

GROWTH RESPONSE AND SURVIVAL RATE OF *CIRRHINUS MRIGALA* CULTURE IN BIOFLOC SYSTEM WITH DIFFERENT FEEDING TRIALS

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Background: Fish is a vital source of high-quality animal protein, essential nutrients, and livelihoods in developing countries, contributing significantly to food security and local economies. Fish growth performance is strongly influenced by feeding regimen, dietary protein level, nutrient utilization efficiency and growing conditions. Unlike column-feeding carps, *Cirrhinus mrigala* primarily feeds near the pond bottom, which may alter its interaction with suspended microbial communities and organic solids under intensive culture conditions. These species-specific dietary and habitat preferences of *Cirrhinus mrigala* suggest that the response to advanced aquaculture systems may differ from that of other carps, requiring targeted investigation. **Objectives:** The aim of this study was to systematically evaluate the response of *Cirrhinus mrigala* to growth, survival, feed utilization, and water quality in biofloc-reared fish with varying dietary protein levels. By clarifying the interactions between feeding strategies and biofloc systems in *Cirrhinus mrigala*, this study aims to generate new species-specific data to advance scientific understanding of efficient and sustainable biofloc-based carp production methods. **Methods:** To achieve this objective, three experimental treatment groups were established under controlled rearing conditions: (i) *Cirrhinus mrigala* cultured in a conventional clear-water system and fed a diet containing 25% protein; (ii) fish cultured in a control system and fed a 30% protein diet; and (iii) fish cultured in a biofloc-based system and fed a 30% protein diet. Growth performance was evaluated using standard length, fork length, total length, total weight gain, standard growth rate, and survival rate. **Results:** Fish reared in the biofloc system with a 30% protein diet exhibited significantly higher growth across all measured parameters, achieving a final total length of 13.0 cm and a biomass increase from 117.4 g to 130.9 g (specific growth rate: 11.49%), with 100% survival. In contrast, fish receiving the same protein level without biofloc showed reduced growth (final length: 12.4 cm; growth rate: 7.12%), despite equivalent survival. The lowest performance was recorded in the 25% protein clear-water treatment, which showed reduced survival (92%) and minimal growth (6.17%). **Conclusion:** These findings demonstrate, for the first time in *Cirrhinus mrigala* culture, that biofloc technology provides a distinct growth advantage beyond dietary protein enhancement alone, confirming a synergistic interaction between biofloc-derived microbial nutrition and formulated feeds. The results provide new experimental evidence supporting biofloc systems as a biologically efficient strategy for improving carp growth performance while potentially reducing reliance on higher protein inputs.

Keywords: biofloc technology; *Cirrhinus mrigala* (Mrigal carp); growth performance; feeding trials; survival rate; SDG 2.

INTRODUCTION

Fish is a vital source of high-quality animal protein, essential nutrients, and livelihoods in developing countries, contributing significantly to food security and local economies (Balai et al., 2017). Among freshwater aquaculture species, including *Labeo rohita*, *Labeo catla*, and *Cirrhinus mrigala*, form the backbone of semi-intensive and intensive farming systems across South Asia, including Pakistan and neighbouring regions (Muhammad et al., 2018; Dwivedi et al., 2017).

Cirrhinus mrigala (Mrigal carp) is a benthic, detritivorous carp widely cultured for its market value and compatibility in polyculture systems. Its growth performance is strongly influenced by feeding regime, dietary protein level, nutrient utilization efficiency, and rearing environment (Jayasankar et al., 2018). Unlike column-feeding carps, *Cirrhinus mrigala* primarily feeds near the pond bottom, which may alter its interaction with suspended microbial communities and organic solids under intensive culture conditions. These species-specific feeding and habitat preferences suggest that responses to advanced aquaculture systems may differ from those of other carps, necessitating targeted investigation.

Biofloc technology (BFT) has emerged as a sustainable aquaculture approach that improves water quality by converting toxic nitrogenous wastes into microbial biomass through the regulation of the carbon-to-nitrogen (C/N) ratio, typically maintained at 12:1 – 15:1 with minimal water exchange (Emerenciano et al., 2017; Khanjani et al., 2023; Khanjani et al., 2024). The microbial flocs produced serve as an additional protein source and have been shown to enhance growth

performance, feed conversion efficiency, immune response, and stress resistance in several fish species under high-density conditions (Zhang et al., 2023; Adineh et al., 2019; Debbarma et al., 2021; Nageswari et al., 2022).

Despite these advantages, biofloc systems may pose species-specific challenges. Inadequate microbial balance can lead to excessive accumulation of suspended solids and organic matter, potentially impairing gill function, feeding behaviour, and overall fish health, particularly in bottom-dwelling species such as *Cirrhinus mrigala* (Manduca et al., 2020; Debnath et al., 2022; Silva et al., 2022). While BFT has been extensively studied in shrimp and some finfish species, empirical data on its application to *Cirrhinus mrigala* remain limited, especially regarding how different dietary protein levels interact with biofloc dynamics to influence growth and survival. This lack of species-specific evidence represents a critical gap in knowledge and limits the development of optimized, sustainable feeding strategies for mrigal carp culture.

Therefore, the present study was designed to address this gap by systematically evaluating the growth response, survival rate, feed utilization, and water quality performance of *Cirrhinus mrigala* reared under biofloc conditions with different dietary protein levels. The central hypothesis of this study is that biofloc technology can partially compensate for lower dietary protein through microbial protein supplementation, resulting in comparable or improved growth and survival compared to conventional feeding. It is further hypothesized that an optimal protein level exists under BFT conditions that maximizes growth performance while enhancing nutrient recycling and water quality.

By clarifying the interaction between feeding strategies and biofloc systems in *Cirrhinus mrigala*, this study aims to generate new, species-specific insights that contribute to the scientific understanding of biofloc-based carp aquaculture and support the development of more efficient, cost-effective, and environmentally sustainable production practices.

MATERIALS AND METHODS

Experimental design

The experiment was conducted from October 21, 2024, to January 9, 2025, at the Fisheries and Aquaculture Laboratory, Abdul Wali Khan University, Mardan, under controlled indoor conditions. Three identical rectangular glass aquarium (length × height × width: 60 × 53 × 49 cm) were used: one

treatment without biofloc and with a 25% protein diet, a second control treatment without biofloc and with a 30% protein diet, and a third treatment with biofloc and a 30% protein diet. Before the introduction of the stocking, all aquariums were disinfected with potassium permanganate (KMnO₄), thoroughly rinsed, and air-dried to eliminate any potential pathogens. The aquarium was placed on a laboratory bench near a large window to benefit from indirect natural lighting. To prevent direct sunlight, a translucent white polyethylene sunshade (reducing light exposure by 50%) was installed over the aquarium. This device provides sufficient ambient light to stimulate microbial activity while preventing excessive algae growth and significant temperature fluctuations. Figure 1 illustrates the aquarium setup, the shading device, the aeration system, and the lighting conditions.



Figure 1. Experimental aquarium setup

To further stabilize environmental conditions, LED lamps (6500 K, 12 h/12 h light cycle) were used during periods of low light to ensure consistent illumination throughout the experiment. Water temperature was rigorously controlled between 24 and 26 °C using an electric immersion heater (accuracy: ±0.5 °C), and daily readings were taken with a digital thermometer. Each aquarium was equipped with two 220 V air pumps for continuous aeration, ensuring homogeneous mixing and maintaining dissolved oxygen concentration above 5.0 mg/L, an optimal value for the growth of *Cirrhinus mrigala* and the stability of their bioflocs.

Water quality management and feeding regimes

All aquariums were filled with de-chlorinated freshwater seven days before stocking to allow microbial acclimatization. In the biofloc treatments, heterotrophic bacterial growth was promoted following standard biofloc protocols, whereas no carbon source was added to the control aquarium. Baseline water quality parameters, including temperature, dissolved oxygen, total dissolved solids (TDS), and nitrite concentration were recorded prior to fish introduction and monitored regularly throughout the experiment using standard digital instruments and test kits. These controlled conditions minimized environmental stress and ensured that differences in growth and survival were primarily attributable to feeding regimes and biofloc presence.

The fish were fed commercial pellets according to their weight, with regular sampling to adjust the ration. Excess feed and debris were visually monitored to maintain water quality without water changes. Figure 2 shows daily operations, including feeding, aeration, temperature control, and biofloc formation, thus providing visual validation of the experimental setup and environmental management measures.

Biofloc preparation

The biofloc inoculum was prepared 3 days before inoculation by continuous aeration of the system for 72 to 94 hours to promote the formation of microbial flocs. A readily available carbon source (sugar) was added to adjust the carbon-to-nitrogen (C/N) ratio and stimulate the growth of heterotrophic bacteria. Calcium carbonate (CaCO₃) was used to buffer the pH and maintain alkalinity, while sodium chloride (NaCl) was used at a low concentration to maintain osmotic balance and microbial stability. Commercial probiotics containing *Bacillus* spp. were introduced to enhance the beneficial microbial community and promote nutrient cycling. Freshly squeezed orange juice was used as the sole source of organic carbon and micronutrients to accelerate initial microbial activation. All additives were selected based on their recognized role in stabilizing biofloc systems and improving water quality.

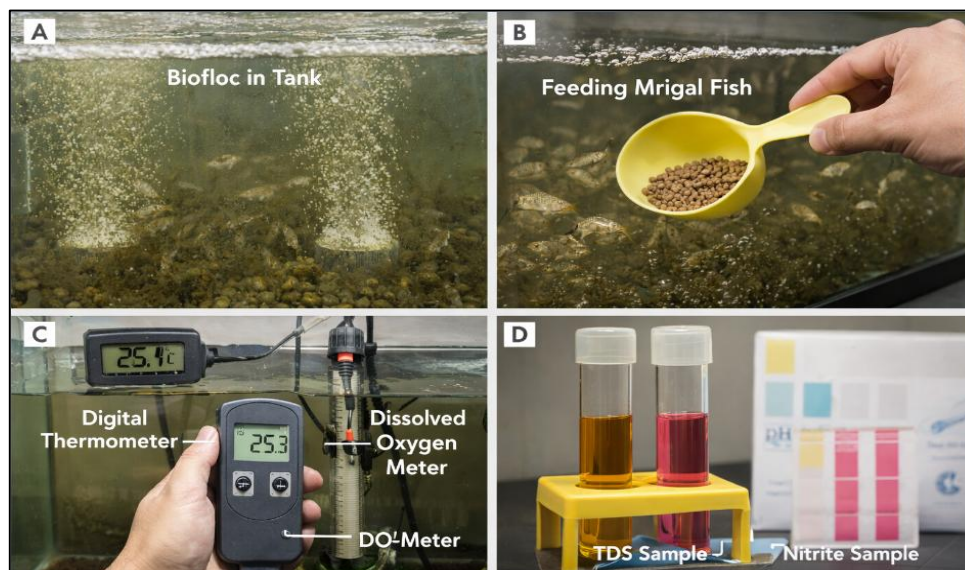


Figure 2. Experimental aquarium setup

This study used a 40-litre glass aquarium (Figure 3) to prepare the biofloc stock solution. The aquarium was first disinfected with a potassium permanganate solution and then thoroughly rinsed with clean water. Clean, uncontaminated water was then used to prepare the stock solution. The protocol was as follows: 1.2 g of probiotics, 4 g of sugar, 40 g of salt, and 2 g of calcium carbonate were added to the water, vigorously mixed with a sterile glass rod, and aerated using a double pump. Aeration was carried out in the shade, away from direct sunlight. After aeration, significant biofloc formation was observed, and the stock solution was ready for use in the experimental aquarium.

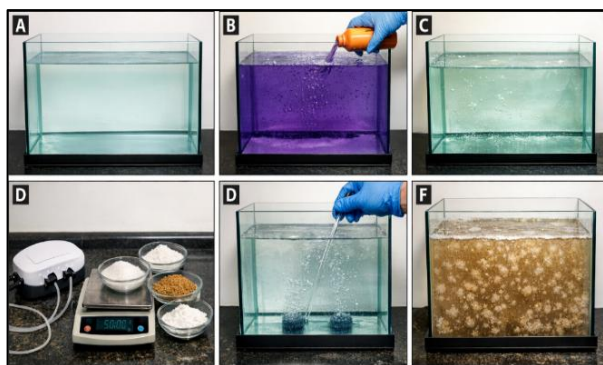


Figure 3. Preparation of biofloc stock solution: A – 40-litre glass aquarium; B – disinfection of the aquarium with potassium permanganate solution; C – weighing of probiotic bacteria, sugar, salt, and calcium carbonate; D – mixing the reagents thoroughly using a sterile glass rod; E – aeration of the stock solution with a double air pump; F – formation of visible bioflocs

Fish collection

This study used the *Cirrhinus mrigala*, also known as Mori Mori as an experimental model. The fish were obtained from a local fish farm in Chalbada Tehsil, Katlang district, Mardan. A total of 120 *Cirrhinus Mrigala* of uniform size and length were counted. Each fish was individually weighed before the experiment. They were fry measuring approximately 2 to 4 cm. All fish were disinfected with potassium permanganate, a chemical compound commonly used for disinfection and water treatment to control the growth of certain pathogens and fungi.

Fish acclimatization

Before the start of the experiment, all fish were given three days to acclimate to their new environment. Each aquarium contained one group of fish. In each group, one fish was placed for every two litres of water, thus maintaining the appropriate stocking density. A total of 25 fish were placed in each aquarium for eight weeks. The fish were fed daily with commercial pellets, at a rate of 5% of their body weight, divided into two feedings: 1.5% at 9 a.m. and 1.5% at 4 p.m., until satiation.

Water quality parameters

During the experiment, water quality parameters, such as temperature, dissolved oxygen, pH, and total alkalinity, were recorded twice daily (once in the morning and once in the evening) for both the bio-flocculation system and the control group. Water temperature was measured using a thermometer, and pH was determined using a freshwater test kit and a pH meter. Dissolved oxygen concentration in the aquarium was estimated and measured using a chemical test kit and a DO meter. Total alkalinity was also measured using a chemical test kit and a titration kit. Total concentrations of ammonia, nitrous, and nitric nitrogen, as well as water hardness, turbidity, and floc volume (FV), were measured twice weekly. Ammonia, nitrous, and nitric nitrogen concentrations, and water hardness were measured using a complete freshwater analysis kit, in accordance with standardized methods. Floc volume, reflecting the amount of biological floc in the treatment basin, was measured using the Imhoff cone method, described by Avnimelech & Kochba (2009).

Growth parameters

Fish were collected weekly from all experimental tanks to analyse their percentage weight gain and specific growth rate (SGR). Survival rate (SR) was also calculated. Fish were weighed using an electronic balance (Denver Instruments, MXX-412, 0.01 g accuracy). The following formulas were used to analyse fish growth parameters:

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \cdot 100, \quad (1)$$

$$\text{SGR} = \frac{\text{Final weight} - \text{Initial weight}}{\text{No. of culture days}} \cdot 100, \quad (2)$$

$$\text{SR} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \cdot 100. \quad (3)$$

Proximate analysis

A preliminary analysis was performed on the experimental diets to determine their nutritional composition, including their moisture contents, crude protein, crude fat, crude fibre, and ash content. This analysis was conducted according to the standardized methods of the American Association of Analytical Chemists (AOAC, 1995). Water content was determined by taking approximately 5 g of feed sample and drying it to constant weight in an oven at 105 °C (Ahn et al., 2014; Quirino et al., 2023). Crude protein content was determined by the Kjeldahl method (Adeyemi et al., 2015), which includes digestion, distillation, and titration. Total nitrogen content was then determined and multiplied by a factor of 6.25. Crude fat content was determined by Soxhlet extraction with petroleum ether. Crude fibre content was calculated by acid-base digestion of defatted samples, followed by drying and incineration. Ash content was determined by calcining a known mass of sample in a muffle furnace at 550 °C for 4 to 6 hours, until a white or light grey residue was obtained. All samples were analysed in triplicate to ensure the accuracy and reliability of the results.

Statistical analysis

Growth performance, biomass changes, and survival data of *Cirrhinus mrigala* obtained from the three experimental treatments were recorded throughout the experimental period. All measurements were compiled separately for each trial within a treatment. Mean values were calculated for morphometric parameters (standard length, fork length, and total length), total weight (initial and final), standard growth rate, and survival rate.

All calculations, including percentage growth and survival rates, were performed using Microsoft Excel software (version 2016). The results are presented as descriptive statistics to compare trends among treatments and feeding trials under controlled rearing conditions. No inferential statistical tests were applied, as the study focused on treatment-wise comparative evaluation based on observed growth and survival responses.

RESULTS

Growth performance and survival rate of *Cirrhinus Mrigala* under low protein and no-biofloc feeding trials

Table 1 showed the growth performance and survival rate of *Cirrhinus mrigala* fed a 25% protein diet under biofloc-free conditions in three feeding trials. In all trials, the standard body length, fork length and total length of the fish increased continuously from the first sampling to the final sampling, indicating positive growth under the experimental conditions.

The standard body length increased in trial 1 from an initial 2.5 – 8.1 cm to a final 2.9 – 8.6 cm, and fork length increased from 2.7 – 8.8 cm to 3.4 – 9.1 cm. Similarly, total length increased from 3.1 – 9.8 cm to 3.9 – 10.0 cm. A total of 25 fish were stocked, of which only one was recorded as dead, resulting in 24 fish surviving at harvest, a survival rate of 96%. The total weight increased from an initial 68.0 g to 72.2 g at the end of the experiment, corresponding to a standard growth rate of 6.17%.

In the second trial, the standard body length increased from an initial 2.9 – 8.8 cm to 3.2 – 9.0 cm at the last observation. The fork length increased from 3.3 – 9.3 cm to 3.6 – 9.6 cm,

while the total length increased from 3.9 – 10.2 cm to 4.1 – 10.7 cm. Of the 25 fish that were stocked, 2 died, of which 23 survived, a survival rate of 92%. The total weight increased from 72.2 g to 75.7 g, with a recorded standard growth rate of 4.84%.

In the third trial, standard body length increased from 3.2 – 9.3 cm to 3.6 – 9.9 cm, fork length increased from 3.6 – 10.2 cm to 4.0 – 10.5 cm, and total length increased from 4.1 – 10.7 cm to 4.5 – 11.8 cm. A total of 24 fish were included in this experiment; 3 died, of which 21 survived, a survival rate of 88%. Total biomass increased from an initial 75.7 g to 79.4 g at harvest, corresponding to a standard growth rate of 4.88%.

Overall, under no-biofloc conditions, all three trials showed a gradual improvement in morphological parameters and biomass; however, survival rates decreased from trial 1 to trial 3. Trial 1 showed the highest growth rate and survival rates, while trial 3, despite having the highest final body length range and biomass, had the lowest survival rates.

Growth performance and survival response of *Cirrhinus mrigala* in the control group

Table 2 indicated the morphological growth, standard growth and survival rate of *Cirrhinus mrigala* in three feeding trials conducted under control conditions (no added biofloc, feeding with 30% protein). All trials showed that standard body length, fork length and total length increased continuously from initial to final sampling, indicating normal somatic growth of the fish under control group conditions.

In trial 1, the standard body length of the fish increased from an initial 2.9 – 8.0 cm to a final 3.2 – 8.7 cm, and the fork length increased from 3.2 – 8.5 cm to 3.6 – 9.4 cm. The total length increased from an initial 3.7 – 9.6 cm to 4.0 – 10.3 cm at the end of the experiment. The initial total weight was 78.3 g, which increased to 84.5 g at the end of the trial, corresponding to a standard growth rate of 7.91%. No deaths were recorded during the experiment and the survival rate was 100%.

In the second trial, the growth trend of the fish was similar to that of the first trial, with the standard body length increasing from an initial 3.2 – 8.7 cm to a final observed 3.5 – 9.0 cm. The fork length increased from 3.6 – 9.4 cm to 3.9 – 9.6 cm, while the total length increased from 4.0 – 10.3 cm to 4.4 – 10.7 cm. The total weight increased from 84.5 g to 90.7 g, corresponding to a standard growth rate of 7.33%. Similar to trial 1, no fish deaths were observed and the survival rate remained 100%.

In the third trial, the absolute body length values recorded were relatively high; however, the growth efficiency was relatively low. The standard body length increased from 3.8 – 10.4 cm to 4.2 – 11.2 cm, the fork length from 4.1 – 11.5 cm to 4.6 – 12.0 cm, and the total length from 4.5 – 12.5 cm to 5.0 – 12.9 cm. The total weight increased from 90.7 g to 95.2 g, resulting in a decrease in the standard growth rate to 4.96%. Two deaths were recorded in this trial, reducing the number of live fish to 23, resulting in a survival rate of 92%.

Overall, the control group showed stable morphometric growth in all trials; however, variations in growth rate and survival were observed across trials, with trial I showing the highest growth rate and trials I and II achieving complete survival.

Table 1. Morphometric growth performance, survival rate, and biomass changes of *Cirrhinus mrigala* under no-biofloc conditions (25% protein) in different feeding trials

Parameters	Trial I / Trial II / Trial III					
	Standard length, cm		Fork length, cm		Total length, cm	
	Initial	Final	Initial	Final	Initial	Final
Fish morphometric	2.5 / 2.9 / 3.2	2.9 / 3.2 / 3.6	2.7 / 3.4 / 3.6	3.4 / 3.6 / 4.0	3.1 / 3.9 / 4.1	3.9 / 4.1 / 4.5
	2.8 / 3.0 / 3.3	3.0 / 3.3 / 3.7	3.3 / 3.3 / 3.9	3.3 / 3.9 / 4.1	3.8 / 3.7 / 4.4	3.7 / 4.4 / 4.6
	3.1 / 3.4 / 3.5	3.4 / 3.5 / 3.9	3.4 / 3.6 / 3.9	3.6 / 3.9 / 4.3	3.8 / 4.1 / 4.4	4.1 / 4.4 / 4.8
	3.3 / 3.5 / 3.7	3.5 / 3.7 / 4.0	3.6 / 3.9 / 4.0	3.9 / 4.0 / 4.3	3.9 / 4.3 / 4.7	4.3 / 4.7 / 5.0
	3.4 / 3.9 / 4.1	3.9 / 4.1 / 4.3	3.7 / 4.2 / 4.5	4.2 / 4.5 / 4.7	4.3 / 4.9 / 4.9	4.9 / 4.9 / 5.1
	3.5 / 3.7 / 4.1	3.8 / 4.1 / 4.4	3.8 / 4.3 / 4.6	4.0 / 4.6 / 5.0	4.4 / 5.0 / 5.2	4.5 / 5.2 / 5.6
	3.4 / 4.3 / 4.5	3.7 / 4.5 / 4.9	3.9 / 4.8 / 4.8	4.3 / 4.8 / 5.3	4.6 / 5.5 / 5.2	5.0 / 5.2 / 5.8
	3.8 / 4.4 / 4.6	4.3 / 4.6 / 4.9	4.3 / 4.8 / 5.0	4.8 / 5.0 / 5.3	4.8 / 5.8 / 5.7	5.5 / 5.7 / 6.0
	3.9 / 4.3 / 4.5	4.4 / 4.5 / 4.7	4.3 / 4.6 / 4.9	4.8 / 4.9 / 5.1	4.7 / 5.5 / 5.7	5.8 / 5.7 / 5.9
	4.0 / 4.4 / 4.7	4.3 / 4.7 / 5.0	4.4 / 4.9 / 5.0	4.6 / 5.0 / 5.3	4.9 / 5.6 / 5.5	5.5 / 5.5 / 5.8
	4.0 / 4.3 / 4.7	4.4 / 4.7 / 5.0	4.4 / 4.8 / 5.0	4.9 / 5.0 / 5.4	5.0 / 5.6 / 5.8	5.6 / 5.8 / 5.9
	4.1 / 4.6 / 4.8	4.3 / 4.8 / 5.1	4.7 / 5.0 / 5.1	4.8 / 5.1 / 5.5	5.3 / 5.9 / 5.8	5.6 / 5.8 / 6.0
	4.2 / 4.7 / 5.0	4.6 / 5.0 / 5.4	4.5 / 5.0 / 5.4	5.0 / 5.4 / 6.0	5.0 / 5.6 / 6.0	5.9 / 6.0 / 6.6
	4.4 / 4.7 / 5.0	4.7 / 5.0 / 5.3	4.9 / 5.1 / 5.4	5.0 / 5.4 / 5.9	5.5 / 5.7 / 6.1	5.6 / 6.1 / 6.5
	4.3 / 4.8 / 5.2	4.7 / 5.2 / 5.6	5.3 / 5.3 / 5.7	5.1 / 5.7 / 6.1	5.9 / 5.9 / 6.5	5.7 / 6.5 / 6.9
	4.5 / 5.3 / 5.8	4.8 / 5.8 / 6.1	5.0 / 6.4 / 6.5	5.3 / 6.5 / 6.7	5.6 / 7.0 / 7.4	5.9 / 7.4 / 7.7
	5.0 / 5.4 / 5.9	5.3 / 5.8 / 6.1	5.4 / 5.9 / 6.5	6.4 / 6.6 / 6.8	5.9 / 6.5 / 7.5	7.0 / 7.4 / 7.8
	5.1 / 5.5 / 5.9	5.4 / 5.9 / 6.3	5.6 / 6.0 / 6.8	5.9 / 6.5 / 7.2	6.1 / 6.9 / 7.5	6.5 / 7.5 / 7.9
	5.1 / 5.5 / 6.0	5.5 / 5.9 / 6.3	5.4 / 6.2 / 7.0	6.0 / 6.8 / 7.2	6.0 / 7.0 / 7.9	6.9 / 7.5 / 8.1
	5.2 / 5.7 / 8.1	5.5 / 7.6 / 8.5	6.0 / 6.4 / 8.7	6.2 / 7.0 / 9.4	6.5 / 7.0 / 10.0	7.0 / 7.9 / 10.4
	5.4 / 6.5 / 8.9	5.7 / 7.0 / 9.3	6.0 / 7.0 / 9.5	6.4 / 7.6 / 9.9	6.6 / 7.9 / 10.1	7.0 / 8.8 / 10.5
	6.1 / 7.7 / 8.9	6.5 / 8.1 / 9.4	6.6 / 8.5 / 9.3	7.0 / 8.7 / 10.0	7.4 / 9.6 / 10.6	7.9 / 10.0 / 11.1
	7.2 / 8.4 / 9.0	7.7 / 8.9 / 9.5	8.0 / 9.0 / 9.4	8.5 / 9.5 / 9.8	9.0 / 9.9 / 10.3	9.6 / 10.1 / 11.0
	8.0 / 8.6 / 9.2	8.4 / 8.9 / 9.7	8.5 / 9.1 / 10.0	9.0 / 9.3 / 10.2	9.0 / 10.0 / 10.5	9.9 / 10.6 / 11.4
	8.1 / 8.8 / 9.3	8.6 / 9.0 / 9.9	8.8 / 9.3 / 10.2	9.1 / 9.6 / 10.5	9.8 / 10.2 / 10.7	10.0 / 10.7 / 11.8
Total fishes	25 / 25 / 25					
Dead fish	1 / 2 / 3					
Live fish	24 / 23 / 21					
Initial weight, g	68 / 72.2 / 75.7					
Final weight, g	72.2 / 75.7 / 79.4					
Standard growth rate, %	6.17 / 4.84 / 4.88					
Survival rate, %	96 / 92 / 88					

Enhanced growth and survival of *Cirrhinus mrigala* in biofloc with optimized protein

Table 3 presented the morphological growth response and survival performance of *Cirrhinus mrigala* cultured in a biofloc system in three feeding trials, fed a diet with 30% protein. In all trials, from the initial sampling period to the final sampling period, the standard body length, fork length and total length of the fish showed continuous and gradual increases, indicating that

the biofloc system provided favourable growth conditions.

In trial 1, the standard body length of the fish increased from an initial 3.0 – 10.0 cm to a final 3.4 – 10.5 cm, and the fork length increased from 3.4 – 10.5 cm to 3.9 – 11.0 cm. Similarly, the total length increased from an initial 4.3 – 11.2 cm to 4.5 – 11.7 cm at the end of the trial. The total weight increased from 88.0 g to 97.3 g, corresponding to a standard growth rate of 10.50%.

Table 2. Morphometric growth performance and survival rate of *Cirrhinus mrigala* in the control group (30% protein, no biofloc) under different feeding trials

Parameters	Trial I / Trial II / Trial III					
	Standard length, cm		Fork length, cm		Total length, cm	
	Initial	Final	Initial	Final	Initial	Final
Fish morphometric	2.9 / 3.2 / 3.8	3.2 / 3.5 / 4.2	3.2 / 3.6 / 4.1	3.6 / 3.9 / 4.6	3.7 / 4.0 / 4.5	4.0 / 4.4 / 5.0
	3.0 / 3.4 / 4.0	3.4 / 3.8 / 4.2	3.3 / 3.9 / 4.3	3.9 / 4.1 / 4.6	3.5 / 4.0 / 4.6	4.0 / 4.5 / 4.9
	3.2 / 3.6 / 4.2	3.6 / 4.0 / 4.6	3.5 / 3.9 / 4.7	3.9 / 4.3 / 4.9	3.8 / 4.1 / 5.2	4.1 / 4.6 / 5.4
	3.5 / 3.9 / 4.4	3.9 / 4.2 / 4.8	3.7 / 4.1 / 4.8	4.1 / 4.7 / 5.1	4.5 / 4.9 / 5.3	4.9 / 5.2 / 5.6
	3.5 / 4.0 / 4.3	4.0 / 4.4 / 4.7	3.9 / 4.5 / 4.9	4.5 / 4.8 / 5.0	4.3 / 5.0 / 5.4	5.0 / 5.3 / 5.6
	3.6 / 4.0 / 4.3	4.0 / 4.3 / 4.9	4.0 / 4.4 / 4.8	4.4 / 4.9 / 5.4	4.6 / 4.9 / 5.5	4.9 / 5.4 / 5.9
	3.6 / 3.9 / 4.5	3.9 / 4.3 / 4.9	4.0 / 4.4 / 4.9	4.4 / 4.8 / 5.2	4.5 / 4.9 / 5.3	4.9 / 5.5 / 5.6
	3.7 / 4.1 / 4.8	4.1 / 4.5 / 5.0	4.2 / 4.5 / 5.1	4.5 / 4.9 / 5.4	4.7 / 5.0 / 5.5	5.0 / 5.3 / 5.9
	4.0 / 4.4 / 4.9	4.4 / 4.8 / 5.2	4.4 / 4.8 / 5.3	4.8 / 5.1 / 5.6	4.8 / 5.3 / 5.8	5.3 / 5.5 / 6.1
	4.0 / 4.3 / 5.2	4.3 / 4.6 / 5.6	4.4 / 4.7 / 5.5	4.7 / 4.9 / 5.9	4.9 / 5.3 / 6.0	5.3 / 5.4 / 6.3
	4.1 / 4.5 / 5.4	4.5 / 4.9 / 5.7	4.5 / 4.9 / 5.8	4.9 / 5.3 / 6.0	5.2 / 5.4 / 6.2	5.4 / 5.8 / 6.6
	4.3 / 4.8 / 5.4	4.8 / 5.2 / 5.8	4.6 / 5.1 / 5.7	5.1 / 5.5 / 6.2	5.2 / 5.7 / 6.2	5.7 / 6.0 / 6.5
	4.4 / 4.9 / 5.3	4.9 / 5.4 / 5.6	4.8 / 5.3 / 5.8	5.3 / 5.8 / 6.0	5.3 / 5.9 / 6.4	5.9 / 6.2 / 6.6
	4.5 / 4.8 / 5.6	4.8 / 5.4 / 6.0	5.0 / 5.1 / 6.0	5.1 / 5.7 / 6.4	5.7 / 5.9 / 6.7	5.9 / 6.2 / 6.9
	4.5 / 4.9 / 6.5	4.9 / 5.3 / 6.8	4.9 / 5.4 / 6.9	5.4 / 5.8 / 7.1	5.5 / 6.1 / 7.4	6.1 / 6.4 / 7.6
	4.9 / 5.2 / 6.3	5.2 / 5.6 / 6.9	5.3 / 5.7 / 6.7	5.7 / 6.0 / 7.2	5.8 / 6.4 / 7.4	6.4 / 6.7 / 7.7
	5.5 / 6.1 / 6.5	6.1 / 6.5 / 6.9	5.9 / 6.5 / 7.0	6.5 / 6.9 / 7.3	6.5 / 7.0 / 7.7	7.0 / 7.4 / 7.8
	5.6 / 5.8 / 7.9	5.8 / 6.3 / 8.3	6.1 / 6.4 / 8.2	6.4 / 6.7 / 8.6	6.7 / 7.0 / 9.2	7.0 / 7.4 / 9.4
	5.9 / 6.2 / 8.3	6.2 / 6.5 / 8.6	6.6 / 6.8 / 8.7	6.8 / 7.0 / 9.0	7.3 / 7.5 / 9.6	7.5 / 7.7 / 9.9
	6.3 / 7.6 / 8.1	7.6 / 7.9 / 8.5	6.8 / 7.9 / 8.8	7.9 / 8.2 / 9.0	7.7 / 8.6 / 9.7	8.6 / 9.2 / 9.9
	7.0 / 7.8 / 8.5	7.8 / 8.3 / 8.8	7.8 / 8.3 / 9.0	8.3 / 8.7 / 9.2	8.8 / 9.2 / 10.0	9.2 / 9.6 / 10.3
	7.0 / 7.8 / 8.5	7.8 / 8.1 / 8.9	7.7 / 8.4 / 9.0	8.4 / 8.8 / 9.3	8.8 / 9.3 / 10.2	9.3 / 9.7 / 10.4
	7.5 / 8.1 / 9.0	8.1 / 8.5 / 9.4	8.2 / 8.7 / 9.6	8.7 / 9.0 / 9.8	9.3 / 10.0 / 10.7	10.0 / 10.0 / 11.0
	7.6 / 8.2 / 9.5	8.2 / 8.5 / 10.0	8.2 / 8.8 / 10.3	8.8 / 9.0 / 10.7	9.1 / 9.9 / 11.3	9.9 / 10.2 / 11.9
	8.0 / 8.7 / 10.4	8.7 / 9.0 / 11.2	8.5 / 9.4 / 11.5	9.4 / 9.6 / 12.0	9.6 / 10.3 / 12.5	10.3 / 10.7 / 12.9
Total fishes	25 / 25 / 25					
Dead fish (DF)	0 / 0 / 2					
Live fish	25 / 25 / 23					
Initial weight, g	78.3 / 84.5 / 90.7					
Final weight, g	84.5 / 90.7 / 95.2					
Standard growth rate, %	7.91 / 7.33 / 4.96					
Survival rate, %	100 / 100 / 92					

In the second trial, a significantly faster growth rate was observed in the fish. The standard body length increased from 3.4 – 10.5 cm to 3.8 – 10.9 cm, the fork length from 3.9 – 11.0 cm to 4.2 – 11.4 cm, and the total length from 4.5 – 11.7 cm to 4.9 – 12.1 cm. The total weight of the fish increased from 97.3 g at the beginning of the experiment to 107.8 g at the end, which corresponds to a standard growth rate of 10.79%.

Trial 3 showed the best growth among all treatment groups. Standard body length increased from 3.8 – 11.9 cm to 4.2 – 12.4 cm, fork length increased from 4.2 – 12.7 cm to

4.7 – 12.9 cm, and total length increased from 4.9 – 13.3 cm to 5.4 – 13.7 cm. Total weight increased from 105.8 g to 117.4 g, with the highest standard growth rate of 10.96%.

In all three trials, the survival rate was 100% and no deaths were recorded throughout the trial period, indicating that the biofloc system provided a stable and favourable culture environment. Overall, the results showed that the biofloc technology combined with a 30% protein diet effectively promotes the growth and survival of *Cirrhinus mrigala*, with trial three showing the most significant improvement in morphological growth and biomass accumulation.

Table 3. Morphometric growth performance and survival rate of *Cirrhinus mrigala* cultured in a biofloc system fed with 30% protein diet under different feeding trials

Parameters	Trial I / Trial II / Trial III					
	Standard length, cm		Fork length, cm		Total length, cm	
	Initial	Final	Initial	Final	Initial	Final
Fish morphometric	3.0 / 3.4 / 3.8	3.4 / 3.8 / 4.2	3.4 / 3.9 / 4.2	3.9 / 4.2 / 4.7	4.3 / 4.5 / 4.9	4.5 / 4.9 / 5.4
	3.3 / 3.7 / 4.2	3.7 / 4.2 / 4.6	3.7 / 4.2 / 4.5	4.2 / 4.5 / 5.0	4.4 / 4.8 / 5.2	4.8 / 5.2 / 5.7
	3.4 / 3.9 / 4.3	3.9 / 4.3 / 4.7	3.8 / 4.4 / 4.9	4.4 / 4.9 / 5.1	4.7 / 5.0 / 5.5	5.9 / 5.5 / 6.6
	3.5 / 3.9 / 4.4	3.9 / 4.4 / 4.9	3.9 / 4.3 / 4.9	4.3 / 4.9 / 5.3	4.6 / 5.0 / 5.4	5.0 / 5.4 / 6.0
	3.7 / 4.1 / 4.5	4.1 / 4.5 / 4.9	4.2 / 4.6 / 5.0	4.7 / 5.0 / 5.4	5.0 / 5.4 / 5.8	5.4 / 5.8 / 6.1
	4.0 / 4.5 / 4.8	4.5 / 4.8 / 5.3	4.4 / 5.0 / 5.1	5.0 / 5.1 / 5.8	5.0 / 5.5 / 5.9	5.5 / 5.9 / 6.5
	4.0 / 4.4 / 4.8	4.4 / 4.8 / 5.2	4.4 / 4.9 / 5.2	4.9 / 5.2 / 5.7	5.2 / 5.7 / 6.1	5.7 / 6.1 / 6.4
	4.1 / 4.4 / 4.9	4.4 / 4.9 / 5.3	4.5 / 5.0 / 5.3	5.0 / 5.3 / 5.8	5.4 / 5.8 / 6.2	5.8 / 6.2 / 6.5
	4.3 / 4.7 / 5.1	4.7 / 5.1 / 5.5	4.8 / 5.2 / 5.5	5.2 / 5.5 / 5.9	5.5 / 5.9 / 6.4	5.9 / 6.4 / 6.7
	4.4 / 4.8 / 5.3	4.8 / 5.3 / 5.7	4.8 / 5.3 / 5.6	5.3 / 5.6 / 6.1	5.8 / 6.0 / 6.4	6.0 / 6.4 / 6.8
	4.5 / 5.0 / 5.4	5.0 / 5.4 / 5.9	4.9 / 5.5 / 5.8	5.5 / 5.8 / 6.4	5.7 / 6.1 / 6.7	6.1 / 6.7 / 7.1
	4.6 / 5.1 / 5.5	5.1 / 5.5 / 5.9	5.0 / 5.7 / 5.8	5.7 / 5.8 / 6.3	5.8 / 6.3 / 6.7	6.3 / 6.7 / 7.0
	4.9 / 5.3 / 5.6	5.3 / 5.6 / 6.0	5.2 / 5.8 / 6.0	5.8 / 6.0 / 6.4	6.0 / 6.4 / 6.8	6.4 / 6.8 / 7.2
	5.6 / 6.0 / 6.4	6.0 / 6.4 / 6.8	6.0 / 6.5 / 6.6	6.5 / 6.6 / 7.2	6.7 / 7.2 / 7.6	7.2 / 7.6 / 8.0
	6.0 / 6.3 / 6.7	6.3 / 6.7 / 7.0	6.4 / 6.8 / 7.1	6.8 / 7.1 / 7.4	7.0 / 7.4 / 7.8	7.4 / 7.8 / 8.1
	6.6 / 6.9 / 7.3	6.9 / 7.3 / 7.6	7.0 / 7.4 / 7.8	7.4 / 7.8 / 8.0	7.6 / 8.0 / 8.4	8.0 / 8.4 / 8.7
	6.9 / 7.3 / 7.7	7.3 / 7.7 / 8.0	7.3 / 7.8 / 8.1	7.8 / 8.1 / 8.5	7.9 / 8.4 / 8.8	8.4 / 8.8 / 9.2
	7.1 / 7.5 / 8.0	7.5 / 8.0 / 8.4	7.5 / 7.9 / 8.4	7.9 / 8.4 / 8.8	8.1 / 8.5 / 8.9	8.5 / 8.9 / 9.5
	7.4 / 7.7 / 8.1	7.7 / 8.1 / 8.6	7.8 / 8.2 / 8.5	8.2 / 8.5 / 9.0	8.4 / 8.7 / 9.1	8.7 / 9.1 / 9.7
	7.5 / 7.8 / 8.1	7.8 / 8.1 / 8.5	7.9 / 8.3 / 8.4	8.3 / 8.4 / 8.9	8.5 / 8.8 / 9.2	8.8 / 9.2 / 9.6
	7.8 / 8.0 / 8.5	8.1 / 8.5 / 8.9	8.3 / 8.6 / 8.9	8.6 / 8.9 / 9.3	8.9 / 9.3 / 9.7	9.3 / 9.7 / 10.1
	8.0 / 8.4 / 8.8	8.4 / 8.8 / 9.3	8.5 / 8.9 / 9.2	8.9 / 9.2 / 9.8	9.1 / 9.5 / 9.9	9.5 / 9.9 / 10.5
	8.4 / 8.9 / 9.3	8.9 / 9.3 / 9.7	8.7 / 9.4 / 9.7	9.4 / 9.7 / 10.1	9.3 / 10.0 / 10.4	10.0 / 10.4 / 10.8
	9.9 / 10.4 / 11.1	10.4 / 10.7 / 11.5	10.5 / 10.9 / 11.9	10.9 / 11.2 / 12.7	11.1 / 11.5 / 12.9	11.5 / 11.9 / 13.1
	10.0 / 10.5 / 11.9	10.5 / 10.9 / 12.4	10.5 / 11.0 / 12.7	11.4 / 11.4 / 12.9	11.2 / 11.7 / 13.3	11.7 / 12.1 / 13.7
Total fishes	25 / 25 / 25					
Dead fish (DF)	0 / 0 / 0					
Live fish	25 / 25 / 25					
Initial weight, g	88.0 / 97.3 / 105.8					
Final weight, g	97.3 / 107.8 / 117.4					
Standard growth rate, %	10.50 / 10.79 / 10.96					
Survival rate, %	100 / 100 / 100					

DISCUSSION

This study aimed to evaluate the growth and survival rate of *Cirrhinus mrigala* reared under different protein diets and biofloc conditions. The results of the various feeding trials revealed significant differences between the experimental groups regarding morphological parameters, weight gain, and survival rate. This part of the study focuses on the interaction between biofloc technology and dietary protein levels on fish growth and analyses the observed differences in light of existing literature. This study examines the biological mechanisms, water quality improvement, and nutrient supply

provided by bioflocs, all factors that can promote fish growth and survival. Comparison with previous studies validates the results and sheds light on the effectiveness of combining biofloc systems with optimized feeding strategies for sustainable aquaculture.

The *Cirrhinus mrigala* is a poikilothermic fish, meaning that the proper functioning of all its biological processes is highly dependent on temperature. Furthermore, compared to other farmed fish, the *Cirrhinus mrigala* has a slower growth rate. Its optimal growth period extends from March to October each year, primarily due to the warmer water temperatures, which

stimulate its metabolic activity, feeding, feed conversion efficiency, and growth rate (Harris & Bodaly, 1998).

Protein is essential for fish growth and development and is the most expensive macronutrient in aquaculture feed (Nurullah et al., 2003). Therefore, the protein content of feed must be sufficient to promote fish growth; excess protein leads to waste and unnecessarily increases feed costs. Protein levels in feed are crucial because they significantly influence fish growth, survival rates, and yield. Extensive research is therefore needed to determine the optimal quantity and quality of protein required for optimal fish growth.

This study demonstrates that, in a biofloc system, the growth of the *Cirrhinus mrigala* is superior to that of the control group (30% protein) and the group raised in pure water (25% protein). As shown in Tables 1 and 2, the weight gains of the *Cirrhinus mrigala* in the biofloc-treated group was consistently higher than that of the control group. Previous studies have also reported similar results, indicating that biofloc systems can improve the growth rate of various fish species. For example, studies on tilapia (*Oreochromis niloticus*) have shown that biofloc systems can improve growth by providing an additional food source and increasing feed conversion ratio (Crab et al., 2012). The results of this study corroborate these observations and confirm the effectiveness of BFT in promoting the growth of *Cirrhinus mrigala*. Fish raised in tanks using biofloc exhibited significantly greater average weight gain than the control group (Crab et al., 2010).

Among the various growth indicators used in aquaculture research experiments, the standardized growth rate (SGR) is a reliable indicator. Compared to the pure water group and the control group (4.88% and 4.96%, respectively), the SGR of the *Cirrhinus mrigala* in the biofloc-treated group was higher (10.96%) (Table 3). Furthermore, the SGR of this group was also higher than that of the pure water group and the control group.

Several factors have contributed to the improved growth performance of *Cirrhinus mrigala* raised in biofloc systems. First, in addition to traditional feeds, microbial flocs provide a reliable and readily available source of supplemental nutrients. This improves nutrient availability, which can potentially lead to higher growth rates and feed conversion ratios. Second, thanks to improved water quality, fish raised in biofloc systems experience less stress, allowing them to dedicate more energy to growth rather than coping with adverse environmental conditions. Third, from an immunological perspective, bioflocs help protect the fish against diseases and infections, thus promoting their overall development.

The average survival rate of *Cirrhinus mrigala* reared with bioflocs was significantly higher than that of groups reared in pure water and the control group. This indicates that BFT not only promotes fish growth but also improves their overall health. The higher survival rate observed in the biofloc system can be attributed to better water quality, as concentrations of ammonia and other toxic nitrogen compounds are generally lower in the biofloc environment than in conventional aquaculture methods. Water pollution is considered one of the major stressors for fish and can lead to increased mortality (Ekasari et al., 2014).

Furthermore, biofloc systems also have positive effects on other factors, such as fish immunity. The presence of microorganisms associated with bioflocs can strengthen the immune system of fish. Numerous studies have shown that bioflocs have probiotic effects, improving the gut microbiota and enhancing immune responses, thereby reducing disease incidence and mortality (Kim et al., 2015). Another advantage of using bioflocs lies in

their probiotic properties, which contribute to improving the overall health and well-being of fish.

The higher survival rates observed in biofloc systems represent a significant advantage for aquaculture. These higher rates mean that more fish reach market size, thus improving overall productivity and profitability. This is particularly important in commercial aquaculture, where high mortality rates can lead to substantial economic losses. By improving survival rates, biofloc technology helps stabilize production and increase the efficiency of aquaculture systems. Based on the results of this study, it can be concluded that biofloc technology effectively improves the survival and growth rates of *Cirrhinus mrigala*. Future research should explore optimal nutritional strategies for *Cirrhinus mrigala* in biofloc systems, which will contribute to more profitable and sustainable aquaculture practices.

Our results are consistent with previous studies on the effectiveness of BFT in aquaculture. Numerous studies have highlighted that BFT is an effective strategy for improving water quality, optimizing growth, and increasing the survival rate of various farmed fish species. For example, research on *Litopenaeus vannamei* has demonstrated that biofloc systems can significantly reduce the feed conversion ratio (FCR) while improving growth and survival rates (Emerenciano et al., 2013). Furthermore, the application of biofloc technology under different environmental conditions (such as variations in salinity and temperature) has demonstrated its adaptability and effectiveness in various aquaculture systems. This study also confirms that biofloc technology can be successfully applied to the aquaculture of *Cirrhinus mrigala*, a highly valued species both globally and in Pakistan.

The economic benefits of biofloc technology are closely linked to its potential to improve feed conversion ratios and reduce feed costs. In traditional aquaculture systems, feed costs represent a significant portion of total production costs, and any measure aimed at improving feed conversion ratios can generate substantial savings. Biofloc technology can provide supplemental nutrition, thereby significantly reducing reliance on commercial feeds and, consequently, overall feed expenditures. Furthermore, it can improve the profitability of aquaculture by promoting growth, increasing survival rates, and boosting yields.

Biofloc technology has been the subject of numerous studies due to its many advantages; however, its implementation presents several challenges and limitations. One of the main challenges lies in the establishment and daily management of biofloc systems. Establishing a stable and effective biofloc environment requires close monitoring of water quality parameters, especially during the initial phases. To ensure proper system operation, adequate aeration must be maintained, along with an appropriate balance between carbon and nitrogen sources. Another issue is the risk of excessive biofloc formation, which, if not properly managed, can negatively impact water quality. An excess of biofloc can lead to water turbidity, decreased oxygen levels, and consequently, harm to fish health and growth. Therefore, regular monitoring and effective management of biofloc levels are essential to prevent these problems.

CONCLUSION

This study successfully achieved its research objectives, demonstrating that combining biofloc technology with a diet containing specific protein levels can significantly improve the growth and survival rate of *Cirrhinus mrigala*. The study provides new experimental evidence showing that, compared to a diet containing the same amount of protein without biofloc, a diet using a biofloc system enriched with 30% protein results in superior growth indicators and a higher survival rate. This study

fills a significant gap in freshwater carp culture by quantifying the synergistic effect of biofloc technology and feed protein optimization on *Cirrhinus mrigala*, an area that has been under-researched. The results demonstrated that biofloc systems can reduce reliance on protein-rich feeds while improving production efficiency, thus offering a practical and sustainable strategy for freshwater aquaculture. These findings open the way for the development of cost-effective feeding programs and environmentally friendly biofloc methods for large-scale carp farming.

Author's statements

Contributions

Conceptualization: M.W.; Data curation: M.D.K.; Formal Analysis: M.W., M.D.K.; Investigation: M.W., M.D.K.; Methodology: M.W.; Validation: M.W., M.D.K.; Writing – original draft: M.W., M.D.K.; Writing – review & editing: M.W., M.D.K. Approval of the version of the manuscript to be published by all co-authors.

Declaration of conflicting interest

All authors of this manuscript agree to the terms and policies of the journal, and they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Financial interests

The authors declare they have no financial interests.

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Data availability statement

All authors of this manuscript confirmed that the data supporting the findings of this study are available within the manuscript, and all the required data are available and easily accessible.

AI Disclosure

The authors declare that generative AI was not used to assist in writing this manuscript.

Ethical approval declarations

All applicable international, national, and institutional guidelines for the care of both human and animal studies were followed in this research work.

Consent to participate

All participants involved in this study provided informed consent before their participation. They were fully briefed on the purpose, procedures, and potential implications of the study. Participation was entirely voluntary, and participants were informed of their right to withdraw from the study at any time without any repercussions. Confidentiality of the participants' information was strictly maintained throughout the research process.

Additional information

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