



Influence of Baker's Yeast and *Aspergillus oryzae* on Growth and Microbial Composition of Silver Carp (*Hypophthalmichthys molitrix*)

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Received: 09.08.2025

Accepted: 19.09.2025

Published: 25.12.2025

How to Cite: Maann et al. (2025). Influence of Baker's Yeast and *Aspergillus oryzae* on Growth and Microbial Composition of Silver Carp (*Hypophthalmichthys molitrix*). *VZS*, 1(2), 131-144. DOI: <https://doi.org/10.64614/vzs-16>

Abstract: This research was conducted to evaluate the growth performance and microbial composition of *Hypophthalmichthys molitrix* (*H. molitrix*) after baker's Yeast (*Saccharomyces cerevisiae*) and Koji Mold (*Aspergillus oryzae*) administration. Both of these feeds contain good levels of beta-glucans and are used for their immunostimulatory effects. *A. oryzae* is an important source of organic compounds, such as glutamic acid, glucoamylase, and proteases. The cellular content of yeast contain proteins, glucans, and minerals that can enhance the growth of fish. About 60 fingerlings were taken and divided into 3 experimental groups with randomized placements to ensure unbiased results. The experimental groups were fed at 2% body weight with baker's yeast (T₁), *A. oryzae* (T₂), and control group with 100% commercial feed (T₀) during the 8-week trial period. For growth estimation, the length and weight of fish were measured weekly. After completion of trial fish weight gain (WG), specific growth rate (SGR), survival rate, and feed conversion ratio (FCR) were measured. The statistical results collected from treatments were compared by using a single-factor ANOVA under Tukey test. There was significant increase in T₁ and T₂ growth indicators as T₀<T₁ <T₂ with p<0.05. Also, samples were collected from gills and intestine of fish for microbial evaluation, and analysis were performed on nutrient agar (NA) media. In comparison to T₀, the bacterial count in T₁ and T₂ treatment was less, resultantly. The results highlighted the potential of baker's yeast and *A. oryzae* as feed supplements, providing encouraging opportunities for enhancing fish overall health and efficiency.

Keywords: *Aspergillus oryzae*, bacterial counting, baker's yeast, growth performance, immunomodulation, fish feed

INTRODUCTION

Aquaculture, particularly pond aquaculture, plays a significant role in Pakistan economy by increasing fish production and contributing to economic development. The fishing industry contributed 0.41% of the gross domestic product (GDP) of 2.12% of the total agriculture GDP of Pakistan (Mohsin et al., 2015). With an average annual output rate of 3,57,903 MT, Pakistan's total fisheries production from 1950 to 2017 was

2,43,37,449 MT. In 2017, aquaculture contributed 23,56,257 MT, while catch fisheries contributed 2,19,81,192 MT.

Fish, crustaceans, aquatic plants, and molluscs can all be raised through a practice known as aquaculture. Fresh, brackish, and seawater habitats are all suitable for growing these organisms. The rapid food production practice of aquaculture primarily depends on culturing and harvesting freshwater fish stock (Bostock et al., 2010). The most affordable and highly nutritious protein sources are aquatic animals, adding to the food surplus available for poor people providing vital nutrients like vitamins, proteins, and minerals (Pradeepkiran, 2019). Aquaculture, on the other side, is facing serious challenges, like cost of feed, labor-intensiveness protocols and environmental pollution (Yue and Shen, 2022).

Silver carp (*Hypophthalmichthys molitrix*), widely used carp specie with great aquaculture potential, is present in reservoirs, lakes, streams, and ponds naturally (Zu et al., 2023). Silver carp, due to their large requirement in aquaculture, plankton control, human consumption and in capture fisheries enhancement, has been widely distributed all over the world (Paker et al., 2013). The temperatures ranges from 6 to 28°C at young age of fish and 22 to 32°C during adult age (Ahmed et al., 2019). Adult Silver carp are filter feeders with unique gill rakers that create a filtering system like a sponges. They consume zooplankton, phytoplankton, debris, and bacteria (Calkins et al., 2012).

In Pakistan, two Chinese carp species, the Grass Carp, *Ctenopharyngodon idella* (Valenciennes) and the Silver carp, *H. molitrix*, are raised extensively in a semi-intensive culture system together with some Indeginous carps like *Labeo rohita* and *Cirrhinus mrigala* with great potential (Iqbal and Asghar, 2012).

The microbiota, formerly known as the microbial biota or microflora, is defined as the diverse population of microorganisms that live on peripheral body surfaces and cavities that are exposed to the atmosphere. The outermost layer of fish body and all other bony skeleton are invaded by many microbes before birth, and these organisms create associations with their hosts (Spor et al., 2011). Fish gills and intestines may have diverse range of microbiota that might play significant role in food digestibility, growth, and immune development. The complex microbial population in the gastrointestinal (GI) tracts of organisms comprises all; bacteria, viruses, yeast, protozoans, and archaeans (Zarkasi et al., 2016).

Baker's Yeast (*Saccharomyces cerevisiae*) is a type of yeast commonly utilized in the baking industry. It contains a variety of immunostimulants, including β -glucans, nucleic acids and mannan oligosaccharides that has been shown effective in improving both immune responses and fish growth performance (Abdel-Tawwab et al., 2008). Yeast are single-celled members of fungi kingdom, ranging from 6 to 10 μ m in size. Yeast cells are composed of two primary fractions, the cell wall and intracellular components (Shurson, 2018).

Approximately 10-30% of the biomass of yeast cells is made up of yeast. For example, the usual composition of the cell wall of *S. cerevisiae* is 5% protein and 95% polysaccharides, with 35-40% mannoprotein, 5-10% β -1,6-glucans, 50-55% β -1,3-glucans, and 2-5% is chitin (Hansen et al., 2021). The ruminal bacteria includes peptides, amino acid, organic acid, and B vitamins that provide growth-promoting substrates by yeast cultures. Yeast uses sugar in the processes of fermentation (anaerobic respiration) and aerobic respiration during lab experiment. Anaerobic fermentation produces a number of metabolites, such as peptides, alcohols, esters, which may be beneficial for an animal's nutrition and health in addition to releasing carbon and net ATP gains. Additionally, there are nutrient-rich yeast supplement options available in the form of Baker's and Brewer's yeast (Baker et al., 2022).

There are various species of filamentous fungus in the genus *Aspergillus*. Koji Mold (*Aspergillus oryzae*) is extensively used for medicine and food production. The two most important fungi for biotechnology applications are *A. oryzae* and *Aspergillus niger*, which have a long history of strain improvement (Hu et al., 2011). In addition to fish skin, *Aspergillus* can also be discovered in fish gills and intestines (Saleemi et al., 2020). Fish fed with dietary *A. oryzae* revealed improved immunological response, resistance to various diseases, better feed utilization, and growth performance (Dawood et al., 2020).

Various studies have proved the potential of *A. oryzae* and *S. cerevisiae* but its efficacy especially in *H. molitrix* health is underexplored. This study aimed to investigate the impact of both feed components on the growth and microbial composition of silver carp.

MATERIAL AND METHOD

Research Field and Duration

This experiment was conducted for a period of 2 months from 20/11/2023 to 20/01/2024 in aquarium designated as Control-T₀, Treatment-T₁ and Treatment-T₂ at Department of Zoology, Wildlife, and Fisheries, University of Agriculture, Faisalabad.

Experimental Design

Two aquarium were alienated into two replicates of experimental aquarium designated as Control- T₀, Treatment-T₁ and Treatment-T₂ with 20 fishes in each tank. Local Ethics Committee principles have been followed with ethical approval number 1224 dated 29-01-2024, University of Agriculture, Faisalabad.

Pre-stock Management

The experimental design of the aquarium considered various factors such as aquarium size, aeration, fish type, water chemistry, and overall design. The aquarium size was chosen based on the number and size of fish to be kept, and a suitable filtration system was selected accordingly. The type of fish chosen was compatible with each other and ideal for the aquarium's size. The aquarium was set up by filling it with water, adding a de-chlorinator, and setting up the aeration system.

Fish Stocking and Management

Each Aquarium was stocked with 20 *H. molitrix*, fed with different experimental feed and control with commercial feed. Healthy Silver carp with an average weight of 90-110g were obtained from freshwater earthen ponds of the University of Agriculture, Faisalabad, and acclimatized for 12 days. During the trial, the fish were kept in 120-litre aquaria with three replicates at a density of 10 fish per aquarium. Water was continuously aerated and maintained at $25 \pm 1^{\circ}\text{C}$, pH 7.5 ± 0.5 , and dissolved oxygen levels of 5-7 mg/L. The fish were fed twice a day with commercial feed and experimental feed.

Feed Preparation

T₀; Control group were given 100% commercial feed according to 2.5% of body weight. T₁ treatment group were fed with Baker's yeast and T₂ treatment group with *Aspergillus oryzae* as supplementary feed. Baker's yeast diet was prepared using chopped bread and yeast which is collected from local area of Pakistan. Add few drops of distilled water, slightly mix and spread this material in the trays for one hour. The oven was preheated at 40°C and the material were dried for 24 hours until crumbled. *A. oryzae*

fungal strain was prepared using an alternative way via solid-state fermentation of okra diluted to about 60% and incubated for 7 days at 30°C (Devanathi et al., 2024). The fermented mass obtained after incubation was harvested as a feed supplement once dried and ground.

Sample Collection

For microbiological analysis, 10 samples from each treatment groups' fish were taken from gills and intestine portion using sterilized apparatus. The samples were placed in an eppendorf tube filled with 0.9% saline solution (9mg of NaCl dissolved in 100ml H₂O), homogenized it well and labelled accordingly as C for control and E1 for first experimental treatment and E2 for second.

Microbial Media: Preparation and Counting

Dissolve 18.75g of nutrient agar (NA) (OXOID LTD., Basingstoke, Hampshire, England) in distilled water and adjust the volume to 250 ml. Thoroughly mix the solution and cover the flask using aluminium foil. Place the flask in an autoclave at a partial pressure of 15 lbs and a temperature of 121°C for 15 minutes. After removing the flask from the autoclave, allow it to cool at 40-45°C and pour the solution into petri dishes, allowing it to solidify under sterilized conditions. The petri plates will exhibit a creamy yellow-colored media. An average of three was considered an accurate count in standard lab analysis. So, three petri dishes were prepared for sample under each treatment with agar media spreading uniformly.

For microbial count nutrient agar was used that supported the successful cultivation of various types of bacteria. It contains essential minerals and nutrients that are necessary for the growth of most bacteria, fungi, and yeasts. Agar is incorporated into the medium as a solidifying agent, allowing it to be poured onto petri plates, creating a suitable surface for microbial growth (Ogunshe and Olabode, 2009). The pH of the nutrient agar is approximately 7.4±0.2 at 25°C. Typical formula (g/L) of nutrient agar used:

Lab-lemco powder 1.0; Yeast extract 2.0; Peptone 5.0; Sodium chloride (NaCl) 5.5; Agar 15.0 red 0.025; Agar 15.0.

Bacterial Examination

For the identification/characterization of bacteria, gram staining was performed using staining property of gut microbes. On petri plates, the colony formation on nutrient

agar media were firstly observed with naked eye. After that, single-celled bacterial cell shapes like bacilli, filamentous and coccus etc.; colony assemblage like chains, clusters etc., were observed and noted after staining the slide containing bacterial colony, microscopically.

For confirming the identified isolates at basic genus level, selective biochemical tests viz., catalase, indole and carbohydrate fermentation test were performed.

Determination of Growth Parameters

Throughout the trial period, various growth parameters were measured to assess the development of the fish. These measurements consist of body weight documented in grams and length documented in centimeters. Additionally, key metrics such as the specific growth rate (SGR), feed conversion ratio (FCR), length and weight gain and survival percentage were calculated by using the same method and formula's used to check the growth performances (Abdel-Aziz et al., 2021).

Limnological Parameters of Water

Consistent monitoring of the water parameters was ensured by maintaining regulatory control with regular timings of 9:00-10:00 AM. Water temperature and pH of treatment groups were noted using HANNA-HI8520 meter. A 'DO (Dissolved Oxygen) meter' (HI-9146) was used after adjusting its unit "ppm" to note the dissolved oxygen in fish tanks.

Statistical Analyses

Statistical inference as Single factor ANOVA under Tukey test were used for final results.

RESULT

Growth Performance

The findings showed that, after eight weeks of T₀ (control group), silver carp average weight at the beginning of the trial was 3.16 g, and it increased to 4.99 g at the end. In contrast, T₁ had an average weight of 3.56 g at the beginning of the trial, which increased to 6.48 g at the end of trial. While T₂ had an average weight 7.23 at the end of the trial. As a result, treatment T₁ and T₂ showed higher growth performance than treatment T₀. The control group gave maximum 8.1 and 5.9 cm length in final record showing the experimental feeds significant result (Table 1).

Table 1. Growth performance of fishes under different treatments

Parameters	T0	T1	T2	p-value	Significant/ Non significant
Average weight	1.95±0.348 ^b	2.77±0.843 ^{ab}	2.92±0.859 ^a	0.02	Significant
Gain in weight	0.12±0.143	0.36±0.329	0.31±0.364	0.02	Significant
Average length	4.54±0.958 ^b	6.0±1.612 ^{ab}	6.47±1.868 ^b	0.004	Significant
Gain in length	0.37±0.138	0.671±0.16	0.75±0.113	0.004	Significant
SGR	1.81±2.039 ^a	5.12±4.704 ^b	4.5±5.18 ^{ab}	0.03	Significant
FCR	2.44±0.172 ^a	2.157±0.098 ^a	2.15±0.098 ^a	0.07	Non-Significant
Survival rate	13.33±9.87	13.33±11.54	13.33±11.55	90%	

The letters (a), (b) and (ab) showed that means that do not share a letter are significantly different. SGR= Specific Growth Rate; FCR= Feed Conversion Ratio

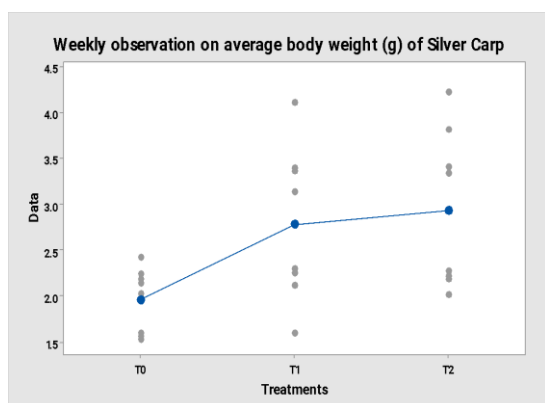


Figure 1. Average body weight (g)

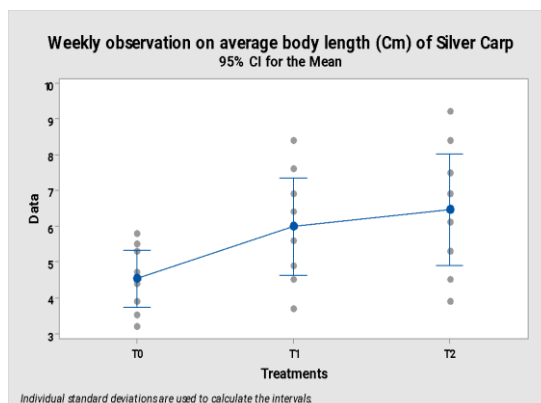


Figure 2. Average body length (cm)

The experimental results showed that there is significant change in silver carp growth rate p -value (<0.05). Silver carp showed positive result as long as fed with the baker's yeast and *A. oryzae* as compared to control group, which was fed with commercial feed. Figure 1 and Figure 2 showed the mean differences in treatment groups average body weight and average body length, respectively.

Total Bacterial Count

Nutrient agar (NA) media was used for culturing the intestine and gills sample of silver carp where maximum and minimum number of colonies were noted and estimated

along with their CFU value. Mean colony forming unit values of each treatment under study on NA showed significant increase in microbial population (Table 2).

Table 2. Mean±SD of colony forming unit of different treatments on nutrient agar (NA)

Treatments	T0	T1	T2	p-value	Significant/ Non-Significant
	Mean±SD	Mean±SD	Mean±SD	0.0001	Significant
CFU of intestine	151.4±39.65	229.7±36.01	88.7±22.36	0.0001	Significant
CFU of gills	138.4±45.74	204.5±25.85	86.2±14.61	0.0001	Significant

SD: Standard Deviation

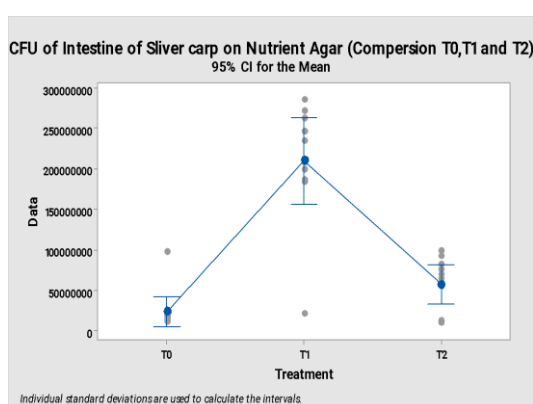


Figure 3. NA CFU comparison of intestine

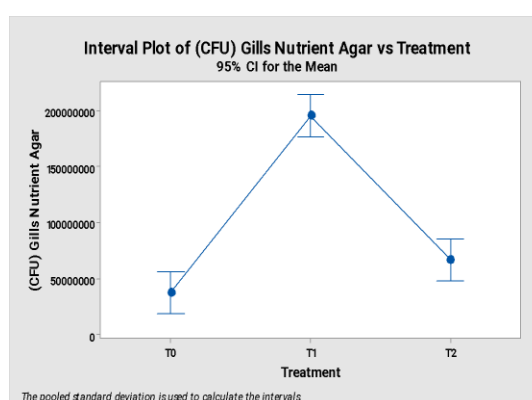


Figure 4. NA CFU comparison of gills

Figure 3 and Figure 4 showed the comparative difference of colony forming unit (CFU) under three treatments for intestine and gill samples, respectively.

Identified Bacterial Strains

After incubation period of petri plates for 24 hours, visual observations were made. The media showed slightly opalescent to yellowish gel with visible elevations (flat and convex) of bacterial cells forming colonies as circular, irregular and rhizoid. The colony morphology depicted the bacterial strains as *Staphylococcus*, *Escherichia coli*, *Bacillus* spp., and *Bifidobacterium*. The microscopic investigation further revealed the presence of gram-negative rods and gram-positive cocci.

Biochemical tests confirmed the isolates as *Staphylococcus* and *Bacillus* spp., were gram-positive cocci and catalase negative while *E. coli* were gram-negative rods and indole positive. *Bifidobacterium* presence was confirmed as it fermented the carbohydrate in test.

Limnological Parameters

Water is the vital commodity of life of fish that directly affect its feeding behavior. Thus, to ensure the optimal survival conditions for fish under each treatment group were observed, maintained, and noted regularly. The Mean values for temperature, pH, and dissolved oxygen from 20/10/2023 to 20/01/2024 determined are shown in Table 3. The readings indicated the presence of optimal vital conditions for fish under study.

Table 3. Limnological parameters in each treatment group

Parameters	Concentrations			
	Groups	Minimum	Maximum	Mean±SD
Temperature	T ₀	20	30	25±3.08
	T ₁	20	30	25±3.08
	T ₂	20	30	25±3.08
pH	T ₀	6.6	7.2	6.9±0.41
	T ₁	6.3	7.6	6.95±0.63
	T ₂	6.5	7.9	7.2±0.67
DO	T ₀	6.4	7.8	7.1±0.69
	T ₁	6.4	7.3	6.85±0.35
	T ₂	6.2	7.1	6.65±0.33

DO: Dissolved Oxygen; SD: Standard Deviation

DISCUSSION

This study was conducted at the Microbiology and Immunology Laboratory, Department of Zoology, Wildlife, and Fisheries, University of Agriculture in Faisalabad. Its primary objective was to examine the effect of baker's yeast and *A. oryzae* on the gut microbes and growth parameters of Silver carp.

Saccharomyces cerevisiae is a significant natural bioproduct which contain chitin oligonucleotides and β -glucan which had positive effect on fish growth and development. About 85–90% of the dry weight of the cell is composed of polysaccharides, which make up the cell wall, which might make up 20–25% of the cell (Hassaan et al., 2018). Adineh et al. (2011) reported that Larvae from the species *Hypophthalmichthys molitrix* larvae are vital for freshwater aquaculture. He worked to elucidate the effects of feeding silver carp larvae a combination of probiotic bacilli bacteria (*Bacillus licheniformis* and *Bacillus circulans*) and Baker's yeast (*Saccharomyces cerevisiae*) and showed promising results. Baker's yeast used as higher production in industry and also enhance the growth as diet.

An essential live food, *Artemia urmiana nauplii* (*A. urmiana*) became a vehicle for providing probiotic bacillus to the digestive system of silver carp larvae. For bioencapsulation *A. urmiana* had high potential to carry beneficial effect to carry digestive tract for the cultivation of fish larvae. According to the experiment, fish fed experimental treatments had much higher feeding and growth data than fish fed control diets, but there was no noticeable distinction in the survival rate. On silver carp larvae yeast bioencapsulation and *Bacillus* had great effect on the growth rate. *A. urmiana* had major effect and food conversion ratio decrease (Adineh et al., 2011).

In aquaculture probiotic practices is important to increase immunity level, and resistance against disease. Gram positive *Bacillus*, *Bifidobacteria* and many other are the member in aquaculture as probiotics. It was proved by many researcher adding *A. oryzae* in the diet of fish, pig and poultry to improve the health and immune responses. On the other side, *A. oryzae* and b-glucan increased the growth in Nile tilapia (Dawood et al., 2020).

Intestinal enzyme output can be diminished when pathogenic microorganisms disrupt the standard shape of the intestinal mucosa (Li et al., 2012). There was a significant $p\text{-value} < 0.05$ decline in number of total bacteria, like *Escherichia coli*, Enterobacteriaceae, in the stomach and intestines of silver carp as their diets were supplemented with *A. oryzae*. This decrease might be linked to a bactericidal process to adjust the aqueous pH or oxidation state for optimal element solubility (Williams et al., 2011).

Fish growth was evaluated on growth parameters like length in (cm) and weight gain (g), SGR, FCR in control group T_0 as well as in baker's yeast treatment T_1 and *A. oryzae* treatment T_2 . The growth performance of silver carp fingerlings fed as experimental diet for 2 months were shown in Table 1. The findings showed that, after eight weeks of T_0 , silver carp average weight at the beginning of the trial was 1.52g, and it increased to 2.41 g at the end. In contrast, T_1 had an average weight of 1.58g at the beginning of the trial, which increased to 4.10 g at the end of trial, and T_2 had average weight 2 g at the start and 4.21 g at the end of trail. As a result, treatment T_1 T_2 showed higher growth performance than treatment T_0 . By calculating the weight of each treatment, the specific growth rate findings were also determined. The data from the control group showed the lowest SGR, while the data from T_1 , T_2 indicated the highest $T_0 < T_1 < T_2$.

The initial average length of silver carp was 3.2 cm, although final average total length was measured as 5.8 cm in control T₀, which was fed with commercial feed. The initial average total length of T₁ was 3.7 cm, while the was recorded as 8.4 cm in treatment, which was treated with 100% baker's yeast and T₂ had 3.9 cm initial length and 9.2 cm was final length with *A. oryzae*, a significant relation exists between treatments and average length. About 90 % survival was noted in T₁ and T₂ as compared to T₀. So, there was a significant increase in fish WG, SGR and survival rate were noted in T₁, T₂, as compared to T₀ (p<0.05).

The findings of the study suggest that baker's yeast supplementation can be an effective nutritional strategy to improve the growth performance and feed consumption efficiency of silver carp in aquaculture settings. The observed changes in feed utilization and gut microbial communities indicate a potential mechanism through which baker's yeast and *A. oryzae* reduce FCR and enhance overall health in fish. In physicochemical parameter survival, reproduction, growth, and other factors are influenced by water temperature (Hussain et al., 2021). Another important limiting factor for growth is pond pH (Uzoka et al., 2012). Physiological activities, growth rate, and metabolic activities reduce due to acidic pH (Uzoka et al., 2015). During the experiment, pH vary from 6.3 to 7.6 in T₁ and 6.5 to 7.9 in T₂ group, and play a significant role in fish production. The physico-chemical parameters such as pH and DO approve with the findings of Okomoda et al. (2016) and Dixit et al. (2015) who measure all these parameters of different types of fish species in pond water. Concerning the finding of Nakkina (2016) and Hura et al. (2018) it may due to different hematological and physico-chemical indices that had selected for the sake of study.

CONCLUSION

Conclusively, this study offered valuable insights into the usage of baker's yeast and *Aspergillus oryzae* in aquaculture feed industry and also highlighted their potential effects on the growth and microbial communities specifically for silver carp. Both these feeds proved their efficacy as dietary supplement in improving the sustainability and profitability of aquaculture practices by enhancing the overall health via microbial modulations and growth performance of fish.

Conflict of Interest

There is no conflict of interest stated by authors.

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