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The role of Biofloc technology in modulating carotenoid levels and pigmentation in Goldfish, *Carassius auratus*

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ABSTRACT

This study compares the effects of Biofloc and Clear Water Culture systems on the growth performance and pigmentation of goldfish, *Carassius auratus* over 60 days. Goldfish in the Biofloc system demonstrated significantly enhanced growth, with a maximum body length of 10.38 ± 0.2 cm and weight of 11.18 ± 0.11 g, compared to 7.96 ± 0.03 cm and 7.77 ± 0.3 g in the control. The Specific Growth Rate (SGR) was also significantly higher in Biofloc (2.27 ± 0.03) than in the control (1.23 ± 0.08). Survival rates were 100% in Biofloc, while the control group exhibited 96.6%. Carotenoid content, which influences pigmentation, increased substantially in Biofloc-treated goldfish, from 3.24 ± 0.01 µg/g to 39.9 ± 0.05 µg/g, compared to a modest increase in the control to 14.6 ± 0.05 µg/g. These findings demonstrate that biofloc technology significantly improves growth and pigmentation in goldfish, highlighting its potential as a sustainable and efficient aquaculture system for ornamental fish production.

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1. Introduction

The global market value of ornamental fish is also largely determined by their coloration, which is a primary indicator of their commercial value (Saxena, 1994). Naturally vibrant fish in intensive culture, however, may show faded pigmentation. In ornamental fish, intense colouration plays a pivotal role in courtship, spawning, and sexual communication and acts as a defence mechanism against predators (Endler, 1991).). Like most animals, fish are incapable of synthesising carotenoids de novo, and thus rely on dietary intake for pigmentation development (Halten et al., 1997). Consequently, the aquaculture industry frequently employs dietary colour enhancing agents to promote the pigmentation of fish skin and muscle.As colouration is central to the market appeal of ornamental fish, achieving optimal pigmentation remains a significant challenge in their production. Reproducing natural hues under captive conditions has long been a key issue in the ornamental fish industry, as pigment fading leads to economic losses due to diminished marketability (Sawant et al., 2020). Though many experiments with pigments have been tried, carotenoid pigments are still the most successful and dependable solution for enhancing pigmentation. Carotenoids are lipid-soluble pigments found naturally in plants, animals, and microbes, and synthetic forms are also available. Beyond their pigmentation role, carotenoids have been shown to confer immunological benefits, including enhanced resistance against bacterial and fungal infections, underscoring their broader biological relevance (Shahidi et al., 1998).

Despite substantial advances in both dietary carotenoid supplementation and biofloc technology (BFT), their combined application in ornamental fish culture remains poorly explored. The joint effects of these strategies on pigmentation and growth performance have rarely been investigated together, particularly under controlled experimental conditions. Biofloc systems, originally developed to recycle nitrogenous waste via heterotrophic bacterial growth, simultaneously improve water quality and generate nutritive microbial biomass that can contribute to fish nutrition and health (Avnimelech, 1999; Crab et al., 2012). Recent experimental and review studies indicate that BFT can influence coloration and production performance in ornamental and food fishes (da Cunha et al., 2020; Besen et al., 2021), while updated reviews on carotenoids reaffirm the value of targeted carotenoid sources (natural and formulated) for enhancing colouration and immune status (Elbahnaswy et al., 2023). However, most studies have examined either (a) BFT effects on growth/pigmentation or (b) dietary carotenoid effects in conventional systems; few have evaluated carotenoid-enriched BFT systems that simultaneously test pigmentation, growth, feed efficiency, and physiological stress/immunity under standardized conditions.

Microorganisms in biofloc systems synthesize different pigments such as carotenoids, melanins, flavins, monascins, violacein, and indigo (Venil et al., 2020)These pigments, which are controlled by environmental conditions, are known for their oxidative damage protection and antimicrobial activities (Ciapara et al., 2004). Of these, carotenoids are the most frequently occurring pigment. The pigment-producing bacterial consortia in biofloc play a major role in increased pigmentation in ornamental fish, and hence BFT is a useful tool for culturing high-value species (Menaga et al., 2021). Different research proved that fish species such as *Cyprinus carpio* (Antonyraj, 2016), *Xiphophorus helleri* (Kuhn et al., 2010), and *Oreochromis niloticus* (Martínez-Córdova et al., 2015) have validated the effectiveness of BFT to enhance carotenoid levels and quality.

Despite the promising outcomes of BFT in ornamental aquaculture, there remains a significant gap in understanding the specific carotenoid profiles within biofloc and their assimilation mechanisms in fish tissues, particularly in goldfish (*Carassius auratus*) (Das & Biswas, 2016; Menaga et al., 2021). While studies have reported enhanced pigmentation (Cunha et al., 2020; Faizullah et al., 2015), few have characterized the types of carotenoids produced by microbial communities in biofloc or tracked their deposition across different tissues such as skin, muscle, liver, and fins. Furthermore, the contribution of microbial-derived pigments versus dietary sources in BFT remains poorly delineated. These knowledge gaps limit the full utilization of biofloc systems for targeted pigmentation enhancement in ornamental fish.Accordingly, the current research explores the use of biofloc technology to improve the growth performance and carotenoid pigmentation in goldfish, *Carassius auratus*. The study focuses on the usefulness of BFT as a natural method of maximizing the performance, coloration, and productivity of ornamental fish farming. We hypothesise that biofloc technology (BFT) significantly enhances carotenoid deposition and growth performance in goldfish compared to conventional clear-water culture systems.

2. Materials and Methods

2.1 Biofloc Production

Biofloc development was the pioneer step of the experiment and it was conducted according to the protocol provided by Avnimelech (2009). Biofloc was cultured in an experimental indoor FRP tank with 80 L of water-holding capacity. Tanks were disinfected using bleaching powder (33% active chlorine) and left to dry for three days. Post-disinfection, the tanks were washed with water and chlorinated using chlorine (30 ppm). After de-chlorination, fish feed (32% protein) was added as Nitrogen source. (Avnimelach, 2009). An inoculum from a previously established biofloc tank was introduced to initiate microbial activity. Prior to use, the microbial composition of the inoculum was assessed to confirm the presence of beneficial heterotrophic bacteria and absence of pathogenic strains. Continuous aeration, provided by an air compressor (Hailea HAP-120), facilitated floc formation and maintained it in suspension. Fermented jaggery was used as the carbon source to maintain a C: N ratio of 20:1 in the biofloc tank. Fermentation was carried out for 24 hours using yeast at a rate of 1:100 of the jaggery used. The physicochemical parameters were measured (APHA, 2008). Imhoff cones were used to collect the floc samples and were measured for floc development (Avnimelech, 2009). The floc volume once reached 40 ml/L, floc was divided into the experimental tanks.

2.2 Experimental Design:

The experiment was performed for 60 days in the wet laboratory at the Department of Aquatic Biology and Fisheries, University of Kerala. Six glass tanks (60×30×33cm, 60 L) were used for the experiment and were maintained as an indoor system. Using the random design, triplicate treatments of clear water (CW) and a biofloc (BFT) were maintained. For the biofloc treatments, 40 L of dechlorinated tap water was mixed with 20 L of inoculum and added to each tank, while the control tanks received 60 L of dechlorinated tap water. This ensured an initial water volume of 60 L in all tanks (both control and biofloc treatments). The air blower (capacity 125W; Hailea Hi-Blow Air Pump) was used to provide aeration and movement to the water, and each tank was provided with air stones for continuous aeration. Carbon sources were added at 20 times the ammonia concentration to maintain a C: N ratio of 20:1, ensuring optimal biofloc production and system stability. About 30% of culture water volume was exchanged daily in CW treatment, whereas no water exchange occurred with the biofloc system. However, to compensate for evaporation, water was added whenever essential.

2.3 Fish Acclimatization

Juveniles of goldfish were acquired from the Government Fish Hatchery located in Neyyar, Thiruvananthapuram. The strain used in the study was the Common Goldfish (*Carassius auratus*), widely recognized for its uniform coloration and adaptability to experimental conditions. The juveniles were acclimated for seven days before the start of the experiment. The fish were kept in RAS-equipped 500L tanks for conditioning throughout this time. Prior to the experiment, all individuals were visually inspected and found to be free of external lesions or signs of disease, with active swimming behavior and normal feeding response, indicating a healthy pre-experimental status. Feed was provided twice a day using Optimum™ 3 in 1 commercial feed, which contained 36% crude protein. The feed is formulated for ornamental fish and includes added pigment sources such as spirulina and astaxanthin. However, it was used uniformly across both experimental and control groups to ensure that any differences in pigmentation could be attributed to the biofloc system rather than dietary variation. . Following the period of acclimation, 150 goldfish juveniles (mean length: 5.8±0.24 cm, mean weight: 4.19 ± 0.21 g) were evenly divided among 6 glass tanks (60 L). The fish were hand-fed 4% of the total biomass twice a day (at 9:00 and 17:00). In addition to replenishing one-third of the water in the control treatment, the feed residues and excreta were removed every day.

2.4 Water quality monitoring

Daily observations were taken for water temperature (°C), pH, and ammonia concentration. Dissolved oxygen (DO) (mg/L), alkalinity, nitrate, and nitrite levels were measured weekly. Temperature was measured with a digital thermometer, and pH was measured with a benchtop pH meter (HANNA® Microprocessor pH Meter). Ammonia, dissolved oxygen, alkalinity, nitrate, and nitrite concentrations were determined according to the APHA (2008) standard procedures. Biofloc volume (ml/L) was measured weekly by sampling 1 L of water from the biofloc tanks into Imhoff cones and allowing it to settle undisturbed for 25–30 minutes, as explained by Avnimelech and Kochba (2009) and Haridas et al. (2021).

2.5 Growth Performance:

Before stocking length and weight of the fish were recorded.

Every 7-day interval fish were randomly collected from each replicate length and weight were recorded.

$$\text{Specific Growth Rate (\%)} = \frac{Ln(\text{final weight}) - Ln(\text{initial weight})}{\text{Days of culture}} \times 100$$

$$\text{Food Conversion Ratio (FCR)} = \frac{\text{Total feed intake per fish}}{\text{Wet weight gain per fish}}$$

$$\text{Survival Rate (\%)} = \frac{\text{Final fish count}}{\text{Initial fish count}} \times 100$$

2.6 Carotenoid Extraction and Quantification:

Carotenoid estimation was conducted at 10-day intervals following the method described by Olson (1984) to monitor changes in pigmentation over time.

2.7 Preparation of the tissues

Two fish per tank were randomly selected at each sampling, ensuring similar size range to reduce bias in carotenoid measurements. Their skin, scales, liver, muscle and fin were dissected through a shallow ventral incision. All these tissues were collected, pooled, weighed and kept in a deep freezer till analysis.

2.8 Extraction and estimation of total carotenoids

About 1 g of the fresh tissue was weighed on a small piece of aluminium foil and placed in labelled clean, dry screw capped 10 ml vials, containing 2. 5 g of an anhydrous sodium sulphate. The tissues were gently mashed using a glass rod. 5 ml of chloroform was added to each borosil vial to extract all the carotenoids from the sample. And the vials were sealed and kept overnight at 0°C in the dark. The blank contained only 5ml of chloroform. An aliquot of 0.3 ml chloroform extract from each vial was diluted to 3 ml with absolute ethanol. The blank was also treated similarly. The optical density was read with a spectrophotometer at 380, 450, 475 and 500 nm. The wavelength with maximum absorption was used for calculation (Fig.1). The total carotenoid content in µg carotenoid/g in the tissue was calculated as follows.

$$\text{Total carotenoids} = \frac{\text{Absorption at maximum wavelength X dilution factor}}{\text{0.25 X sample weight in gm}}$$

Here; dilution factor = 10; Extinction co-efficient = 0.25

2.9 Statistical Analysis:

SPSS version 25 was utilized in the statistical analysis of all data. The data were presented as mean ± standard deviation (SD), and the independent sample t-test was utilized in testing the significance of differences between the two treatments.

3. Results and Discussion

3.1 Water Quality

Most water quality parameters (Table 1), including pH, ammonia, nitrite, nitrate, and dissolved oxygen (DO), remained within the optimal range for goldfish in both the control and biofloc groups and showed no significant variation ($p > 0.05$). This suggests the suitability of both systems for goldfish culture. However, a statistically significant temperature difference was observed ($p = 0.008$), though the variation was minimal, and unlikely to affect fish performance.

A notable and highly significant increase in alkalinity was observed in the biofloc group (128.22 ± 8.56 ppm) compared to the control (62.78 ± 6.77 ppm) ($p < 0.001$). The elevated alkalinity in the biofloc system is attributed to the buffering requirement for microbial processes, particularly nitrification. In biofloc systems, continuous microbial activity, especially from nitrifying bacteria, consumes alkalinity. If not replenished, this leads to reduced pH and compromised microbial performance. Regular supplementation of carbonates or bicarbonates, particularly sodium bicarbonate, helps stabilize alkalinity, supporting both microbial and fish health (Ebeling et al., 2006; Furtado et al., 2011).

Floc volume, measurable only in the experimental tanks, averaged 18.45 ± 1.53 ml/L, confirming the development of biofloc. The addition of carbon sources in the experimental tanks promoted heterotrophic bacterial proliferation, contributing to nitrogen assimilation and improved water quality (Avnimelech, 2009; Wang et al., 2015). Overall, these results demonstrate that BFT offers stable and favourable water conditions for rearing goldfish without requiring frequent water exchanges.

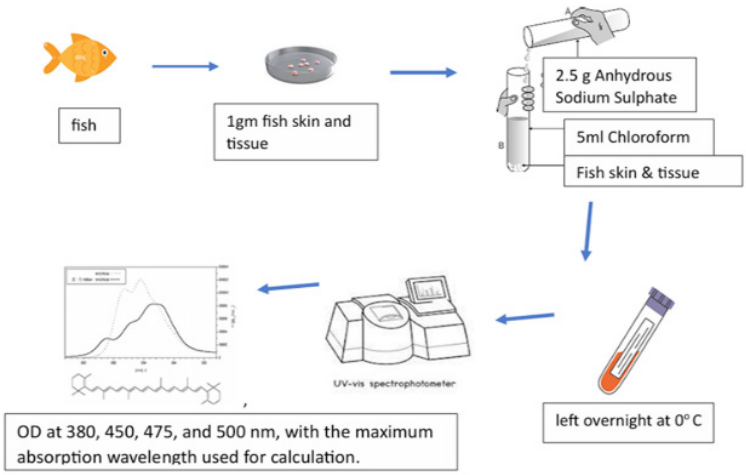


Fig. 1. Procedure for carotenoid analysis

Table 1. Water quality parameters summarised throughout 60 days of rearing goldfish

Variables	Water Quality Analysis				
	Control (Mean ± SD)	Experiment (Mean ± SD)	t-value	p-value	Inference
pH	7.4 ± 0.2	7.8 ± 0.18	-2.45	0.316	NS
Temperature (^{O}C)	27.97 ± 0.44	27.82 ± 0.38	4.91	0.008	**
Ammonia (ppm)	0.32 ± 0.31	0.39 ± 0.41	-0.22	0.189	NS
Nitrite (ppm)	0.24 ± 0.31	0.23 ± 0.23	0.04	0.944	NS
Nitrate (ppm)	2.74 ± 2.14	3.21 ± 1.76	-0.30	0.918	NS
DO (ppm)	5.228 ± 0.81	4.91 ± 0.61	0.52	0.877	NS
Alkalinity (ppm)	62.78 ± 6.77	128.22 ± 8.56	-13.16	<0.001	***
Floc Volume (ml/L)	--	18.45 ± 1.53		--	--

Values are mean ± SD; NS = not significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

3.2 Growth Performance

Fish in the biofloc group displayed significantly better growth performance. There was no significant difference in initial length and weight between the groups, indicating uniformity in the baseline. However, by day 60, fish in the biofloc system achieved greater final length and weight, showing significantly higher specific growth rate (SGR) and weight gain ($p < 0.001$). Additionally, the feed conversion ratio (FCR) was substantially lower in the biofloc group (1.18 ± 0.02) than in the control (2.59 ± 0.02), indicating superior feed utilization (Table 2).

These results confirm previous studies (Avnimelech, 2015; Ekasari et al., 2016; Wang et al., 2015) that demonstrate enhanced growth and feed efficiency in BFT due to the continuous availability of microbial proteins, better water stability, and improved gut health. The high nutritional value of microbial flocs—including proteins, amino acids, fatty acids, and polysaccharides—supports growth and immunity (Tabarrok et al., 2020; Bakhshi et al., 2018; Khanjani et al., 2023).

The carotenoid concentration in goldfish exhibited a progressive increase throughout the culture period in both the control and biofloc groups (Fig. 2–4). However, goldfish reared under biofloc technology (BFT) consistently demonstrated significantly higher carotenoid levels across all sampling intervals compared to the control group.

Initial carotenoid levels were comparable between groups, measuring 3.21 ± 0.1 µg/g in the control and 3.76 ± 0.1 µg/g in the biofloc group. By Day 10, a significant increase was observed in the biofloc group (6.39 ± 0.15 µg/g), nearly doubling from the initial concentration, while the control group reached only 3.93 ± 0.12 µg/g. The disparity widened further with time. On Day 20, carotenoid content in the biofloc group reached 12.77 ± 0.2 µg/g, double that of the control (6.4 ± 0.18 µg/g). This trend continued, with biofloc-fed fish achieving 25.8 ± 0.25 µg/g on Day 30 versus 9.06 ± 0.2 µg/g in the control. Subsequent sampling points (Days 40, 50, and 60) recorded 28.43 ± 0.3 µg/g, 31.50 ± 0.35 µg/g, and 40.2 ± 0.4 µg/g, respectively, in the biofloc group, while the control group showed a gradual and less pronounced increase, peaking at 14.2 ± 0.3 µg/g by Day 60. These results clearly indicate that BFT significantly enhances carotenoid accumulation in goldfish over time.

This enhancement is attributable to the natural availability of carotenoids within the biofloc matrix, comprising diverse microbial communities including heterotrophic bacteria, algae, and protozoa. Many of these microorganisms are capable of synthesizing bio-pigments such as carotenoids, flavins, violaceins, and melanins as adaptive or defensive mechanisms in their ecological niches (Duffosse, 2006; Manon Mani et al., 2015). Compared to plant sources, microbial pigments offer advantages such as consistent yield, ease of extraction, and high bioavailability under controlled culture conditions (Joshi et al., 2003; Radha, 2015).

Although only total carotenoid content was quantified in the present study, literature indicates that biofloc systems commonly contain β-carotene and astaxanthin as predominant pigments, derived from microalgae, cyanobacteria, and pigment-producing bacteria (Venil et al., 2020; Lim et al., 2018). Given that the feed in this study also contained astaxanthin, the observed pigmentation is likely due to a synergistic effect between dietary and biofloc-derived carotenoids. Future studies employing chromatographic techniques such as HPLC could identify

Table 2. Growth parameters summarized throughout 60 days of rearing goldfish

Variables	Growth Performance				
	Control (Mean ± SD)	Experiment (Mean ± SD)	t-value	p-value	Inference
Initial Length (cm)	5.65 ± 0.26	5.64 ± 0.29	0.143	0.887	NS
Final Length (cm)	8.82 ± 0.36	10.69 ± 0.31	-21.53	<0.001	**
Length Gain (cm)	3.17 ± 0.1	5.05 ± 0.03	-11.828	<0.001	**
Initial Weight (g)	4.18 ± 0.16	4.17 ± 0.13	0.181	0.857	NS
Final Weight (g)	8.62 ± 0.29	11.36 ± 0.33	-34.201	<0.001	**
Weight Gain (g)	4.44 ± 0.13	7.19 ± 0.2	-21.999	<0.001	**
SGR (%)	1.21 ± 0.09	1.67 ± 0.08	-21.82	<0.001	**
FCR	2.59 ± 0.02	1.18 ± 0.02	14.087	<0.001	**
Survival (%)	96.67 ± 5.77	100 ± 0	-1	0.374	NS

Values are mean ± SD; NS = not significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

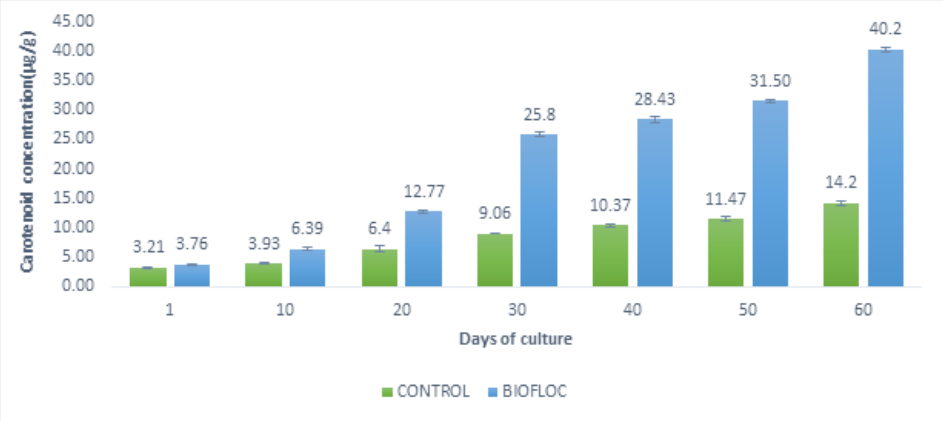


Fig. 2. Total Carotenoid Content (µg/g) of *C. auratus*

and quantify specific carotenoid types to better understand their relative contributions.

Carotenoids such as astaxanthin, β-carotene, and lutein are essential for pigmentation in ornamental fish but cannot be synthesized de novo by fish and must be obtained through diet (Gomes et al., 2002; Gouveia et al., 2003). In this context, biofloc serves not just as a protein-rich live feed alternative but also as a sustainable and natural source of dietary pigments. The current study confirms that goldfish cultured in biofloc systems exhibited more vivid and uniform coloration, supporting the hypothesis that biofloc-associated carotenoids are assimilated effectively by fish and contribute directly to pigment deposition in skin and fins.

Supporting literature has shown similar results in various ornamental species. Epa and Perera (2023) reported intensified coloration in *Poecilia reticulata* under BFT, while Lim (2018) and Castro-Castellón et al. (2020; 2023) observed enhanced pigmentation in *Xiphophorus maculatus*, *Astronotus ocellatus*, and *Heros severus*, respectively. In *Carassius auratus*, Faizullah et al. (2015) and Cunha et al. (2020) documented similar outcomes, emphasizing BFT's role in augmenting ornamental quality. Da Silva et al. (2018) further extended these findings to *Danio rerio* and *Archocentrus nigrofasciatus*, underscoring the broad applicability of BFT.

Apart from coloration, carotenoids in biofloc impart critical physiological benefits. Acting as potent antioxidants, they mitigate oxidative stress, enhance immune responses, and contribute to reproductive performance (Yuan et al., 2011; Xu & Pan, 2013). For example, astaxanthin has been shown to stimulate immune-related gene expression and improve stress resistance (Nakano et al., 1995; Amar et al., 2001), making biofloc an ideal multi-functional input in aquaculture.

Mechanistically, dietary carotenoids are absorbed in the intestine, transported via lipoproteins in the bloodstream, and deposited in chromatophores such as xanthophores

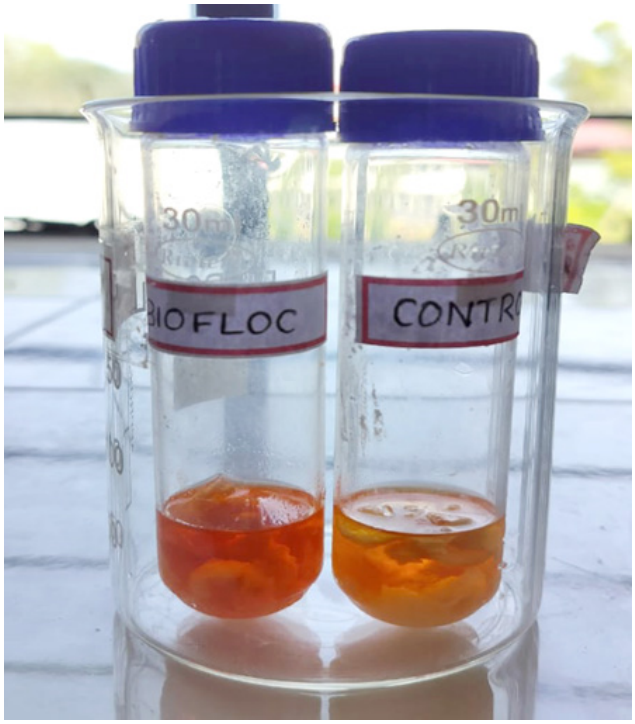


Fig. 3. Pigment extract from the experimental fish



Fig. 4. Photographs of goldfish after 60 days of trial

and erythrophores (Torrissen et al., 1989). The continuous presence of biofloc in the water column ensures uninterrupted carotenoid ingestion, unlike conventional feeding with intermittent supplementation. This likely explains the sustained and homogeneous pigmentation observed in the BFT group. Studies by Sefc et al. (2014) and Da Cunha et al. (2020) similarly reported stable color morph expression under biofloc regimes.

While the current results affirm the pigmentation benefits of BFT, further investigation is warranted to dissect the specific types of carotenoids present in biofloc, the influence of microbial diversity on pigment biosynthesis, and the molecular mechanisms governing carotenoid uptake and retention in ornamental fish. Advanced analytical tools and transcriptomic studies could elucidate these pathways and optimize the biofloc formulation for maximum ornamental and health benefits.

The present study demonstrates that biofloc technology significantly enhances carotenoid deposition and pigmentation in *Carassius auratus*, offering a sustainable, health-promoting, and visually appealing alternative to synthetic pigment supplementation. These findings highlight the potential for integrating BFT into ornamental aquaculture systems to improve product quality, reduce input costs, and promote eco-friendly practices.

4. Conclusion

This study demonstrates that biofloc technology (BFT) significantly improves the growth performance, feed efficiency, and pigmentation of goldfish (*Carassius auratus*) under indoor culture conditions. Fish reared in the biofloc system exhibited significantly higher final length, weight, specific growth rate, and total carotenoid content, along with a lower feed conversion ratio, compared to those in clear-water systems. The consistent increase in carotenoid levels and the vibrant skin coloration observed in the biofloc group indicate the potential of biofloc as a natural source of pigmentation enhancing compounds. Although both groups exhibited high survival rates, the biofloc treatment achieved 100% survival, further supporting its health promoting effects.

While this study quantified total carotenoid accumulation, the specific types of carotenoids and their microbial sources were not identified this remains a limitation. Future studies should focus on characterizing the dominant carotenoid producing microorganisms within the biofloc and determining the specific pigments contributing to tissue deposition. Comparative trials between biofloc based and synthetic pigment enriched diets would also help assess commercial feasibility. In addition, long-term economic evaluations should be undertaken to quantify potential feed and pigment cost reductions, especially in commercial ornamental hatcheries.

Collectively, the findings position BFT as a sustainable and cost-effective technology that not only improves the aesthetic and market value of ornamental fish but also reduces environmental and operational burdens in aquaculture systems.

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