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Fermented vs. Non-Fermented Biofloc: Growth Performance, Biochemical Composition and Water Quality Parameters in Indoor Culture of Goldfish, *Carassius auratus*

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Abstract

This study assessed the growth performance, water quality, and biochemical composition of goldfish (*Carassius auratus*) reared in biofloc technology (BFT) systems. The experiment was conducted over a period of 56 days in 80 L indoor tanks with three treatment groups: control, fermented biofloc, and non-fermented biofloc, each in triplicates. Jaggery served as the carbon source, and biofloc fermentation was achieved using yeast for 24 hours. All treatments were fed a diet containing 36% crude protein (CP) at a daily feeding rate of 5% of the total fish biomass per tank. Juvenile goldfish were stocked at uniform densities with an average initial weight of 0.12±0.01 g and an initial length of 1.12±0.13 cm. The fermented biofloc treatment demonstrated superior growth performance, including a higher specific growth rate (SGR) and a lower feed conversion ratio (FCR) compared to the non-fermented biofloc and control treatments. Protein levels in the fermented biofloc were significantly higher than those in the non-fermented biofloc. Additionally, the study revealed adequate levels of essential amino acids (EAA) and non-essential amino acids (NEAA) in the biofloc, highlighting the role of fermentation in enhancing its nutritional quality. These findings suggest that fermented biofloc improves the nutritional profile and promotes the overall well-being and growth of *C. auratus.*

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Introduction

The ornamental fish industry is a rapidly expanding sector within global aquaculture, driven by increasing consumer interest in keeping fish as pets for their aesthetic appeal and low maintenance requirements. In 2023, the global ornamental fish market was valued at USD 5.95 billion and is projected to reach USD 11.69 billion by 2032, growing at a compound annual growth rate (CAGR) of 7.8% during the forecast period (2024–2032) (Straits Research, 2024). This growth is fueled by advancements in aquarium technology, the expansion of e-commerce, and a rising demand for vibrant, exotic fish species in both home and commercial settings. Ornamental fishes are often called 'living jewels' due to their distinctive colour, shape, behaviour and other aesthetic traits (Jayasankar, 1998; Das, Sarma, & Das, 2005). The ornamental fish-keeping hobby is emerging as one of the most popular hobbies across the world, second only to photography (Nayar, 1996; Kurup, Premlal, Thomas, & Anand, 2003; Singh & Dey, 2003). Some recent comprehensive reviews on ornamental fish production techniques highlight the global interest in ornamental fish (Vanderzwalmen et al., 2019; Chen, Zeng, Jerry, & Cobcroft, 2020).

New and sustainable rearing techniques could boost the aquaculture business and reduce the depletion of natural resources in many developing nations as they strive to meet this growing demand (Hoseinifar et al., 2023). The primary goal of aquaculture is to achieve the fastest growth rate at the lowest cost of production. Several techniques have been developed to improve growth rates and achieve this objective.

As technological advancements continue to shape the industry, integrating biofloc systems into ornamental aquaculture presents new opportunities for cost-effective and environmentally friendly fish production. One such popular technology is biofloc technology, which involves a zero- or minimal-water-exchange culture system. Biofloc is an aggregation of algae, bacteria, protozoan, faecal matter, and uneaten feed, which are held together in a loose matrix by the secretion of filamentous microorganisms or by electrostatic attraction (Hargreaves, 2013). This technology works by maintaining the carbon to nitrogen ratio in the culture system, utilizing the dense microbial biomass to strip ammonia from the water (Schneider, Sereti, Eding, & Verreth, 2005).

Ornamental fish have traditionally been fed live feed, which in often nutritionally inadequate and serve as a vector for disease transmission (Faizullah, Rajagopalasamy, Ahilan, & Francis, 2015). The ornamental aquaculture industry faces significant challenges, including low survival rates, limited availability of suitable live feed, difficulties in water quality management, and frequent disease outbreaks. These challenges have accelerated the adoption of innovative technologies beyond traditional culture systems to enhance production and productivity (Faizullah et al., 2015). Consumption of biofloc offers various benefits for shrimp and fish, including improved growth rate (Wasielesky, Atwood, Stokes, & Browdy, 2006), lower FCR, and reduced feed costs (Burford, Thompson, McIntosh, Bauman, & Pearson, 2004). Biofloc technology (BFT) also enhances fish survival by promoting beneficial microorganisms that outcompete pathogenic bacteria, thereby reducing disease outbreaks (Samocha et al., 2007; Megahed, 2010; Pérez-Fuentes, Pérez-Rostro & Hernández-Vergara, 2013). These microorganisms contribute to better digestion, increased feed efficiency, and enhanced immune responses, ultimately leading to higher survival rates.

However, in a biofloc-based system, the selection of carbon sources can have a significant impact on animal performance, water quality, biofloc productivity and its nutritional value. When jaggery was utilized to produce biofloc in shrimp culture and produced very high protein levels in the flocs. Sakkaravarthi and Sankar (2015) identified jaggery as a novel and effective carbon source, noting that it also encourages the growth of bacteria, fungi, and heterotrophic bacteria. In addition, jaggery meets key criteria such as biodegradability, affordability, accessibility, bacterial assimilation, and the ability to generate nutritious flocs (Jackqulinwino et al., 2024).

The freshwater ornamental fish industry primarily comprises exotic species cultured for the local market. To ensure its sustainability, it is essential to develop effective rearing methods that guarantee continuous production (Deocampo, Fenol, Jimenez, Paguntalan, & Caipang, 2022). One such innovative approach is the application of biofloc technology (BFT). Unlike fish cultured for human consumption, ornamental fish often incur significantly higher production costs (Satam et al., 2018). Therefore, improving profitability requires effective strategies to reduce mortality throughout the production cycle. This can be achieved by creating an optimal environment tailored to the species' specific needs and by providing cost-effective, high-quality nutrition (Pleeging & Moons, 2017).

Biofloc technology has been successfully employed in aquaculture systems for various species, including freshwater prawns (Crab, Chielens, Wille, Bossier, & Verstraete, 2010), brackish-water and marine shrimp (Decamp, Moriarty, & Lavens, 2008; Ju, Forster, Conquest, & Dominy, 2008; Emerenciano, Cuzon, Arévalo, & Gaxiola, 2014; Khanjani, Sajjadi, Alizadeh, & Sourinejad, 2017), and finfish (Avnimelech, 2007; Najdegerami et al., 2012; Mahanand, Moulick, & Rao, 2013; Ekasari et al., 2014; Luo et al., 2014; Yusuf, Elfghi, Zaidi, Abdullah, & Khan, 2015; Minabi, Sourinejad, Alizadeh, Ghatrami, & Khanjani, 2020). However, its application in ornamental fish culture remains limited, despite its potential to enhance water quality and improve production performance (Wang et al., 2015; da Cunha et al., 2020; Deocampo et al., 2022).

Goldfish (*C. auratus*), belonging to the family Cyprinidae, are among the earliest domesticated and most widely kept ornamental fish species, renowned for its adaptability, vibrant pigmentation, and diverse morphologies. As a cornerstone of the global ornamental fish trade, optimizing growth and survival continues to be a key focus in aquaculture. While traditional aquaculture systems often rely on fishmeal-based feeds, the increasing need for sustainability and cost-efficiency has driven interest in alternative approaches, such as biofloc technology (BFT). Previous studies have demonstrated that culturing goldfish larvae in biofloc systems can improve growth, survival rates, and skin pigmenta-

tion (Faizullah et al., 2015; da Cunha et al., 2020; Besen et al., 2021). The present study evaluated the potential of using fermented and non-fermented jaggery as carbon sources in biofloc systems to culture goldfish (*C. auratus*). By examining growth performance, survival, and the biochemical composition of biofloc, this study seeks to provide insights into the impact of fermentation on biofloc composition and its subsequent benefits for ornamental aquaculture. The findings aim to strengthen the case for biofloc technology as a sustainable, cost-effective, and health-enhancing approach to goldfish aquaculture.

Materials and Methods

The process described by Avnimelech (2009) was followed for the development of biofloc. Biofloc was produced separately for each carbon source, fermented and raw jaggery, using two experimental indoor tanks with a water holding capacity of 80 L each. After being disinfected with Clorox, the tanks were left to dry for three days. Following disinfection, water was added to the tanks, and chlorine was added at a concentration of 30 ppm. Once the chlorine had properly evaporated after three days, feed (32% protein) was added to facilitate ammonia development (Avnimelech, 2009). An inoculum from a previously established biofloc tank was introduced to initiate microbial activity. Continuous aeration, provided by an air compressor (Hailea HAP-120), facilitated floc formation and kept the particles in suspension. Jaggery and fermented jaggery were used as carbon sources to maintain a C:N ratio of 20:1 in each tank. Based on CHN analysis, the jaggery used contained 34.7% carbon, 6.7% hydrogen, and 0.9% nitrogen. Fermentation was carried out for 24 hours using yeast at a 1:100 ratio relative to the amount of jaggery used. Physicochemical parameters were measured according to standard methods (APHA, 1998). Imhoff cones were used to collect biofloc samples, which were then measured to assess floc development and formation (Avnimelech, 2009).

A lot of 400 juvenile common goldfish were procured from Reeba Fisheries, Chennai, with a mean length of 1.12±0.13 cm and a mean weight of 0.12±0.01 g. The fish were acclimatized in a preconditioned 500 L capacity FRP tank. During the acclimation period, the fish were fed a commercial feed containing 36% protein (Optimum), twice daily to satiation, for one week prior to the start of the experimental trials. Additionally, 50% of the tank water was replaced every three days to maintain optimal water quality.

Nine indoor tanks in the department laboratory were assigned to three treatments: Control, Fermented Biofloc, and Non-Fermented Biofloc, with each treatment maintained in triplicate over a period of 56 days. The tanks were positioned within an indoor laboratory facility, receiving diffused natural sunlight through laboratory windows during daylight hours. This setup supported limited algal growth within the biofloc systems without the risk of overheating or uncontrolled algal blooms. Prior to the experiment, all tanks were thoroughly cleaned, dried, and filled with 80 L of water to a depth of 0.5 meters. The tanks were left undisturbed for one week to allow for natural dechlorination. Continuous aeration was provided 24 hours a day throughout the experimental period to facilitate biofloc formation and maintain water circulation. One day before stocking the fish, biofloc from the inoculum tank was introduced into the experimental tanks. For the biofloc treatments, 60 L of dechlorinated tap water was mixed with 20 L of inoculum and added to each tank, while the control tanks received 80 L of dechlorinated tap water. This ensured an initial water volume of 80 L in all tanks (both control and biofloc treatments). Carbon sources were added at 20 times the measured ammonia concentration to maintain a C:N ratio of 20:1, ensuring optimal biofloc production and system stability.

In the present experiment, the carbon to nitrogen (C:N) ratio was maintained at 20:1, meaning 20 grams of carbon source were supplied for every gram of ammonia detected in the culture units. Two types of carbon sources were used: fermented jaggery and non-fermented jaggery. For the fermented treatment, jaggery was fermented for approximately 24 hours using *Saccharomyces cerevisiae* (baker's yeast), at a ratio of 1:100 (yeast to jaggery by weight). The fermentation process involved dissolving pre-weighed jaggery in dechlorinated water, adding the specified amount of yeast, and incubating the mixture at room temperature.

For both treatments, the pre-weighed carbon sources (fermented or non-fermented jaggery) were mixed in glass beakers with water collected from the respective culture tanks and the mixture was subsequently added into the culture systems. The

addition of carbohydrates led to the formation of froth, which gradually dissipated over several days. This process stimulated the development of suspended aggregates, known as bioflocs, resulting from the proliferation of heterotrophic bacteria (Hari, Kurup, Varghese, Schrama, & Verdegem, 2004; Ebeling, Timmons, & Bisogni, 2006; Avnimelech & Kochba, 2009; Crab et al., 2010).

All 9 tanks were stocked with 40 goldfish (1.12±0.13 cm & 0.12±0.01 g), with each of the three treatments (control, fermented, and non-fermented biofloc) replicated in triplicate. The fish were fed the same commercial fish feed used during acclimation at a rate of 5% of their body weight. Feed amounts were adjusted weekly based on the weekly weight of the fish samples. In the BFT treatments, jaggery (with and without fermentation) was added at a carbon-to-nitrogen ratio of 20:1, following the methods of de Schryver, Crab, Defoirdt, Boon, and Verstraete (2008). While the BFT treatments were designed to function as a zero-exchange culture system, the control treatment underwent a 50% water exchange every three days to prevent ammonia accumulation. The floc volume in the biofloc tanks was measured daily by filling an Imhoff cone with 1 L of water and, after allowing the biofloc to settle for 20 minutes, the volume was recorded.

During the experimental period, water quality parameters such as temperature, dissolved oxygen, pH, and ammonia, were recorded daily in the culture systems. Temperature was measured using a digital thermometer, and pH was determined using a pH meter (Eutech - PC testr 35). Floc volume (FV) was measured following the method used by Avnimelech and Kochba (2009) using Imhoff cones. Modified Winkler's titration method (APHA, 1998) was adopted to estimate the dissolved oxygen, total ammonia-N was measured using the Phenol Hypochlorite method (Richards & Kletch, 1964), nitrite-N by the NNED-Sulphanilamide method (APHA, 1998), and nitrate-N by the Resorcinol method (APHA, 1998), assessed on a weekly basis.

Before stocking, the length and weight of the fish were recorded. Every 7 days, fish were randomly collected from each replicate, and their length and weight were recorded.

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

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

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

At the end of the experiment, biofloc was extracted from each biofloc treatment tank using a 60 μm mesh-size plankton net. Any excess water was drained, and the wet weights were measured. The samples were then oven-dried overnight at 50°C, and the biofloc dry weights were recorded. The ash content of the dried floc was determined by burning it in a muffle furnace at 600°C and weighing after cooled. The protein, carbohydrate, moisture, and lipid contents were analysed following the standard protocols of AOAC (2012). Amino acid profiling was measured at ICAR-CIFT, Cochin, using the High Performance Liquid Chromatography (HPLC) pre-column derivatisation method.

All data are presented as mean ± standard deviation. Before doing statistical analysis, the results were tested for normality and homogeneity of variances. One-way ANOVA and independent samples t-test were applied for data analysis. If significant differences were detected ($p<0.05$), a Tukey's test was used to identify differences among the treatments.

Results and Discussion

The water quality parameters monitored over the 8-week rearing period of goldfish (*Carassius auratus*) juveniles in control, non-fermented biofloc, and fermented biofloc systems are summarized in Table 1. Temperature remained consistent across all treatments, but statistically significant differences were observed ($p<0.001$). Similarly, pH values ranged from 7.5±0.06 in the control system to 7.82±0.05 in the fermented biofloc system, but no significant differences were observed between treatments.

Ammonia levels were significantly higher in both biofloc systems (0.51±0.37 ppm for non-fermented and fermented systems) compared to the control (0.003±0.004 ppm) ($p<0.001$). Nitrate concentrations followed a similar trend, with significantly elevated levels in the non-fermented (0.44±0.37 ppm) and

fermented biofloc systems (0.45±0.22 ppm) compared to the control (0.001±0.004 ppm). The nitrite concentrations remained lower in all the treatments. Alkalinity was markedly higher in the biofloc treatments, maintaining levels below 100 ppm. Dissolved oxygen (DO) levels were lower in the biofloc systems, with 4.87±0.46 ppm and 4.99±0.22 ppm recorded in the non-fermented and fermented systems, respectively, compared to 5.59±0.12 ppm in the control. These differences were statistically significant ($p<0.001$).

Floc volume had no effect in the control system and was recorded only in the biofloc treatments. It was slightly higher in the fermented biofloc system (20.34±6.37 ml/L) compared to the non-fermented system (18.86±5.36 ml/L). Floc volume was maintained below 20 ml/L with the help of sludge removal at proper intervals. In general, the floc volume increased with the age of biofloc culture, but the rate of increase varied among treatments. The total plate count (TPC) on the final day revealed significant microbial proliferation in the biofloc treatments ($p<0.001$). The control group exhibited the lowest microbial density of $5\times10^5\pm1\times10^5$ cfu, while the non-fermented biofloc system recorded $2\times10^7\pm0.5\times10^7$ cfu, and the fermented biofloc system showed the highest TPC of $3\times10^8\pm0.2\times10^8$ cfu.

Overall, the results indicate that biofloc systems, particularly those utilizing fermented carbon sources, significantly enhanced microbial activity and altered key water quality parameters.

The growth performance of *C. auratus* juveniles reared in control, non-fermented biofloc, and fermented biofloc systems are summarized in Table 2.

Initial length and weight measurements of the juveniles were similar across all treatments, with no significant differences ($p>0.05$), confirming that the groups were comparable at the start of the experiment. However, the final growth metrics (length, weight, and specific growth rate) showed significant differences ($p<0.01$) among the treatments.

The final length of goldfish in the control group (3.89±0.18 cm) was significantly lower compared to both the non-fermented biofloc (5.58±0.19 cm) and fermented biofloc groups (6.35±0.19 cm). The control group exhibited the lowest length gain (2.47±0.14 cm), while the non-fermented and fermented biofloc treatments showed significantly higher length gains of 4.46±0.15 cm and 5.23±0.11 cm, respectively. These differences in final length and length gain were highly significant ($p<0.01$), highlighting the limited growth potential of goldfish in the control group compared to the biofloc systems.

Table 1. Water quality parameters summarised throughout 8 weeks of rearing Goldfish (*C. auratus*) juveniles in different culture systems. Values are presented as the mean ± standard deviation.

Sl. No.	Parameters	Treatments			P Value
		Control	Non-fermented	Fermented	
1	Temperature (° C)	27.53±0.19^a	27.75±0.23^b	27.74±0.31^b	<0.001
2	pH	7.5±0.06^a	7.74±0.05^a	7.82±0.05^a	<0.001
3	Ammonia (ppm)	0.003±0.004^a	0.51±0.37^b	0.51±0.37^b	<0.001
4	Nitrite (ppm)	0.04±0.004^a	0.06±0.04^a	0.07±0.02^a	0.063
5	Nitrate (ppm)	0.001±0.004^a	0.44±0.37^b	0.45±0.22^b	0.002
6	Alkalinity (ppm)	40.88±1.25^a	149.56±22.7^b	159.63±18.2^b	<0.001
7	DO (ppm)	5.59±0.12^a	4.87±0.46^a	4.99±0.22^b	<0.001
8	Floc Volume (ml/L)	—	18.86±5.36	20.34±6.37	0.185
9	Total Plate Count (cfu) of final day	$5\times10^5\pm1\times10^{5a}$	$2\times10^7\pm0.5\times10^{7b}$	$3\times10^8\pm0.2\times10^{8c}$	<0.001

All values are expressed in Mean ± SD. Different superscript letters in the same row indicate statistical significance at a 1% level

Table 2. Growth Performance summary of Goldfish (*C. auratus*) juveniles reared for 8 weeks in different culture systems. Values are presented as the mean ± standard deviation.

Parameters	CONTROL	NON -FERMENTED	FERMENTED	P value
Initial length (cm)	1.12±0.14^a	1.12±0.13^a	1.12±0.1^a	
Final length (cm)	3.89±0.18^c	5.58±0.19^b	6.35±0.19^a	p<0.01
Length Gain (cm)	2.47±0.14^c	4.46±0.15^b	5.23±0.11^a	p<0.01
Initial Weight (g)	0.12±0.01^a	0.12±0.01^a	0.12±0.01^a	
Final Weight (g)	0.78±0.008^c	1.55±0.055^b	2.12±0.06^a	p<0.01
Weight gain (g)	0.66±0.16^c	1.43±0.04^b	2±0.04^a	p<0.01
SGR3.38 ± 0.02^c	5.06±0.01^b	5.83±0.01^a	p<0.01	
FCR1.59 ± 0.014^a	1.06±0.002^b	0.91±0.004^c	p<0.01	
Survival (%)	60.83±1.15^c	90±1.73^b	91.6^a	p<0.01

All values are expressed in Mean ± SD. Different superscript letters in the same row indicates statistical significance at a 1% level

Similarly, weight gain was also significantly lower in the control group (0.66±0.16 g) compared to the biofloc treatments, with the non-fermented and fermented biofloc systems resulting in 1.43±0.04 g and 2.00±0.04 g of weight gain, respectively. The final weight of goldfish in the control group (0.78±0.008 g) was notably lower than in the biofloc treatments, with the non-fermented and fermented biofloc groups achieving significantly higher final weights (1.55±0.055 g and 2.12±0.06 g, respectively). The differences in final weight and weight gain were highly significant (p<0.01). Specific Growth Rate (SGR) was also lowest in the control group (3.38±0.02), compared to the non-fermented biofloc (5.06±0.01) and fermented biofloc treatments (5.83±0.01). These differences in SGR were highly significant (p<0.01), further indicating the limitations of the control treatment in promoting optimal growth rates.

Feed Conversion Ratio (FCR) was significantly higher (p<0.01) in the control group (1.59±0.014), indicating inefficient feed utilization compared to the non-fermented (1.06±0.002) and fermented biofloc groups (0.91±0.004). Finally, the survival rate of fish in the control group (60.83±1.15%) was significantly lower than those in the biofloc treatments, with survival rates reaching 90±1.73% for non-fermented biofloc and 91.6% for the fermented biofloc.

Analyses of the proximate composition (Table 3) showed no significant difference in carbohydrate and moisture contents of floc meal between the non-fermented and fermented BFT treatments (p>0.05). However, significant differences were observed in the protein, lipid, and ash contents among the treatments (p<0.05).

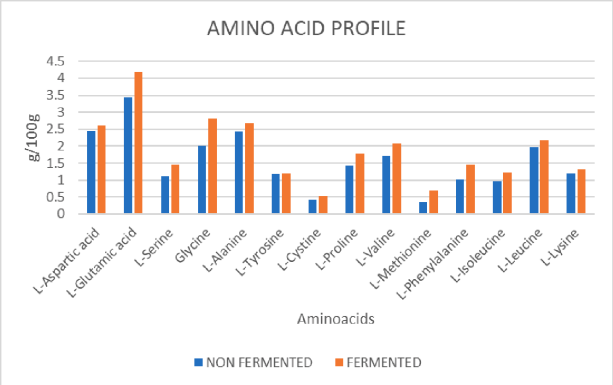

Fig. 1. Amino acid profile of Biofloc meal

Fig. 1 displays the amino acid composition of the biofloc meal for each type of biofloc. Arginine, lysine, histidine, arginine, phenylalanine, valine, leucine, isoleucine, methionine, tryptophan, and threonine are among the essential amino acids, while others are non-essential amino acids. The fermented floc meal showed slightly higher values for these amino acids compared to the non-fermented floc meal.

The present study explores the effect of carbon source fermentation in biofloc production and goldfish culture in biofloc system. For this experiment C:N ratio was maintained at 20:1, which facilitated to take inorganic nitrogen for microbial

protein assimilation. Previous research has shown that, compared to traditional culture systems, biofloc technology enhances growth and survival rates in goldfish (Faizullah et al., 2015; da Cunha et al., 2020; da Cunha et al., 2022). The current study demonstrates that fermented biofloc can improve the nutritional quality of the culture system, positively influencing both the performance of the cultured goldfish and the physico-chemical parameters of the system. Factors such as probiotics, a reduced number of harmful algae, and an increase in zooplankton abundance may all contribute to the advantages of fermenting carbon sources (Silva et al., 2013). While biofloc technology is well known for its effectiveness, little is known about its application for goldfish culture and the effect of fermented carbon sources on performance. Therefore, this study aimed to investigate and compare factors related to water quality, growth, and survival rates of goldfish cultured in fermented and non-fermented biofloc environments. The results indicated that use of fermented biofloc leads to significant improvements in water quality, growth, and survival rates.

In this study, water quality parameters remained within the optimal range for *C. auratus* culture, fostering improved growth and survival, which is consistent with previous studies (Faizullah et al., 2015). No significant differences in water quality parameters were observed for between the fermented and non-fermented biofloc treatments. Average temperature values were consistent across all treatments and aligned with prior research on goldfish culture. pH and alkalinity levels were maintained at higher levels in the biofloc tanks, facilitated by the addition of sodium bicarbonate. Maintaining optimal pH and alkalinity is crucial for the proper functioning of biofloc systems, with ideal alkalinity levels typically recommended to be between 100–150 ppm (Furtado et al., 2014; Zhang, Wang, & Yu, 2017). Variations in pH and alkalinity within the biofloc treatments were likely due to the addition of carbon sources, which can lower pH and promote the nitrification process (Wasave et al., 2020b).

Compared to the control, biofloc treatments exhibited lower dissolved oxygen levels. Similar observations have been reported by several researchers, who noted that biofloc systems generally have reduced dissolved oxygen levels due to the higher oxygen demand of the heterotrophic microbial community (Visscher, Quist, & van Gemerden, 1991; Avnimelech, 1999; Harini, Rajagopalasamy, Kumar, & Santhakumar, 2016). In biofloc technology (BFT), nitrification is directly influenced by dissolved oxygen levels, as nitrifying bacteria are obligate aerobes. Therefore, maintaining adequate oxygen concentration is essential to support these bacterial activity and ensure efficient nitrification (Lekang, 2007). Although, ammonia, nitrite, and nitrate concentrations were slightly elevated in the biofloc treatments compared to the control, they remained within acceptable limits due to proper system management. Effective management practices and the tolerance levels of the cultured organisms play a critical role in mitigating the potential toxicity of these chemicals (Genes et al., 2019). Thus, despite higher nitrogen compound levels in the biofloc systems, they did not negatively impact the health or growth of *C. auratus* under the controlled conditions of this study.

The positive effects of biofloc technology on growth performance observed in this study align with previous research findings across various species, including *Oreochromis niloticus* (Tilapia) (Avnimelech, 2007), *Cyprinus carpio* (common carp) (Dinda, Mandal, & Das, 2020), *Clarias gariepinus* (African catfish) (Putra, Fauzan, & Wulandari, 2017), *Paracheirodon innesi* (Blue morph) (Harini et al.,

Table 3. Proximate composition and amino acid composition of Biofloc meal

Proximate Composition	Non-fermented	Fermented	p-value
Protein	31.3±0.35	36.23±0.25	<0.001
Lipid	3.43±0.03	4.26.0±0.1	<0.001
Carbohydrate	22.83±0.29	23±0.56	0.669
Ash	24.4±0.36	20.97±1.19	0.009
Moisture	18.93±0.82	19.08±0.38	0.784

All values are expressed in Mean ± SD, with statistical significance at a 1% level

2016), and *Carassius auratus* (goldfish) (Faizullah et al., 2015; Wang et al., 2015). In the present study, both biofloc treatments significantly enhanced growth, yielding specific growth rates (SGR) of 5.06±0.01 and 5.83±0.01, alongside lower feed conversion ratios (FCR) of 1.06±0.002 and 0.91±0.004, and improved survival rates of 90.8% and 91.6% for non-fermented and fermented biofloc systems, respectively. These values were notably higher than those recorded in the control group. The fermented biofloc treatment, in particular, demonstrated significantly superior growth performance, corroborating findings from earlier studies (Suresh & Lin, 1992; Faizullah et al., 2015; Harini et al., 2016). Previous studies have also reported that fermentation enhances the nutritional quality of biofloc, compared to non-fermented biofloc (Hapsari, 2016; Wasave et al., 2020a). The improved performance observed in the fermentated biofloc can likely be contributed to this enhanced floc quality, reinforcing the potential benefits of incorporating fermented biofloc systems into aquaculture practices.

The proximate composition of biofloc is influenced by various factors, including the carbon source used, the carbon-nitrogen (C/N) ratio of the culture water (Xu & Pan, 2014), the concentration of bacteria and phytoplankton, as well as the age and composition of biofloc aggregates (Kuhn, Boardman, Craig, Flick, & McLean, 2008; Avnimelech, 2015). In the present study, fermented biofloc exhibited significantly higher protein levels (36.23±0.25%) compared to non-fermented biofloc (31.2±0.35%). Fermentation was carried out using yeast (*Saccharomyces cerevisiae*) to ferment the carbon source, which in this case was jaggery. Previous studies have demonstrated that inclusion of yeast in supplemental diets can improve both water quality and the growth performance of various cultivated species (Abdel-Tawwab, Abdel-Rahman, & Ismael, 2008). Similarly, Supriyati, Angkawijaya, and Wibowo (2015) reported that the fermentation of carbon sources significantly increased the crude protein content of biofloc.

Regarding lipid content, the fermented biofloc showed higher lipid levels (4.26±0.1%) than the non-fermented biofloc (3.43±0.03%). These findings are consistent with the research of Hende, Vervaeren, and Boon (2012), who observed microbial lipid accumulation in biofloc as a result of an abundance of carbon and reduced nitrogen availability. Ekasari et al. (2014) also reported enhanced lipid content in biofloc systems that utilized organic carbon sources such as jaggery, rice bran, and molasses, thereby increasing the nutritional value of the biofloc for aquaculture species. Lipids are essential for the structural and metabolic processes of microorganisms, serving as a source of energy storage, supporting metabolism, and maintaining cell membrane integrity (Avnimelech, 2009). The carbohydrate content in fermented biofloc (23±0.56%) was slightly higher than that in non-fermented biofloc (22.83±0.29%), although no significant differences were observed between the two treatments. These findings are consistent with previous studies by Emerenciano et al. (2014). Additionally, the ash content of fermented biofloc was lower (20.97±1.19%) than that of non-fermented biofloc (24.4±0.36%), which follows a trend similar to that reported by Ju et al. (2008) & Maicá, de Borba, and Wasielesky (2012).

Biofloc is rich in essential amino acids (EAA), and the findings of the present study align with previous reports on the overall EAA and NEAA content in biofloc, which were reported to range from 11.69–24.49 g/100g dry sample and 13.21–24.61 g/100g dry sample, respectively (Moreno-Arias et al., 2018). These results further support the nutritional value of biofloc as an important source of amino acids for aquaculture species.

In conclusion, the present study highlights that both fermented and non-fermented biofloc systems positively influence the growth performance, survival, and nutritional profile of goldfish, with the fermented biofloc system yielding significantly superior outcomes. The fermentation process enhanced the protein and lipid content of the biofloc, contributing to improved growth parameters such as specific growth rate (SGR), feed conversion ratio (FCR), and survival rates. While water quality parameters remained within acceptable limits, slight variations were observed in dissolved oxygen, ammonia, nitrite, and nitrate levels, with biofloc systems maintaining a balanced environment through proper management. The nutritional composition of fermented biofloc, particularly its higher protein and lipid content, underlines its potential as a more efficient and beneficial nutritional resource in aquaculture. These findings highlight the positive impact of biofloc technology, particularly when incorporating fermented carbon sources, on aquaculture practices, offering a sustainable and nutritionally enriched alternative to conventional aquaculture systems.

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References

Abdel-Tawwab, M., Abdel-Rahman, A. M., & Ismael, N. E. M. (2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for Fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. *Aquaculture, 280*(1-4), 185-189. https://doi.org/10.1016/j.aquaculture.2008.03.055.

AOAC. (2012). *Official methods of analysis* (19th ed.). AOAC International, Gaithersburg, MD.

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed.). American Public Health Association, Washington DC.

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture, 176*(3-4), 227-235. https://doi.org/10.1016/S0044-8486(99)00085-X.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture, 264*(1-4), 140-147. https://doi.org/10.1016/j.aquaculture.2006.11.025.

Avnimelech, Y. (2009). *Biofloc technology: A practical guide book* (1st ed.). World Aquaculture Society, Baton Rouge.

Avnimelech, Y., & Kochba, M. (2009). Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using ^{15}N tracing. *Aquaculture, 287*(1-2), 163-168. https://doi.org/10.1016/j.aquaculture.2008.10.009.

Avnimelech, Y. (2015). *Biofloc technology: A practical guide book* (3rd ed.). World Aquaculture Society, Baton Rouge.

Besen, K. P., da Cunha, L., Delziovo, F. R., Melim, E. W. H., Cipriani, L. A., Gomes, R., Skoronski, E., & Fabregat, T. H. P. (2021). Goldfish (*Carassius auratus*) larviculture in biofloc systems: Level of *Artemia* nauplii, stocking density and concentration of the bioflocs. *Aquaculture, 540*, Article 736738. https://doi.org/10.1016/j.aquaculture.2021.736738.

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H., & Pearson, D. C. (2004). The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high intensity, zero-exchange system. *Aquaculture, 232*(1-2), 525-537. https://doi.org/10.1016/S0044-8486(03)00541-6.

Chen, J. Y., Zeng, C., Jerry, D. R., & Cobcroft, J. M. (2020). Recent advances of marine ornamental fish larviculture: Broodstock reproduction, live prey and feeding regimes, and comparison between demersal and pelagic spawners. *Reviews in Aquaculture, 12*(3), 1518-1541. https://doi.org/10.1111/raq.12394.

Crab, R., Chielens, B., Wille, M., Bossier, P., & Verstraete, W. (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research, 41*(4), 559-567. https://doi.org/10.1111/j.1365-2109.2009.02353.x.

da Cunha, L., Besen, K. P., de Oliveira, N. S., Delziovo, F. R., Gomes, R., da Cruz, J. M., Picoli, F., Gisbert, E., Skoronski, E., & Fabregat, T. H. P. (2022). Fermented soybean meal can partially replace fishmeal and improve the intestinal condition of goldfish juveniles reared in a biofloc system. *Aquaculture Research, 53*(18), 6803-6815. https://doi.org/10.1111/are.16147.

da Cunha, L., Besen, K. P., Ha, N., Uczay, J., Skoronski, E., & Fabregat, T. H. P. (2020). Biofloc technology (BFT) improves skin pigmentation of goldfish (*Carassius auratus*). *Aquaculture, 522*, Article 735132. https://doi.org/10.1016/j.aquaculture.2020.735132.

Das, M., Sarma, S., & Das, A. K. (2005). Status of ornamental fishes of Assam. *Fishing Chimes, 25*(3), 13-15.

Decamp, O., Moriarty, D. J. W., & Lavens, P. (2008). Probiotics for shrimp larviculture: review of field data from Asia and Latin America. *Aquaculture Research, 39*(4), 334-338. https://doi.org/10.1111/j.1365-2109.2007.01664.x.

Deocampo, J. E., Fenol, J. T., Jimenez, A. G. M., Paguntalan, G. S., & Caipang, C. M. A. (2022). Production of ornamental fish in a biofloc-based system using sweetpotato (*Ipomoea batatas*) waste as carbon source. *IOP Conference Series: Earth and Environmental Science, 1118*, Article 012017. https://doi.org/10.1088/1755-1315/1118/1/012017.

de Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture, 277*(3–4), 125-137. https://doi.org/10.1016/j.aquaculture.2008.02.019.

Dinda, R., Mandal, A., & Das, S. K. (2020). Neem (*Azadirachta indica* A. Juss) supplemented biofloc medium as alternative feed in common carp (*Cyprinus carpio* var. communis Linnaeus) culture. *Journal of Applied Aquaculture, 32*(4), 361-379. https://doi.org/10.1080/10454438.2019.1645076.

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of ammonia nitrogen in aquaculture production systems.

Aquaculture, *257*(1–4), 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.

Ekasari, J., Azhar, M. H., Surawidjaja, E. H., Nuryati, S., de Schryver, P., & Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish & Shellfish Immunology*, *41*(2), 332-339. https://doi.org/10.1016/j.fsi.2014.09.004.

Emerenciano, M., Cuzon, G., Arévalo, M., & Gaxiola, G. (2014). Biofloc technology in intensive broodstock farming of the pink shrimp *Farfantepenaeus duorarum*: spawning performance, biochemical composition and fatty acid profile of eggs. *Aquaculture Research, 45*(10), 1713-1726. https://doi.org/10.1111/are.12117.

Faizullah, M., Rajagopalasamy, C. B. T., Ahilan, B., & Francis, T. (2015). Impact of biofloc technology on the growth of goldfish young ones. *Indian Journal of Science and Technology*, *8*(13), Article 49. https://doi.org/10.17485/ijst/2015/v8i13/54060.

Furtado, R. N., Carneiro, M. S. S., Candido, M. J. D., Gomes, F. H. T., Rogerio, M. C. P., & da Silva, D. S. (2014). Nitrogen balance and ruminal assessment in male and female sheep fed rations containing castor cake under different treatments. *Semina-Ciencias Agrarias, 35*(6), 3237-3247. https://doi.org/10.5433/1679-0359.2014v35n6p3237.

Genes, J. E. A., Buelvas, V. M. P., Velásquez, C. A. J., Araujo, J. A. E., Atencio-García, V. J., & Prieto-Guevara, M. J. (2019). Planktonic and bacterial communities associated with the cultivation of bocachico Prochilodus magdalenae with biofloc technology. *MVZ Córdoba Magazine*, *24*(2), 7209-7217. https://doi.org/10.21897/rmvz.1648.

Hapsari, F. (2016). The effect of fermented and non-fermented biofloc inoculated with bacterium *Bacillus cereus* for catfish (*Clarias gariepinus*) juveniles. *Aquaculture, Aquarium, Conservation & Legislation, 9*(2), 334-339.

Hargreaves, J. A. (2013). Biofloc production systems for aquaculture. *Southern Regional Aquaculture Center Publication, 4503*, 1-12.

Hari, B., Kurup, B. M., Varghese, J. T., Schrama, J. W., & Verdegem, M. C. J. (2004). Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture*, *241*(1–4), 179–194. https://doi.org/10.1016/j.aquaculture.2004.07.002.

Harini, C., Rajagopalasamy, C. B. T., Kumar, J. S. S., & Santhakumar, R. (2016). Role of biofloc in the growth and survival of blue morph, *Pseudotropheus saulosi*. *Indian Journal of Science and Technology, 9*(8). https://doi.org/10.17485/ijst/2016/v9i8/75237.

Hende, S. D., Vervaeren, H., & Boon, N. (2012). Flue gas compounds and microalgae: (Bio-)chemical interactions leading to biotechnological opportunities. *Biotechnology Advances*, *30*(6), 1405–1424. https://doi.org/10.1016/j.biotechadv.2012.02.015.

Hoseinifar, S. H., Maradonna, F., Faheem, M., Harikrishnan, R., Devi, G., Ringø, E., van Doan, H., Ashouri, G., Gioacchini, G., & Carnevali, O. (2023). Sustainable ornamental fish aquaculture: The implication of microbial feed additives. *Animals*, *13*(10), Article 1583. https://doi.org/10.3390/ani13101583.

Jackqulinwino, A., Ahilan, B., Chidambaram, P., Uma, A., Cheryl, A., & Ruby, P. (2024). Effect of jaggery-based biofloc meal supplementation on growth, digestive enzymes, and disease resistance in *Penaeus vannamei* in a recirculatory aquaculture system. *Animal Nutrition and Feed Technology*, *24*(1), 105-119. http://dx.doi.org/10.5958/0974-181X.2024.00008.8.

Jayasankar, P. (1998). Ornamental fish culture and trade: Current status and prospects. *Fishing Chimes, 17*(12), 9-13.

Ju, Z. Y., Forster, I., Conquest, L., & Dominy, W. (2008). Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. *Aquaculture Nutrition, 14*(6), 533-543. https://doi.org/10.1111/j.1365-2095.2007.00559.x.

Khanjani, M. H., Sajjadi, M. M., Alizadeh, M., & Sourinejad, I. (2017). Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. *Aquaculture Research, 48*(4), 1491- 1501. https://doi.org/10.1111/are.12985.

Kuhn, D. D., Boardman, G. D., Craig, S. R., Flick, G. J., & McLean, E. (2008). Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, *Litopenaeus vannamei*, in recirculating aquaculture systems. *Journal of Aquaculture Society, 39*(1), 72-82. https://doi.org/10.1111/j.1749-7345.2007.00145.x.

Kurup, B. M., Premlal, P., Thomas, J. V., & Anand, V. (2003). Bottom trawl discards along Kerala coast: A case study. *Journal of the Marine Biological Association of India, 45*(1), 99-107.

Lekang, O. I. (2007). *Aquaculture Engineering*. John Wiley & Sons Ltd., Iowa.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment, 473-474*, 619-641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

Mahanand, S. S., Moulick, S., & Rao, P. S. (2013). Water quality and growth of Rohu, *Labeo rohita*, in a biofloc system. *Journal of Applied Aquaculture, 25*(2), 121–131. https://doi.org/10.1080/10454438.2013.788898.

Maicá, P. F., de Borba, M. R., & Wasielesky, W. (2012). Effect of low salinity on microbial floc composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared in a zero-water-exchange super-intensive system. *Aquaculture Research, 43*(2), 361-370. https://doi.org/10.1111/j.1365-2109.2011.02838.x.

Megahed, M. E. (2010). The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. *Journal of the Arabian Aquaculture Society, 5(2)*, 119–142.

Minabi, K., Sourinejad, I., Alizadeh, M., Ghatrami, E. R., & Khanjani, M. H. (2020). Effects of different carbon to nitrogen ratios in the biofloc system on water quality, growth, and body composition of common carp (*Cyprinus carpio* L.) fingerlings. *Aquaculture International, 28*, 1883–1898. https://doi.org/10.1007/s10499-020-00564-7.

Moreno-Arias, A., López-Elías, J. A., Martínez-Córdova, L. R., Ramírez-Suárez, J. C., Carvallo-Ruiz, M. G., García-Sánchez, G., Lugo-Sánchez, M. E., & Miranda-Baeza, A. (2018). Effect of fishmeal replacement with a vegetable protein mixture on the amino acid and fatty acid profiles of diets, biofloc and shrimp cultured in BFT system. *Aquaculture, 483*, 53-62. https://doi.org/10.1016/j.aquaculture.2017.10.011.

Nayar, M. P. (1996). *Hotspots of Endemic Plants of India, Nepal and Bhutan*. Tropical Botanic Garden Research Institute, Trivandrum, India.

Najdegerami, E. H., Ngoc-Tran, T., Defoirdt, T., Marzorati, M., Sorgeloos, P., Boon, N., & Bossier, P. (2012). Effects of poly-â-hydroxybutyrate (PHB) on Siberian sturgeon (*Acipenser baerii*) fingerlings performance and its gastrointestinal tract microbial community. *FEMS Microbiology Ecology*, *79*(1), 25-33. https://doi.org/10.1111/j.1574-6941.2011.01194.x.

Pérez-Fuentes, J. A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2013). Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. *Aquaculture, 400-401*, 105-110. https://doi.org/10.1016/j.aquaculture.2013.02.028.

Pleeging, C. C. F., & Moons, C. P. H. (2017). Potential welfare issues of the Siamese fighting fish (*Betta splendens*) at the retailer and in the hobbyist aquarium. Vlaams Diergeneeskundig Tijdschrift, *86*, 213-223. https://doi.org/10.21825/vdt.v86i4.16182.

Putra, I., Rusliadi, R., Fauzi, M., Tang, U. M., & Muchlisin, Z. (2017). Growth performance and feed utilization of African catfish *Clarias gariepinus* fed a commercial diet and reared in the biofloc system enhanced with probiotic. *F1000 Research, 22*(6), Article 1545. https://doi.org/10.12688/f1000research.12438.1.

Richards, F. A., & Kletsch, R. A. (1964). The spectrophotometric determination of ammonia and labile amino compounds in fresh and sea water by oxidation to nitrite. In Y. Miyake, & T. Koyama (Eds.), *Recent Researches in the Field of Hydrosphere, Atmosphere and Nuclear Geochemistry* (pp. 65-81). Maruzen, Tokyo.

Sakkaravarthi, K., & Sankar, G. (2015). Identification of effective organic carbon for biofloc shrimp culture system. *Journal of Biological Sciences, 15*(3), 144-149.

Samocha, T. M., Patnaik, S., Speed, M., Ali, A. M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A., & Brock, D. L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering*, *36*(2), 184-191. https://doi.org/10.1016/j.aquaeng.2006.10.004.

Satam, S. B., Sawant, N. H., Ghughuskar, M. M., Sahastrabuddhe, V. D., Pagarkar, A. U., Chogale, N. D., Metar, S. Y., Sadawarte, V. R., Sawant, A. N., Singh, H., Sawant, P. A., Patil, V. K., Rane, A. D., Haldankar, P. M., & Bhattacharyya, T. (2018). Ornamental fisheries: A new avenue to supplement farm income. *Advanced Agricultural Research & Technology Journal, 2*(2), 193-197.

Schneider, O., Sereti, V., Eding, E. H., & Verreth, J. A. J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. *Aquaculture Engineering, 32*(3-4), 379–401. https://doi.org/10.1016/j.aquaeng.2004.09.001.

Silva, C. F., Vilela, D. M., Cordeiro, C. S., Duarte, W. F., Dias, D. R., & Schwan, R. F. (2013). Evaluation of a potential starter culture for enhanced quality of coffee fermentation. *World Journal of Microbiology and Biotechnology, 29*, 235–247. https://doi.org/10.1007/s11274-012-1175-2.

Singh, T., & Dey, V. K. (2003). Ornamental fish trade runs into billions. *Infofish International,* 22(5), 55-60.

Straits Research. (2024). *Ornamental fish market share, trends, forecast analysis report 2032*. Retrieved from https://straitsresearch.com/report/ornamental-fish-market.

Supriyati, E., Haryati, T., Susanti, T., & Susana, W. R. (2015). Nutritional value of rice bran fermented by *Bacillus amyloliquefaciens* and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian-Australasian Journal of Animal Sciences, 28*(2), 231-238. https://doi.org/10.5713/ajas.14.0039.

Suresh, A. V., & Lin, C. K. (1992). Effect of stocking density on water quality and production of red tilapia in a recirculated water system. *Aquacultural Engineering, 11*(1), 1–22.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., & Sloman, K. A. (2019). The use of feed and water additives for live fish transport. *Reviews in Aquaculture, 11*(1), 263–278. https://doi.org/10.1111/raq.12239.

Visscher, P. T., Quist, P., & van Gemerden, H. (1991). Methylated sulfur compounds in microbial mats: In situ concentrations and metabolism by a colorless sulfur bacterium. *Applied and Environmental Microbiology, 57*, 1758–1763. https://doi.org/10.1128/aem.57.6.1758-1763.1991.

Wang, G.., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L., & Zheng, Z. (2015). Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, C*arassius auratus*. *Aquaculture, 443*, 98-104. https://doi.org/10.1016/j.aquaculture.2015.03.015.

Wasielesky, W., Atwood, H., Stokes, A., & Browdy, C. L. (2006). Effect of natural production in a zero exchange suspended microbial floc-based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture, 258*(1-4), 396-403. https://doi.org/10.1016/j.aquaculture.2006.04.030.

Wasave, S., Chavan, B. R., Pawase, A. S., Shirdhankar, M., Mohite, S., Pai, R., & Wasave, S. (2020a). Effect of carbon sources and water requirement in rearing of genetically improved farmed tilapia (*Oreochromis niloticus*) fry in biofloc system. *Journal of Entomology and Zoology Studies, 8*(5), 1361–1365.

Wasave, S. S., Chavan, B. R., Pawase, A. S., Shirdhankar, M. M., Mohite, A. S., Pai, R., Wassave, S. M., & Naik, S. D. (2020b). Growth performance of GIFT tilapia (*Oreochromis niloticus*) fry in a biofloc system. *International Journal of Fisheries and Aquatic Studies, 8*(4), 206-211.

Xu, W. J., & Pan, L. Q. (2014). Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. *Aquaculture, 426-427*, 181–188. https://doi.org/10.1016/j.aquaculture.2014.02.003.

Yusuf, M., Elfghi, F. M., Zaidi, S. A., Abdullah, E. C., & Khan, M. A. (2015). Applications of graphene and its derivatives as an adsorbent for heavy metal and dye removal: A systematic and comprehensive overview. *RSC Advances, 5*(5), 50392–50420. https://doi.org/10.1039/C5RA07223A.

Zhang, S., Wang, D., & Yu, S. (2017). Precise large deviations of aggregate claims in a size-dependent renewal risk model with stopping time claim-number process. *Journal of Inequalities and Applications, 2017*, 43-49. https://doi.org/10.1186/s13660-017-1364-5.