), with the lowest feed conversion ratio (2.75 ± 0.09) ($p < 0.05$). Gut microbial analysis revealed higher *L. sporogenes* counts in T2 (21,366.67 ± 185.59) and T4 (12,100.00 ± 152.75), while *S. cerevisiae* was highest in T4 (232,333.33 ± 6,661.92) and T3 (198,333.33 ± 1,201.85) ($p < 0.05$). *S. cerevisiae* colonized the gut after 15 days, but *L. sporogenes* did not. The findings suggest that dietary supplementation of *L. sporogenes* and *S. cerevisiae* at 2% each enhances growth and gut health in Silver barb.

Keywords: Dietary supplementation, gut health, Silver barb, Growth performance, Feed.

INTRODUCTION

Fish is one of the most widely available, cost-effective, and nutritious sources of animal protein, playing a crucial role in addressing protein-calorie malnutrition. According to the FAO (2020), global fish production reached 179 million tons in 2018. Fish consumption patterns vary based on geographic location, demographics, socio-cultural factors, and local availability. Over the past two decades, capture fisheries have remained relatively stable, while the decline in global fish stocks has led to increased reliance on aquaculture to meet the nutritional demands of a growing population. Sustainable development, management, and regulation of aquatic resources are essential to ensure long-term economic, ecological, and social benefits. Understanding the geographical distribution of native fish species is vital for their conservation and protection from further decline. Effective management strategies and advanced scientific approaches are increasingly being applied in aquaculture to enhance production and maintain ecological balance.

In cash of growing demand for fish, the disease out breaks due to many reasons remains a constraint in the path of blue revolution to get more profits. Now-a-days, the paradigm shifting of world aquaculture from traditional farming to modern scientific approaches has made the aquacture as a global standard. Again the intensive aquaculture has created many issues mainly by diseases outbreaks and subsequently, huge economical losses. Even though, the application of antibiotics and chemotherapeutics has reduced the diseases in many ways, but constraint lies in the production of drug-resistant pathogens, inhibition of the aquatic animals' immune system and environmental vulnerability. In recent times, the uses of probiotics and immune-stimulants have become an important option rather than the use of antibiotics and chemotherapiteusin controlling the fish diseases.

Approximately 200 probiotic strains have been identified for use in various animal species (Palod and Singh 2004). It is now widely recognized that lactic acid bacteria (LAB) are part of the gut microbiota in fish from the early stages of life (Ringo, 2004; Ringo *et al*., 2005). A major challenge for researchers is establishing a stable indigenous microbiota in fish. According to Ringo *et al*. (2005), the protective effects of gut microbiota against pathogens are likely due to competition for nutrients and adhesion sites, along with the production of metabolites such as organic acids, hydrogen peroxide (H_2O_2), and bacteriocins. A key question in understanding the protective role of gastrointestinal microbiota is whether the fish GI tract

could serve as an entry point for pathogens. Over the past 25 years, several studies have shown that the GI tract is involved in infections caused by *Aeromonas* and *Vibrio* species (Groff and La Patra 2000; Harikrishnan and Balasundaram 2005). This suggests that lactic acid bacteria and other beneficial microbes colonizing the gastrointestinal tract might protect fish from pathogen attacks by producing protective substances such as bacteriocins. Among the various probiotic strains used in aquaculture, lactic acid-producing bacteria have gained significant importance due to their adaptability to diverse environmental conditions and their widespread presence in nature.

Extensive research has demonstrated that probiotics play a crucial role in aquaculture by enhancing fish growth rates and improving immune responses. To meet the growing global demand for animal protein, the use of probiotics in aquaculture has become increasingly important. Probiotics are now widely applied to boost fish production, and even small-scale farmers have started using them. However, for marginal farmers, the high cost of continuous probiotic use remains a significant challenge, increasing overall production expenses. This study aims to reduce the cost of probiotic application, thereby lowering cultivation expenses and increasing farmers' profit margins. In this research, locally available sporolac powder from pharmacies was used as a source of *Lactobacillus sporogenes*, while baker's yeast from local shops served as a source of *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

This study aimed to evaluate the impact of probiotic supplementation, including yeast (*Saccharomyces cerevisiae*), bacteria (*Lactobacillus sporogenes*), and their combination, on the growth performance and gut microbial composition of Silver barb (*Puntius gonionotus*). The research focused on assessing the colonization of the introduced microorganisms in the fish gut and their influence on overall growth. The materials and methods used in the study are outlined in detail below.

Experimental site and Design. The feeding trial was performed for 90 daysfrom 1st January 2020 to 31st march 2020 in the FRP tanks rearing systems at the Wet laboratory, College of Fisheries (OUAT), Rangailunda, Odisha, India. The feeding experiment was held for 13 weeks in the FRP tanks rearing systems at the Wet laboratory, College of Fisheries (OUAT), Rangailunda, Odisha, India. Twelve numbers of FRP tanks of 500-L capacity each, provided with seasonal and conditioned water (obtained from borewell) were used for the study. Standard pre-stocking procedure was followed before the initiation of the study including proper cleaning, washing and drying etc. Four different feeding trials were designed in triplicates such as T1 (Control), T2 (*Lactobacillus sporogenes*), T3 (*Saccharomyces cerevisiae*) and T4 (*L. sporogenes* + *S. cerevisiae*) following a completely randomized design (CRD). A detail regarding the experimental design was given below:

- Treatment 1 (T_1): Feeding with commercially floating pelleted feed.
- Treatment 2 (T_2): Dietary inclusion of *L. sporogenes* probiotic @4% in the commercially floating pelleted feed.
- Treatment 3 (T_3): Dietary inclusion of *S. cerevisiae* probiotic @4% in the commercially floating pelleted feed.
- Treatment 4 (T_4): Dietary inclusion of *L. sporogenes* and *S. cerevisiae* @2% each in the commercial feed.

For this study, baker's yeast (Angel) and sporolac powder were used as live sources of *Saccharomyces cerevisiae* and *Lactobacillus sporogenes*, respectively. The baker's yeast contained approximately 15 billion viable cells per gram, while the sporolac powder provided no less than 150 million spores of *Lactic Acid Bacillus* (*L. sporogenes*) per gram.

Experimental fish. Silver barb (*Puntius gonionotus*) yearlings, with an average weight of 63.76 ± 0.67 g and a length of approximately 14.06 ± 2 cm, were sourced from Gopinath Aquafarm, a private fish seed supplier in Bhubaneswar, Odisha, for this study. Upon arrival, the fish were acclimated under controlled conditions with proper management practices for 15 days. During the acclimation period, they were fed a commercial pellet feed containing 32% crude protein (CP). After acclimation, 12 yearlings were stocked into each FRP tank filled with well-conditioned, aerated water. The fish were then fed the respective experimental diets (Growell pellet feed, CP: 32%) at 3% of their body weight, divided into two equal portions daily, for 90 days. To maintain water quality, 30% of the tank water was replaced every two weeks to remove waste and fecal matter.

Growth performance and gut microbial counts were monitored through fortnightly sampling from each treatment group. Additionally, one fish from each tank was sacrificed periodically for gut microbial colony analysis. Fish in the probiotic treatment groups were alternately fed probiotic-supplemented and control diets in 15-day cycles. Specifically, they were fed probiotic-incorporated feed for the first 15 days, followed by the control feed for the next 15 days, to evaluate the consistency of probiotic effects over time. This feeding cycle was maintained for the entire 90-day trial period, with regular sampling conducted to assess growth and gut health.

Experimental feed. A commercially available pelleted floating fish feed (Growell pelleted floating feed) with 32% crude protein, 5% fat, and 5.5% fiber was used for both the acclimatization period and the feeding trial. The experimental diets were formulated by incorporating probiotics at 2% and 4% levels into the commercial feed using carboxymethyl cellulose (CMC) as a binder. Three different probiotic treatments were prepared: *Lactobacillus sporogenes* at 4% of the total

feed, *Saccharomyces cerevisiae* at 4%, and a combination of *L. sporogenes* and *S. cerevisiae* at 2% each.

To prepare the experimental feeds, the specified quantities of probiotics were mixed with the commercial feed in a container. The calculated amount of CMC was then added and thoroughly mixed to form a uniform dough. The dough was processed into 2 mm diameter pellets using a hand pelletizer. The pellets were spread on a clean surface for air drying and then oven-dried at 40–50°C overnight. Once dried, the pellets were packed into airtight polythene bags, labeled, and stored at 4°C until use.

Growth study. Sampling was conducted every two weeks over a 90-day period to evaluate fish growth performance. All fish from each experimental tank were carefully collected and weighed in bulk using an electronic balance, ensuring no water was included in the measurement. Fish length was recorded using a standard measuring scale. At the conclusion of the study, all fish were harvested using a hand net, and the tanks were completely drained. The final survival rate (%) for each tank was then calculated. The growth indices, feed qualities and survival (%) were evaluated using the following formulae:

Growth parameters:

Weight gain (g) = Mean final body weight of fish – Mean initial bodyweight of fish

$$\text{Weight gain (\%)} = \frac{\text{Final weight of fish} - \text{Initial weight of fish}}{\text{Initial weight of fish}} \times 100$$

$$\text{Specific growth rate (\% day}^{-1}\text{)} = \frac{(\log_e \text{Final body weight} - \log_e \text{Initial body weight})}{\text{Total no. experimental days}} \times 100$$

Survivability (%) = (F/I) × 100

Where, F = Total number of fishes at the time of harvesting

I = Initial number of fishes at the time of stocking

Feed utilization parameters:

Feed conversion ratio (FCR). The following formula calculated the feed conversion ratio

$$\text{FCR} = \frac{\text{Total dry food intake(g)}}{\text{Total live weight gain (g)}}$$

$$\text{FCR} = \frac{\text{Total dry food intake(g)}}{\text{Total live weight gain (g)}}$$

Protein efficiency ratio (PER). The following formula calculated the PER

$$\text{PER} = \frac{\text{Total weight gain (g)(g)}}{\text{Total protein intake (g)}}$$

Water quality analysis. Water quality parameters from each experimental tank such as temperature, pH, dissolved oxygen (DO), total alkalinity, hardness and ammonia-nitrogen (NH_3-N) were monitored at every weekly interval till end, following the standard methods of APHA (2005). The water quality parameters like temperature, pH and dissolved oxygen were calculated daily basis with standard methods. Likewise, water alkalinity, hardness and ammonia-nitrogen (NH_3-N) were measured using standard laboratory methods periodically.

Estimation of probiotic gut microbial load. The microbial load was assessed using the Total Plate Count (TPC) method (APHA, 2005). Initially, the concentration of *Lactobacillus sporogenes* in sporolac powder and *Saccharomyces cerevisiae* in baker's yeast was determined using the TPC method. Sampling was carried out at 15-day intervals. One fish from each tank was carefully transferred to the laboratory under hygienic conditions. To prevent contamination, the fish were cleaned with absolute alcohol. They were then euthanized using an overdose of clove oil (50 µl/l), and the dissection was performed using sterilized scissors. The gut contents were extracted using sterile forceps. Prior to dissection, the fish were starved for 24 hours. Approximately 1 g of gut sample was collected from each fish under aseptic conditions.

The extracted gut samples were homogenized with 0.85% NaCl solution using a sterilized homogenizer. Serial dilutions were prepared from 10^{-3} to 10^{-5} using sterile saline in separate dilution tubes. MRS agar and YPG agar media were used for isolating *L. sporogenes* and *S. cerevisiae*, respectively. Duplicate plates were prepared for each dilution level (10^{-3}, 10^{-4}, and 10^{-5}). A 1 ml sample from each dilution was transferred into Petri plates, followed by the addition of 20 ml of media. After allowing the media to solidify, the MRS agar plates were incubated at 35°C for 24–72 hours. Similarly, YPG agar plates were incubated at room temperature (30°C) for 3–4 days to allow the formation of *S. cerevisiae* colonies. The developed colonies were counted, and the colony-forming units (CFU) were calculated accordingly.

Biochemical test. After confirming the presence of bacteria and yeast using specific media, further identification was carried out through biochemical tests following standard protocols (APHA, 2005). These tests were based on pH changes and substrate utilization. *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* exhibited metabolic activity during incubation, indicated by a color change in the media, which could be observed directly or after adding a reagent.

The identified organisms were first isolated and purified. A colony was carefully picked from the Petri plate using a sterile loop and transferred to a slant containing the appropriate agar media for cultivation. Afterward, a single isolated colony was taken and inoculated into a 5 ml nutrient broth. The inoculated broth was incubated at 35–37°C for 24 hours or until the culture appeared turbid, indicating successful growth.

Statistical interpretation. The recorded values were evaluated statistically through DMRT (Duncan's multiple range tests)by statistical package SPSS version 19.0 (SPSS Incorporation, Chicago, USA). A 5% level of possibility ($p<0.05$) was taken to decide the statistically significant responses between the treatments means. Results are represented as mean ± S.E. (standard error). Moreover, the data arrangements

and graphs were performed by using MS excel sheet 2007.

RESULTS AND DISCUSSION

Water quality parameters. Table 1 depicts the water quality parameters prevailed throughout the study. During the study period water temperature ranged from 26.1 to 30.2°C, dissolved oxygen contents were above 5 mg l^{-1}, total alkalinity varied between 60 to 125 mg $CaCO_3$ l^{-1}, hardness was 60 to 90 mg $CaCO_3$ l^{-1}, free carbon dioxide (CO_2) and ammonia-nitrogen (NH_3-N) ranged between 0.02-0.05 and 0.05-0.12 mg l^{-1}, correspondingly; pH varied between 7.0 to 8.4. The water quality parameters didn't show much variation among the treatments ($p>0.05$).

Growth performance. The growth performance and feed utilization of silver barb (*Puntius gonionotus*) under different probiotic treatments are summarized in Table 2. No significant variation was observed in the initial body weight among the treatments ($p > 0.05$). However, the final body weight, body weight gain, percentage weight gain, and specific growth rate (SGR) were significantly higher in the T4 group compared to other treatments ($p < 0.05$). The body weight gain (%) at the end of the study was 30.55±3.40, 40.64±1.83, 55.91±3.29, and 73.07±1.72 for T1, T2, T3, and T4, respectively. The highest growth, indicated by final body weight (110.31±1.58 g), body weight gain (46.58±1.30 g), and SGR (0.61±0.01% per day), was recorded in the T4 group ($p < 0.05$). The T1 group showed the lowest growth among all treatments ($p < 0.05$).

Feed utilization indices, such as feed conversion ratio (FCR) and protein efficiency ratio (PER), also varied significantly ($p < 0.05$). The lowest FCR (2.75±0.09) was noted in the T4 group, followed by T3, while T1 had the highest FCR. The highest PER (1.35±0.04) was observed in the T4 group ($p < 0.05$). Survival rates did not differ significantly between treatments ($p > 0.05$).

Gut microbial study. The periodic changes in gut microbial counts of *L. sporogenes* and *S. cerevisiae* are shown in Table 3 and 4. Initially, the microbial load was zero. After 15 days, *L. sporogenes* count in T2 was 21,200±173.21 CFU/g, while *S. cerevisiae* was absent since the feed contained only *L. sporogenes*. In T3, *S. cerevisiae* count was 117,666.67±1452.97 CFU/g, while *L. sporogenes* was absent. In T4, both *L. sporogenes* (10,533.33±233.33 CFU/g) and *S. cerevisiae* (145,000.00±1527.53 CFU/g) were present due to the mixed feed.

After 30 days, *L. sporogenes* was absent in T2 as normal feed was used, but *S. cerevisiae* counts in T3 and T4 increased to 126,000.0 ±2081.67 CFU/g and 158,000.00±577.35 CFU/g, respectively. After 90 days, the microbial load in T1 and T2 remained zero, while *S. cerevisiae* counts were 198,333.33±1201.85 CFU/g in T3 and 232,333.33±881.92 CFU/g in T4.

The highest *L. sporogenes* count was observed in T2, followed by T4 ($p<0.05$), while T1 showed significantly lower counts ($p<0.05$). *S. cerevisiae* counts were significantly higher in T4 than in T3 throughout the study ($p<0.05$). The combined microbial load (*L. sporogenes* + *S. cerevisiae*) was significantly higher in T4 across the study period ($p<0.05$).

Biochemical test. The results of different biochemical tests were presented in Table 5.

Table 1: Range of various water quality parameters prevailed during study.

Parameters	T_1	T_2	T_3	T_4
Temperature (°C)	26.1-29.7	26.4-30	26.2-30.2	26.8-30.1
P^H	7.0-8.0	7.1-8.3	7.3-8.0	7.0-8.4
Dissolved oxygen(DO)	5.0-8.0	5.0-7.8	5.3-8.1	5.4-8.5
Total alkalinity (mg $CaCO_3$ l^{-1})	60-115	65-120	70-125	65-120
Hardness (mg $CaCO_3$ l^{-1})	60-70	60-80	65-90	68-85
Ammonia-nitrogen (mg l^{-1})	0.05-0.10	0.08-0.12	0.05-0.10	0.06-0.12
Free carbon dioxide(mg l^{-1})	0.02-0.05	0.03-0.04	0.04-0.05	0.02-0.05

Note: Data are depicted as mean ± SE (r = 3); Values in the same row with dissimilar superscript *i.e.* a, b, c and d varying significantly ($p<0.05$) among the treatments.

Table 2: Growth performance (weight) of Silver barb (*Puntius gonionotus*) in different treatments.

Parameters	T_1	T_2	T_3	T_4	*p-value*
Average initial weight (g)	$63.42±1.13^a$	$64.42±0.66^a$	$63.46±0.62^a$	$63.73±0.28^a$	0.76
Average final weight (g)	$82.76±2.14^a$	$90.58±0.33^b$	$98.90±1.15^c$	$110.31±1.58^d$	0.000
Body weight gain (g)	$19.35±2.04^a$	$26.16±0.93^b$	$35.44±1.75^c$	$46.58±1.30^d$	0.000
Body weight gain (%)	$30.55±3.40^a$	$40.64±1.83^b$	$55.91±3.29^c$	$73.07±1.72^d$	0.000
Specific growth rate (% day^{-1})	$0.29±0.03^a$	$0.38±0.02^b$	$0.49±0.02^c$	$0.61±0.01^d$	0.000
Food conversion ratio	$3.71±0.04^c$	$3.37±0.10^b$	$3.21±0.02^b$	$2.75±0.09^a$	0.000
Protein efficiency ratio	$1.00±0.01^a$	$1.10±0.03^b$	$1.15±0.01^b$	$1.35±0.04^c$	0.000

Note: Data are expressed as mean ± SE (r = 3); mean values in the same row with dissimilar superscript varying significantly ($p<0.05$); small alphabets (*viz.*, a, b, c and d) indicates significance disparity between the treatments.

Table 3: Fortnightly changes in *L. sporogenes* count of Silver barb in different treatments.

Treatments	Initial	15 Days	30 Days	45 Days	60 Days	75 Days	90 Days
T_1	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
T_2	0.00±0.00^a	21200.00±173.21^c	0.00±0.00^a	21233.33±176.38^c	0.00±0.00^a	21366.67±185.59^c	0.00±0.00^a
T_3	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
T_4	0.00±0.00^a	10533.33±233.33^b	0.00±0.00^a	11600.00±115.47^b	0.00±0.00^a	12100.00±152.75^b	0.00±0.00
p-value	NS	0.000	NS	0.000	NS	0.000	NS

Note: Data are expressed as mean ± SE (r = 3); mean values in the same row with dissimilar superscript varying significantly (p<0.05); small alphabets (*viz.*, a, b, c and d) indicates significance disparity between the treatments.
NS : Non significant

Table 4: Fortnightly changes in *S.cerevisiae* count of Silver barb in different treatments.

Treatments	Initial	15Days	30Days	45Days	60Days	75Days	90Days
T_1	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
T_2	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
T_3	0.00±0.00^a	117666.67±1452.97^b	126000.00±2081.67^b	146000.00±1527.53^b	153333.33±4255.72^b	182666.67±1452.97^b	198333.33±1201.85^b
T_4	0.00±0.00^a	145000.00±1527.53^c	158000.00±577.35^c	177000.00±1154.70^c	193666.67±881.92^c	217000.00±1154.70^c	232333.33±881.92^c
p-value	NS	0.000	0.000	0.000	0.000	0.000	0.000

Note: Data are expressed as mean ± SE (r = 3); mean values in the same row with dissimilar superscript varying significantly (p<0.05); small alphabets (*viz.*, a, b, c and d) indicates significance disparity between the treatments.
NS : Non significant

Table 5: Biochemical test for microbes.

Sr. No.	Name of biochemical Test	Results	
		Lactobacillus sporogenes	*Saccharomyces cerevisiae*
1.	Staining	Gram +ve	-
2.	Catalase	+ve	-
3.	Nitrate reduction	-ve	-ve
4.	Motility	Motile	Non motile
5.	VP	+ve	-
6.	Methyl red	+ve	-
7.	Starch	+ve	+ve
8.	Fructose	+ve	+ve
9.	Indole	-ve	-
10.	Lactose	+ve	-ve

Note: +ve sign specifies the positive reaction, -ve sign specifies the negative reaction and - indicates the test is not necessary.

Environmental factors play a crucial role in maintaining a healthy aquatic environment and creating optimal conditions for fish growth (Boyd, 1982; Biswas *et al.*, 2015). The success of aquaculture systems largely depends on effectively managing these environmental factors, which directly or indirectly impact production. Proper management typically involves fertilization strategies combined with maintaining good soil and water quality (Ayyappan *et al.*, 2011). Aquatic organisms maintain a natural balance with potential pathogens and their environment, but any decline in water quality can lead to stress and increased disease vulnerability. Therefore, maintaining optimal water quality is essential for improving aquatic animal production, making it a key factor in successful aquaculture.

In the study, the water temperature in the tanks ranged between of 26.1 to 30.2°C during the study. The other water quality parameters including dissolved oxygen (DO) contents were beyond 5 mg l^{-1}, total alkalinity varied in the range of 60 to 125 mg $CaCO_3$ l^{-1}, free carbon dioxide (CO_2) and ammonia-nitrogen (NH_3-N) ranged between 0.02-0.05 and 0.05-0.12 mg l^{-1}, respectively. The water pH remained alkaline, with the range of 7.0-8.4 in the tanks throughout the study period. The water quality indices *viz.*, water temperature, pH, dissolved oxygen (DO), total alkalinity, hardness, free CO_2 and ammonia showed no marked variation in all the treatments. This phenomenon may be due to periodic management practices including periodically water replenishment and maintenance throughout culturing. The prevailed water quality parameters during the culture period were within thesafe range as prescribed for grow-out rearing of various carps and catfishes (Boyd, 1982; Jena *et al.*, 2007; Rahman *et al.*, 2013; Debnath *et al.*, 2016; Das *et al.*, 2020). Similar observations were also reported in several other carps and catfishes such as sharpunti (Chakraborty *et al.*, 2003), mahseer (Rahman *et al.*,

2005), tengra (Rahman *et al.*, 2013) and pengba (Das *et al*., 2020).

In the present experiment, fish reared in combined probiotics *i.e.*, T_4 (*Lactobacillus sporogenes* + *Saccharomyces cerevisiae*) fed treatment showed significant higher growth as indicated by average final body weight, body weight gain (g) and body weight gain (%) compared to control and other treatments. Likely, the specific growth rate was also found significantly high in T_4 treatments than in other treatments. In line to this, the fish fed with control diets showed significantly lower growth as indicated by body weight gain (g), body weight gain (%) and specific growth rate (% day^{-1}) than in other probiotic fed groups.

The higher growth performance in probiotic supplemented treatments than that of control is due to the positive effect of probiotics, as growth promoter, significantly improved the survival and growth performance (Keysami *et al*., 2007; Parthasarathy and Ravi 2011; Gupta and Dhawan 2013). Further, the better growth performance in T4 groups than those of single probiotic incorporated groups is due to the combined effect of both probiotics as a growth promoter, significantly enhanced the growth and survivability of Silver barb in the study.

Feed conversion ratio (FCR) and Protein efficiency ratio (PER) is one of the important feed quality attributes that determine the profitability of any aquaculture system. Because it measures the amount of flesh produced or protein consumed per kg of feed given. A higher energy requirement can explain the higher FCR for absorption at higher food utilization, normally accompanied through reduced nutrient assimilation and growth (Meyer-Burgdorff and Rosenow 1995; Bonaldo *et al*., 2010). The aquaculture industries are finding suitable options to reduce FCR to the minimum level to reduce the cost associated with the feed.

In the study, the fish fed with combined probiotics diet documented significantly lower in FCR, whereas the control group showed significantly higher in FCR. The lower FCR in T_4 groups than control group is associated better palatability and acceptance of the diet, which subsequently resulted in lower FCR. In addition, the T_4 treatment also reported significantly lower FCR than other single probiotic bacterial incorporated groups, which might be due to the combined effect of probiotics in this group. Similar to the present findings, Saad *et al*. (2009); El-Haroun *et al*. (2006) reported the positive effects of probiotic incorporation on feed utilization in *M. rosenbergii* and Nile tilapia, where lower FCR was reported.

PER is a determinant to know how well fish can consume the protein that is given through feed (Nalawade and Bhilave 2011). Protein is the most costly component in any aquaculture system. Hence, the use of surplus protein in the fish feed is wasted, which subsequently, elevates the operational cost and nitrogen load in the aquatic environment (Ahmad *et al*., 2008). In the present study, the fish fed with combined probiotic incorporated diet received significantly higher in PER compared to control and single probiotic incorporated groups. Similarly, Uma *et al*. (1999) documented a notable improvement in FCR and PER during shrimp larvae rearing when fed diets containing *L. plantarum* probiotics. Ziaei-Nejad *et al*. (2006) also reported enhanced food assimilation in probiotic treatments, leading to better FCR and SGR in *F. indicus*. Comparable findings have been reported by other researchers, including Suralikar and Sahu (2001); Yanbo and Zirong (2006); Gupta and Dhawan (2013).

In general, the incorporation of probiotics in the diet enhances the health status and disease resistance of the animals (Dawood *et al*., 2016). Moreover, the feeding probiotics enhances the elevation of immunological and hematological responses, which subsequently, resulted in better survival. The present study showed no significance difference in survival (%) among the treatments. The possible explanation to these findings might be due to the controlled rearing system with periodical standard managements practices, as evidenced from the water quality parameters which is within the suitable ranges for the fish culture.

Mohapatra *et al*. (2011) studied the outcome of various microbial probiotics on different activities of rohu (*Labeo rohita*) as applied in the diet of fingerlings. Their study counted different heterotrophic bacteria population in CFU-10^{10} kg^{-1} in the gut of fish fingerlings at every fortnightly sampling through different dietary probiotic supplemented groups. Divya *et al.* (2012) studied the colonization of probiotic in the gut and its effect on Rosy barb, *Puntius conchonius*. They enumerated the bacterial count of various microbes in the gut of Rosy barb before and after for 45 days of probiotic treatment.

In the present study, the fish reared in T_2 and T_4 showed significantly higher in *L. sporogenes* and *S. cereviceae* gut microbiota count, respectively. In overall, the fish fed with combined probiotics incorporated diet *i.e.* T_4 treatments showed better results in gut microbiota count (*L. sporogenes* and *S. cereviceae*) than other treatments. Moreover, the T_4 treatment also showed significantly higher in both probiotics (*L. sporogenes* and *S. cereviceae*) in the gut microbiota compared to other two single probiotic incorporated treatments. This supports the better growth performance in T_4 groups rather than other two probiotic incorporated treatments. Several researches also observed colony establishments in the guts in other carp species such as in Rohu (Mohapatra *et al*., 2011) and in other Indian major carps (Nayak and Mukherjee 2011). Therefore, in the present study combined establishment of both bacterial colony in T_4 treatments rather than T_2 and T_3, improved the intestinal health,

thereby increases feed utilization and nutritional profiles of Silver barb.

Selective media were used to verify species, followed by biochemical tests for confirmation. Table 8 indicates that *Lactobacillus sporogenes* tested positive for catalase, lactose, fructose, starch, VP, and methyl red tests, but negative for nitrate reduction and indole. Similarly, *S. cerevisiae* tested positive for fructose and starch but negative for nitrate reduction and lactose.

Venkat *et al*. (2004) conducted biochemical tests for *L. acidophilus* and *L. sporogenes*, while Gobinath and Ramanibai (2012) studied the impact of probiotics on pathogenic bacteria in Nile tilapia. They noted that *Lactobacillus* species were negative for indole, methyl red, VP, nitrate reduction, catalase, and oxidase but positive for citrate utilization, urease, fructose, glucose, and lactose. Dhansekaran *et al*. (2010) reported different biochemical traits of *Lactobacillus* isolates against freshwater fish pathogens. Vaseeharan *et al*. (2011) also documented the effect of probiotics on antibiotic sensitivity and pathogenicity of *Listonella anguillarum* in *Penaeus monodon* culture. Ghosh (2011) analyzed 13 yeast species from *Syzygium cumini* fruit based on biochemical characteristics.

CONCLUSIONS

The study demonstrated that feeding Silver barb yearlings with a combined probiotic diet containing *L. sporogenes* and *S. cerevisiae* at 2% each resulted in notable improvements in growth and feed conversion ratio. The findings suggest that incorporating these probiotics at a total rate of 4% (2% each) in Silver barb diets supports enhanced growth and balanced gut microbiota. Further research is recommended to explore the mechanism behind probiotic supplementation, focusing on its impact on feed digestibility, immune response, and stress tolerance in Silver barb. Further study may also be done for multiple species of probiotic microbes application in Aquaculture to meet the cost effective aquafarming.

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