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Original Article

Optimising feed management for green tiger shrimp (*Penaeus semisulcatus*) in a biofloc system: impacts on growth performance, body composition and antioxidant activities

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Abstract

This study conducted over a 45-day period examined how different feeding strategies affect green tiger shrimp (*Penaeus semisulcatus*) development in biofloc aquaculture systems. The investigation focused on juvenile shrimp with an initial mean weight 2.85 ± 0.22 g, distributed across various experimental conditions. Five distinct treatment groups were considered, each consisting of tanks with a volume of 150 L of filtered seawater. Each tank was stocked with 53 shrimp, maintaining a stocking density of ~1 g L^{-1}. Four groups utilised biofloc technology (BFT) with varying feeding levels: 0% (BFT0), 2% (BFT2), 4% (BFT4) and 6% (BFT6) of body weight per day. One additional group served as control, using a clear water system with feeding level of 6% (CW6) body weight daily. The BFT systems successfully maintained optimal water parameters despite minimal water exchange requirements. Performance metrics indicated that shrimp in the BFT4 and BFT6 groups achieved higher biomass production and survival rates. Biochemical analyses demonstrated enhanced nutritional profiles (protein, lipid and ash) in BFT-treated specimens. Antioxidant enzyme activity was significantly elevated in BFT systems, indicating improved overall health status. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were significantly higher in BFT groups than control. These findings suggest that feeding juvenile green tiger shrimp at 4% body weight within a BFT system represents an optimal strategy, achieving balanced growth performance while maintaining superior water quality and enhanced animal health indicators without compromising survival rates or body composition.

Keywords: antioxidant activity; biofloc system, feeding levels; green tiger shrimp; growth performance

1 | INTRODUCTION

The rapid growth of aquaculture is crucial for meeting the rising global demand for aquatic foods (FAO 2024). Aquaculture has the potential to play a significant role in enhancing food security; therefore, it must prioritise sustainability in its future developments to ensure long-term viability and minimize environmental impacts (Keshavarzifard *et al.* 2020; Emerenciano *et al.* 2025; Haque and Mahmud 2025;). This commitment to sustainability involves not only the efficient use of resources but also the adoption of practices that protect aquatic ecosystems and promote the health of cultured species (Khanjani 2025).

Crustacean production has seen remarkable growth, accounting for 24.6% of the total increase in aquaculture output. In comparison, finfish contributed 58.1%, while molluscs represented 15.6% (FAO 2022). Notably, crustacean production reached approximately 23.11 million tons in 2022, which constitutes 2.9% of global aquaculture production. This growth highlights the increasing relevance of shrimp farming within the broader context of aquaculture, as it plays a critical role in ensuring global food security and improving production efficiency (Sharifinia *et al.* 2025). The significant rise in crustacean production not only reflects the industry's evolution but also highlights the necessity for sustainable practices in shrimp farming to meet the rising demand and enhance the overall effectiveness of aquaculture (FAO 2024).

The Penaeidae family, which includes economically significant shrimp species, plays a crucial role in the aquaculture sector. Among these, the green tiger shrimp (*Penaeus semisulcatus*) stands out as a prominent species in the Persian Gulf and the Sea of Oman. This shrimp is characterised by its extensive distribution, spanning from the Indian Ocean to the Red Sea, and is recognised as a key commercial species in the Persian Gulf region. The high market demand for *P. semisulcatus* not only supports local fisheries but also provides substantial income for fishers involved in its capture and aquaculture (Kaya *et al.* 2020; Mohammad Moradi *et al.* 2023).

Understanding the feeding behaviour of *P. semisulcatus* is essential for optimising its cultivation. Research indicates that this species primarily consumes a diverse diet, including benthic invertebrates, plankton and detritus (Al-Maslamani *et al.* 2007; Mohammad Moradi *et al.* 2023). This opportunistic feeding strategy underscores the importance of providing a balanced diet that meets the nutritional requirements of shrimp in aquaculture settings. Notably, feed costs constitute a significant portion of total aquaculture expenses, accounting for 40 – 50% (Rather *et al.* 2024). This substantial expenditure emphasizes the necessity for innovative technologies that can minimize feed waste and ultimately reduce overall production costs. In this context, the implementation of biofloc technology (BFT) emerges as a compelling solution. BFT not only enhances feed efficiency by promoting the growth of beneficial microorganisms that can be utilised as a supplementary food source but also contributes to a more sustainable aquaculture system. By adopting BFT, aquaculture operations can effectively address the challenges posed by high feed costs while improving productivity and environmental sustainability (Emerenciano *et al.* 2025).

One such innovation is BFT, which has emerged as a promising approach in shrimp farming (Khanjani *et al.* 2025a). BFT is characterised by limited water exchange, which helps maintain water quality while promoting the growth of microbial biomass (Khanjani *et al.* 2024a). This microbial biomass can serve as a supplementary food source for cultured species, thereby decreasing reliance on traditional feed inputs (Khanjani and Alizadeh 2024). The application of BFT in shrimp aquaculture has gained popularity in recent years, with studies demonstrating its effectiveness in enhancing growth and reducing feed costs across various species, including *Penaeus vannamei* (Addo *et al.* 2023), *P. monodon* (Anand *et al.* 2014), *Fenneropenaeus merguiensis* (Khanjani and Sharifinia 2022), *F. indicus* (Effendy *et al.* 2016), *Metapenaeus monoceros* (Kaya *et al.* 2019), *P. aztecus* (Kaya 2025) and *P. semisulcatus* (Kaya *et al.* 2020). Specifically, Megahed (2010) highlighted that BFT could mitigate feed costs while improving water quality in intensive farming systems for *P. semisulcatus*. Implementing BFT for this species can improve cultivation efficiency in land-based production systems (Kaya *et al.* 2020).

An effective feeding strategy is essential in shrimp farming, as it promotes growth through optimal feed utilization while minimising environmental impact (Sharifinia *et al.* 2023a, 2023b; Sharifinia 2025). Understanding the optimal feeding rate is crucial for maximising yield per unit area and ensuring profitable shrimp culture. Although BFT systems offer nutritional benefits, quantifying these contributions can be challenging due to the variability in nutrient profiles based on the carbon sources used (Khanjani and Sharifinia 2024). Current practices in industrial shrimp farming often rely on predetermined feeding schedules based on expected growth patterns, rather than directly monitoring the animals' needs (Ullman *et al.* 2017).

Shrimp exhibit distinct feeding characteristics that significantly influence aquaculture operations. Their opportunistic feeding behaviour, along with external handling of feed before ingestion and substrate-based feeding preferences, results in prolonged feed exposure in water systems (Garlock *et al.* 2020). This biological trait introduces important considerations for BFT systems, where different feeding strategies can have cascading effects throughout the entire cultivation process. Understanding these dynamics is crucial for optimizing feeding practices and enhancing overall shrimp production efficiency.

The differences between the current study and Kaya *et al.* (2020) study is in the initial weight of shrimp, rearing period, stocking density, volume of culture, carbon to nitrogen ratio, percentage of feeding to body weight, and carbon source used in the BFT system, which can affect the system performance. The present study aims to investigate the effects of varying feeding levels on water quality, growth performance, body composition, and antioxidant enzyme activities in the hepatopancreas of *Penaeus semisulcatus* within a BFT system.

By exploring these aspects, this research seeks to provide valuable insights into effective feed management strategies that can enhance the sustainability and productivity of shrimp aquaculture. Specifically, the study will assess how different feeding levels impact the growth rates of *P. semisulcatus*, identifying optimal feeding strategies that maximize biomass production while minimizing feed costs. Additionally, it will analyse the body composition of shrimp under various feeding regimes, focusing on key nutritional parameters such as protein, lipid and moisture content, which are critical for determining both the market value and nutritional quality of the shrimp. This investigation goes beyond basic growth metrics to examine the intricate physiological mechanisms that govern shrimp health. By focusing on antioxidant enzyme activity within the hepatopancreas-a vital organ responsible for digestion, metabolism, and immune function-the research aims to elucidate how different feeding regimens influence the biological stress management systems of *P. semisulcatus*.

2 | METHODOLOGY

2.1 Experimental design and conditions

A comprehensive aquaculture study was conducted at the Kolahi marine shrimp facility in Iran's Hormozgan province, spanning over 45 days in June and August 2024. The research utilised juvenile green tiger shrimp (*P. semisulcatus*) with carefully controlled initial specifications: mean weight of 2.85 ± 0.22 g. Prior to experimentation, specimens underwent acclimatisation in 150 m^2 ponds at a dedicated breeding centre in Jask. For the present experiment, the effect of different feeding levels on green tiger shrimp performance was investigated in two clear water and biofloc systems. The experimental design incorporated 5 distinct treatment groups in 3 repetitions. The experimental setup consisted of 15 specially designed polyethylene tanks, featuring a graduated diameter configuration (70 cm base, 80 cm top and 60 cm height) with 0.38 m^2 of bottom area. Each tank was filled with 150 L of filtered seawater and stocked with 53 shrimp, maintaining a precise density of ~1 g L^{-1}. Control group included CW6 (6% body weight feeding) while BFT treatments comprised BFT6 (6% body weight feeding), BFT4 (4% body weight feeding), BFT2 (2% body weight feeding) and BFT0 (no commercial feed feeding) (Table 1). Water management protocols differed significantly between treatment types, with control group receiving substantial daily water exchanges (35 – 50%) and BFT treatments maintaining minimal water exchange (0.5 – 1% daily); biofloc-based tanks received initial inoculation (0.5 mL biofloc per liter). Feeding schedules were standardised across all groups, with three daily feedings at fixed intervals (08:00, 14:00 and 20:00) using a uniform diet composition containing 38% protein, 9 % lipid and 14 % ash.

Preparation of the BFT system began well in advance of the main experiment, utilizing three large-scale vessels with a 2000 L capacity. For the BFT0 treatment, biofloc was filtered daily using a mesh with 20-micron openings, with a quantity of 5 to 10 mL being added to the culture tanks from the biofloc production tanks. The inoculum development process was initiated 20 days prior to the commencement of feeding trials. To establish optimal microbial growth conditions, starch was selected as the primary carbon source, chosen for its high dry matter content (90.18%) and substantial carbohydrate concentration (98.90%). Following established principles of microbial cultivation, careful attention was paid to achieving a specific carbon-to-nitrogen balance of 12:1, calculated based on nitrogen availability from feed sources (Avnimelech 2012). Throughout the experimental period, environmental conditions were precisely controlled within an indoor facility, maintaining a consistent day-night cycle of 12-hour duration.

Water parameters were closely observed and kept within designated ranges. The initial water quality conditions were as follows: temperature ranged from 30 to 31°C, pH was 8.1, dissolved oxygen measured 6.4 mg L^{-1}, and salinity was 32 ppt. All tanks were aerated and continuously stirred using three air stones linked to an air pump, operating 24-hour a day throughout the experiment to enhance biofloculation.

TABLE 1 Experimental treatments and different feeding levels provided for test days, feeding levels based on body weight (%) and water exchange (WE) for different groups of green tiger shrimp.

No.	No.	Rep.	WE (daily)	1–10 days	11–20 days	21–30 days	31–45 days
Different feeding levels base on body weight (BW %)	CW6	3	35- 50% WE	6	5.5	5	4.5
	BFT6	3	0.5-1% WE	6	5.5	5	4.5
	BFT4	3	0.5-1% WE	4	3.66	3.33	3
	BFT2	3	0.5-1% WE	2	1.83	1.66	1.5
	BFT0	3	0.5-1% WE	Only biofloc, 5–10 ml L^{-1} daily			

biofloc technology; CW, clear water; BFT, Rep., repetition; WE, water exchange

2.2 Water quality assessment

Comprehensive water quality monitoring was implemented through a structured measurement protocol. Temperature readings were taken using digital thermometers, while pH levels were assessed with a Lutron 208 pH meter, and dissolved oxygen measurements were captured using a Lutron 5510 oxygen meter. These parameters were evaluated during two distinct timeframes daily: morning sessions between 08:00 – 09:00 and afternoon measurements from 16:00 – 17:00. Daily salinity assessments were conducted at 09:00 using refractometry techniques.

Water transparency, settleable solids (SS) and total suspended solids (TSS) were evaluated every five days. Water transparency was measured using a Secchi disk apparatus, while SS content was determined by collecting one-litre water samples in imhoff cones and allowing settling for twenty minutes. TSS analysis involved filtering 100 mL tank water samples through Whatman No. 42 filter paper, followed by drying at 105°C for three hours prior to weight-based calculations. The analysis of inorganic nitrogenous compounds was conducted on a near-weekly basis, adhering to the standardised protocols (APHA 2012). Water samples from each tank were filtered through Whatman filter papers to facilitate measurement of key nitrogen compounds, including total ammonia nitrogen (TAN), nitrite (NO_2–N) and nitrate (NO_3–N).

2.3 Growth indices
Throughout the experimental period, comprehensive growth assessments were conducted to evaluate shrimp performance metrics. Initial measurements established baseline values for body weight gain, body weight index, growth rate, biomass gain, specific growth rate and feed conversion ratio. These parameters were systematically monitored on a weekly basis to track developmental progress. Population counts were documented at both the commencement and conclusion of the study to determine survival rates accurately. The following formulas were used for calculations (after Khanjani *et al.* 2025a).

Body weight gain (BWG) (g) = final weight – initial weight

Growth rate (GR) (g/day) = ((final weight – initial weight) / days of experiment)

Biomass gain (BG) (g) = (final weight – initial weight) × survival rate × number of shrimp

Survival rate (SR) (%) = 100 × (final number of shrimp at end of trial period / initial number of shrimp)

Specific growth rate (SGR) (%/day) = 100 × ((ln final weight – ln initial weight)) / days of experiment

Feed conversion ratio (FCR) = feed consumed (dry weight) / live weight gain (wet weight)

2.4 Biofloc collection and chemical analysis
Following the completion of the experimental phase, a detailed characterisation of the biofloc was conducted. Sample collection involved filtering the treatment water through a 20-micrometer mesh screen to isolate the biofloc material. The isolated samples underwent standardized preparation procedures, beginning with a 24-hour desiccation at 105°C, followed by storage at –18°C for preservation. The analysis of chemical composition included four primary metrics: dry matter content, crude protein concentration, crude lipid percentage, and ash content determination. All measurements were performed according to the standardised protocols (AOAC 2012).

2.5 Proximate analysis
At the end of the experimental period, body composition analysis was performed on a representative sample of twenty shrimp from each replicate treatment. The specimens were initially processed by removing the exoskeleton and head, followed by thorough homogenization using a meat grinder. The processed samples were then preserved at –18°C until analysis.

The analytical protocol adhered to the standardized methods established by the Association of Official Analytical Chemists (AOAC 2012) and incorporated multiple complementary techniques. Moisture content was determined by drying samples in porcelain crucibles at 105°C until a constant weight was achieved. Protein content analysis employed the Kjeldahl method for nitrogen estimation, with crude protein calculated by multiplying the nitrogen percentage by 6.25. Lipid extraction was conducted using a Soxhlet apparatus with ether as the solvent, while ash content was determined through incineration in a muffle furnace at 600°C for three hours.

2.6 Hepatopancreas biochemical analysis
Prior to tissue collection, the shrimp underwent a 24-hour fasting period. Specimens were then randomly selected from each replicate (15 per group) and treated humanely according to established protocols (Fernandes and Pedroso 2017). Anesthesia was administered using clove oil at a concentration of 100 ppm, followed by euthanasia in accordance with ethical guidelines by Luedeman and Lightner (1992). The hepatopancreas was carefully isolated on ice, rinsed with a saline solution (0.8% NaCl), and preserved at –80°C for subsequent biochemical analysis.

For enzyme extraction, the frozen hepatopancreas samples were thawed and homogenised in a hydrochloric acid-tris buffer (pH 7.4) at 4°C. The homogenate was then centrifuged at 4000 rpm for 10 minutes at 4°C and the resulting supernatant was collected for further biochemical evaluation (Yang *et al.* 2010).

Antioxidant enzyme activities were quantified using commercial kits from ZellBio GmbH (Germany), following the manufacturer's specified protocols. The analysis included measurements of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Additional enzyme assessments were conducted for alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using kits from Pars Azmun Co. (Tehran, Iran), adhering to established methodologies (Burtis *et al.* 2007).

2.7 Data analysis

Statistical analysis was performed using SPSS version 23.0 software (Chicago, Illinois, USA). The data were evaluated using one-way analysis of variance (ANOVA) to identify significant differences between treatment groups. Post-hoc analysis was conducted using Duncan's multiple range test to compare mean values. Results are presented as mean ± standard deviation, with statistical significance determined at $p < 0.05$. All visual representations were generated using Excel version 2016.

3 | RESULTS

3.1 Water quality

The results of water quality parameters in the green tiger shrimp tanks under varying feeding levels are shown in Table 2. The findings indicate that dissolved oxygen levels (5.64 – 6.00 mg L^{-1}) and pH values (7.96 – 8.06) in the BFT treatments were lower than those observed in the control group, where dissolved oxygen ranged from 6.35 to 6.66 mg L^{-1} and pH values ranged from 8.10 to 8.13 (ANOVA: $p < 0.05$). Additionally, salinity levels in the BFT treatments (33.36 – 33.41 ppt) were significantly higher compared to the control group (32.55 ppt) ($p < 0.05$). The concentrations of SS and TSS were also elevated in the BFT treatments, with the highest values recorded in BFT0 (7.13 ml L^{-1} and 201.8 mg L^{-1}) and BFT6 (6.63 ml L^{-1} and 207.5 mg L^{-1}) compared to other treatments. The variations in SS, TSS and water transparency over the experimental days are illustrated in Figure 1. Differences were observed in nitrogenous compounds among the treatments, with the lowest TAN (0.09 mg L^{-1}) and nitrite (2.02 mg L^{-1}) levels recorded in BFT0. Conversely, the highest nitrate concentration (9.88 mg L^{-1}) was found in BFT6. The fluctuations in TAN, NO_2 and NO_3 levels throughout the experimental period are depicted in Figure 2.

TABLE 2 Water quality parameters in tanks of green tiger shrimp under different feeding levels during 45 days of test.

Parameters	CW6	BFT6	BFT4	BFT2	BFT0
T a.m. (°C)	30.05± 0.6^{a}	30.09± 0.55^{a}	30.10± 0.58^{a}	30.07± 0.46^{a}	30.12± 0.7^{a}
T p.m. (°C)	30.62± 0.61^{a}	30.60± 0.5^{a}	30.59± 0.45^{a}	30.57± 0.51^{a}	30.68± 0.59^{a}
DO a.m. (mg L^{-1})	6.55± 0.2^{a}	5.85 ± 0.28^{b}	5.90 ± 0.18^{b}	6.00 ± 0.25^{b}	5.81± 0.30^{b}
DO p.m. (mg L^{-1})	6.35± 0.25^{a}	5.64 ± 0.38^{b}	5.72 ± 0.33^{b}	5.79 ± 0.29^{b}	5.70± 0.25^{b}
pH a.m.	8.12 ± 0.05^{a}	8.04± 0.04^{b}	8.06 ± 0.03^{b}	8.05 ± 0.04^{b}	8.06± 0.04^{b}
pH p.m.	8.10± 0.05^{a}	7.96± 0.06^{b}	7.99 ± 0.04^{b}	8.00 ± 0.05^{b}	8.00± 0.05^{b}
Salinity (ppt)	32.55± 0.50^{b}	33.41 ± 0.98^{a}	33.38 ± 0.90^{a}	33.40 ± 0.92^{a}	33.36 ± 0.75^{a}
SS (ml L^{-1})	0.94± 0.35^{c}	6.63 ± 2.70^{a}	5.78 ± 2.50^{ab}	4.45 ± 1.95^{b}	7.13 ± 2.75^{a}
TSS (mg L^{-1})	67.40± 23.27^{c}	207.50 ± 89.90^{a}	169.21 ± 66.32^{ab}	137.80 ± 43.46^{b}	201.8 ± 74.24^{a}
Transparency (cm)	39.37± 4.20^{a}	17.33± 8.44^{c}	20.34± 8.02^{bc}	22.89± 7.15^{b}	17.05± 8.3^{c}
TAN (mg L^{-1})	0.75± 0.48^{a}	0.32± 0.31^{b}	0.26± 0.24^{b}	0.20± 0.17^{b}	0.09± 0.05^{c}
NO_2 (mg L^{-1})	7.15± 3.15^{a}	5.90± 2.34^{b}	4.57± 1.87^{c}	3.11± 0.88^{d}	2.02± 1.05^{e}
NO_3 (mg L^{-1})	4.45± 1.57^{c}	9.88± 5.20^{a}	8.36± 4.20^{ab}	6.83± 3.1^{b}	2.12± 0.86^{d}

Values are expressed as mean ± SD. Values in the same row with different superscripts are significantly different ($p < 0.05$). Abbreviations: a.m.: before midday; p.m.: after midday

3.2 Growth performance and survival

The results regarding growth performance, feed utilisation, and survival of green tiger shrimp under different feeding levels are summarised in Table 3. The data indicate that the highest final weight (7.54 g), growth rate (0.10 g day^{-1}), and specific growth rate (2.16% day^{-1}) were achieved in the BFT6 treatment, which showed significant differences compared to all other treatments, except for BFT4. No significant differences were observed in final weight, growth rate, and specific growth rate between BFT4 and the control group CW6.

The feed conversion ratios in the BFT treatments (1.08 – 1.80) were lower than those in the control group (2.20) ($p < 0.05$). Survival rates in the BFT treatments were higher than in the control group, with the lowest survival rate (55.97%) recorded in BFT0, which was significantly different from the other treatments ($p < 0.05$).

3.3 Body composition and biofloc production

The biochemical composition of green tiger shrimp and the biofloc produced in the culture tanks is presented in Table 4. Overall, the results indicate that the protein, lipid, and ash content of shrimp in the BFT treatments were higher than in the control group. The lowest protein (72.90%) and lipid (4.89%) content, along with the highest ash content (15.52% dry weight), were found in BFT0 (without commercial feed), showing significant differences from the other treatments ($p < 0.05$).

Regarding the biochemical composition of the produced biofloc, it was observed that with increasing feeding levels, the dry weight, protein and lipid content of the biofloc increased. The lowest dry weight (20.23%), protein (20.66%) and lipid (0.95%) were recorded in BFT0, while the lowest ash content (29.33%) was observed in the biofloc produced in BFT2.

3.4 Hepatopancreas biochemical parameters

The enzyme activities in the hepatopancreas of green tiger shrimp are illustrated in Figure 3. The highest ALP activity was observed in the BFT6 (63.5 u mg^{-1} protein) and BFT4 (61.4 u mg^{-1} protein) treatments, which showed significant differences from the other treatments. The highest ALT (3.40 u mg^{-1} protein) and AST (3.15 u mg^{-1} protein) activities were recorded in BFT0, indicating significant differences from the other treatments.

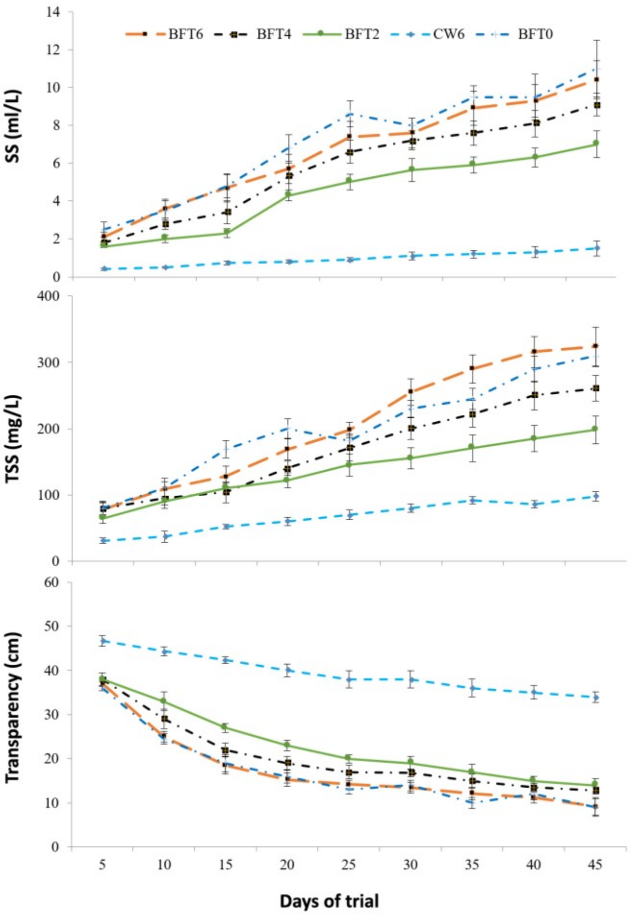

FIGURE 1 Changes in the amount of settleable solids (SS), total suspended solids (TSS) and transparency in the experimental different treatments (mean ± SD).

The antioxidant activities in the hepatopancreas of green tiger shrimp are presented in Figure 4. Overall, the results demonstrated that antioxidant activities in the BFT treatments were higher than in the control group, showing significant differences. The highest CAT activity was observed in BFT4 (0.60 u mg^{-1} protein) and BFT6 (0.58 u mg^{-1} protein), which were significantly different from the other treatments. GPx activity was measured at 3.28, 3.36 and 3.24 in BFT6, BFT4 and BFT2 respectively. The lowest SOD activities were recorded in the control group (2.72), showing differences compared to the BFT treatments.

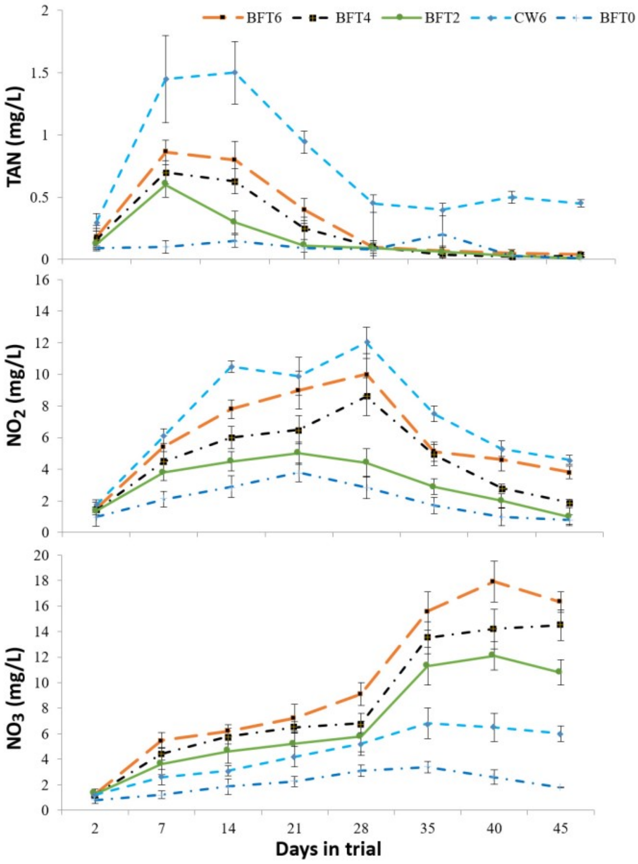

FIGURE 2 Changes in the total ammonia nitrogen (TAN); nitrite (NO_2) and nitrate (NO_3) in experimental different treatments (mean ± SD).

4 | DISCUSSION

4.1 Water quality

The water quality parameters measured in this investigation fell within optimal ranges for green tiger shrimp (Penaeus semisulcatus) cultivation, aligning with established standards reported by Kaya *et al.* (2020) and Khanjani *et al.* (2024b). The lower dissolved oxygen levels observed in the BFT treatments compared to control group can be explained by the enhanced microbial activity within these systems. The limited water exchange and higher TSS in BFT systems create an environment where heterotrophic bacteria thrive. These microorganisms are more efficient at ammonia metabolism than their autotrophic counterparts, consuming oxygen during their respiratory processes, which leads to reduced dissolved oxygen levels in the water column (Schveitzer *et al.* 2024).

The notably lower pH readings in the BFT treatments compared to the control group can be attributed to elevated microbial respiration activity. As heterotrophic bacteria in the BFT system break down organic matter, they produce increased amounts of carbon dioxide as a metabolic byproduct. This heightened CO_2 concentration leads to a decrease in water pH, as the dissolved carbon dioxide reacts with water to form carbonic acid (Miao *et al.* 2017). Additionally, the decline in alkalinity resulting from the metabolism of ammonia by both autotrophic and heterotrophic bacteria may further contribute to the observed reductions in pH (Martins *et al.* 2019; Khanjani *et al.* 2024b).

TABLE 3 Growth performance, survival rate and nutritional performance of green tiger shrimp cultivated under different feeding levels at the end of 45 days of the test period (mean ± SD).

Parameters	CW6	BFT6	BFT4	BFT2	BFT0
FW (g)	7.03± 0.27^{b}	7.54± 0.24^{a}	7.26± 0.38^{ab}	6.15± 0.27^{c}	4.11± 0.24^{d}
BWG (g)	4.18± 0.27^{b}	4.68± 0.24^{a}	4.41± 0.38^{ab}	3.3± 0.27^{c}	1.26± 0.24^{d}
GR (g day^{-1})	0.093± 0.006^{b}	0.10± 0.005^{a}	0.098± 0.008^{ab}	0.073± 0.006^{c}	0.028± 0.005^{d}
BG (g)	183.2± 11.75^{b}	224.76± 11.86^{a}	213.200± 18.5^{a}	125.4± 10.33^{c}	37.44± 7.11^{d}
SGR (% day^{-1})	2.01± 0.083^{b}	2.16± 0.07^{a}	2.07± 0.12^{ab}	1.70± 0.09^{c}	0.81± 0.12^{d}
FCR	2.20± 0.16^{a}	1.80± 0.08^{b}	1.31± 0.13^{c}	1.08± 0.08^{d}	-
SR (%)	82.39± 2.88^{b}	90.56± 1.88^{a}	91.19± 1.09^{a}	71.70± 1.88^{c}	55.97± 1.09^{d}

Values are expressed as mean ± SD. Values in the same row with different superscripts are significantly different ($p < 0.05$). Abbreviations: Final weight (FW), body weight gain (BWG), growth rate (GR), biomass gain (BG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR).

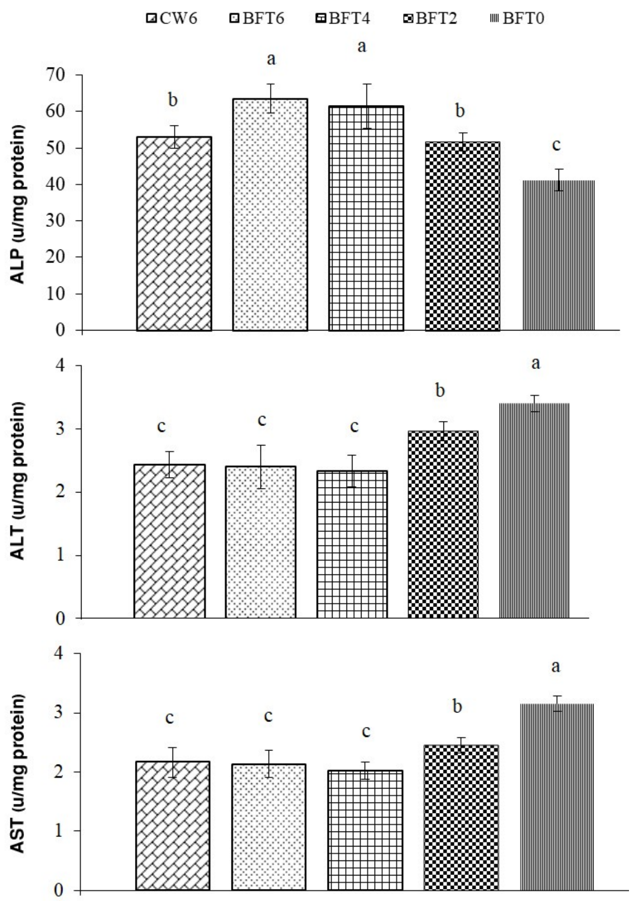

FIGURE 3 Metabolic enzymes activities in the hepatopancreas of green tiger shrimp at the end of the experimental period under different treatments. Values (mean ± SD) in columns with different superscripts are significantly different ($p < 0.05$).

The elevated salinity levels observed in the BFT treatments compared to control group can be explained by two primary mechanisms. The minimal water exchange in BFT systems allows for increased evaporation, which concentrates existing salts in the water column. Furthermore, the accumulation of dissolved salts from uneaten feed contributes to the higher salinity levels, as these nutrients remain in the system rather than being removed through water exchanges (de Oliveira Alves *et al.* 2017).

Nitrogenous compounds, including TAN, NO_2 and NO_3, exhibited trends consistent with previous research (Khanjani *et al.* 2024b). The observed temporal pattern in nitrogen compounds, characterized by initial TAN accumulation followed by sequential conversion to nitrite and nitrate, aligns with established biological processes (Khanjani *et al.* 2024b). This transformation is facilitated by two distinct groups of microorganisms: ammonia-oxidizing bacteria, which catalyze the initial conversion of ammonia to nitrite, and nitrite-oxidizing bacteria, which complete the process by converting nitrite to nitrate (Wang *et al.* 2022).

The study's findings uncovered a significant correlation between carbon availability and nitrogen transformation patterns in aquaculture systems. In systems with high commercial feed inputs, nitrogen compounds tend to accumulate in the water due to insufficient bacterial activity. However, when heterotrophic bacteria dominate the system, they effectively convert nitrogen compounds into bacterial biomass, resulting in lower concentrations of harmful nitrogen compounds. The presence of organic carbon sources, such as starch, provides the necessary substrate for heterotrophic bacteria to efficiently process ammonia, leading to reduced levels of toxic nitrogen compounds as biofloc development increases (Samocha 2019).

The highest TSS (324 mg L^{-1}) was recorded in the

BFT6 treatment, indicating that the added biofloc remained suspended in the water column, contributing to elevated TSS levels (Schveitzer *et al.* 2024). Notably, shrimp in the BFT0 treatment, which were fed exclusively on biofloc, exhibited reduced performance, indicating that green tiger shrimp cannot rely solely on biofloc as their food source. The recommended TSS levels for *L. vannamei* range from 73.3 to 499.14 mg L^{-1} (Huang *et al.* 2023; Prates *et al.* 2023). Overall, the water quality results suggest that moderate biofloc levels can enhance water quality in green tiger shrimp cultivation, aligning with findings from other researchers (Vinasyiam *et al.* 2023; Schveitzer *et al.* 2024).

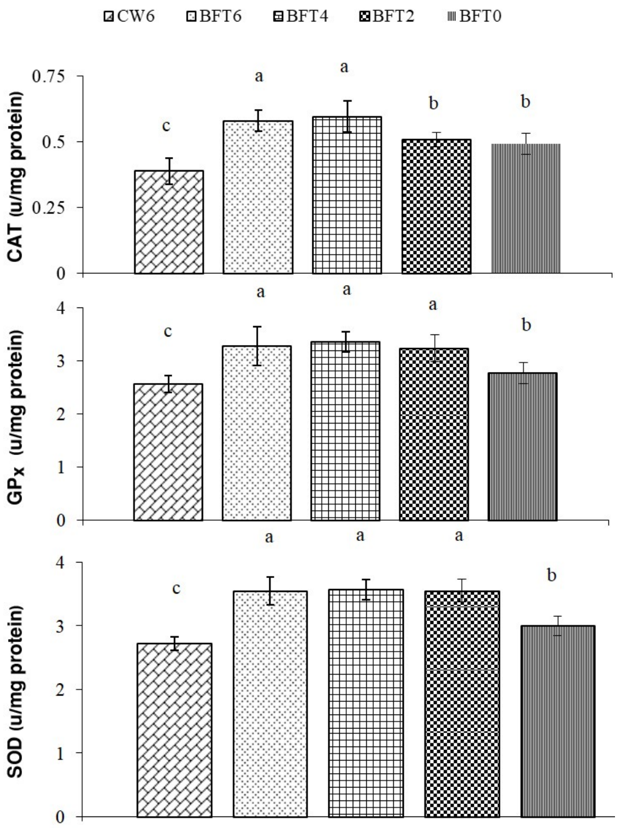

FIGURE 4 Antioxidant enzymes activities in the hepatopancreas of green tiger shrimp at the end of the experimental period under different treatments. Values (mean ± SD) in columns with different superscripts are significantly different ($p < 0.05$).

4.2 Growth performance and survival

Research has demonstrated that incorporating biofloc into shrimp feed enhances growth performance (Barzamini *et al.* 2021; Uawisetwathana *et al.* 2021). Under the conditions of the present study revealed improved survival rates for green tiger shrimp in the BFT6 and BFT4 treatments. Notably, growth performance showed no significant difference between the CW6 (6% body weight) and BFT4 (4% body weight) treatments. However, the BFT6 treatment demonstrated significantly higher BWG and SGR compared to the CW6 group ($p < 0.05$), while no significant differences were observed between BFT6 and BFT4 treatments.

The experimental results revealed distinct patterns in shrimp performance across different treatment groups. The BFT4 treatment showed superior outcomes, with notably higher survival rates and biomass accumulation compared to the clear water control group (CW6). In contrast, the BFT0 treatment, which relied solely on biofloc as a food source, demonstrated the poorest performance metrics, including lower survival rates, BWG and SGR.

Recent research has highlighted that BFT provides a nutrient-rich supplement for shrimp cultivation, containing valuable compounds such as carotenoids, chlorophylls and phytosterols (Ju *et al.* 2008; Khanjani *et al.* 2023). When integrated into commercial feed formulations, these natural compounds create a comprehensive nutritional profile that supports shrimp development. The presence of beneficial microorganisms in BFT systems has been shown to enhance both survival rates and growth performance in shrimp populations, making it a valuable addition to traditional feeding strategies. Also, these findings suggest that while biofloc can be beneficial as a supplementary feed component, its exclusive use may be limiting. Research indicates that high concentrations of microbial products can potentially reduce feed palatability and digestibility (Khatoon *et al.* 2016). Furthermore, biofloc may not provide a complete spectrum of essential micronutrients required for optimal shrimp growth (Khanjani and Sharifinia, 2022; Khatoon et al., 2016), which could explain the observed performance limitations in the BFT0 treatment.

Furthermore, studies have shown that the growth performance of other shrimp species, such as *L. vannamei* (Khanjani *et al.* 2025a), *P. monodon* (Anand *et al.* 2014), *P. aztecus* (Kaya 2025), *F. merguiensis* (Khanjani and Sharifinia 2022), *F. indicus* (Effendy *et al.* 2016) and *M. monoceros* (Kaya *et al.* 2019), is better in BFT systems compared to clear water systems. BFT clearly enhances the growth performance of shrimp and is effective for the proliferation of heterotrophic bacteria (Khanjani *et al.* 2025b; Kumar *et al.* 2018). Most previous studies on shrimp culture have shown that shrimp generally achieve better growth, survival rates, and FCR in well-prepared and controlled BFT conditions compared to clear water conditions (Cavalcanti *et al.* 2019; Panigrahi *et al.* 2019; Khanjani *et al.* 2025a).

The experimental results demonstrated higher survival rates in both BFT4 and BFT6 treatments compared to the control group, likely attributed to enhanced water quality conditions in these systems. This finding aligns with recent research showing that the combination of biofloc processes with carbohydrate supplementation positively impacts shrimp survival rates (Addo *et al.* 2023;

Huang *et al.* 2023; Yu *et al.* 2024). The notably lower survival rate of 55.97% observed in the BFT0 treatment, which utilised biofloc as the sole nutritional source, highlights the limitations of relying exclusively on biofloc for shrimp nutrition.

The FCR results revealed significant variations across treatment groups, with BFT2 achieving the most efficient feed utilization at 1.08, while CW6 showed the highest FCR at 2.20. Research has consistently demonstrated that integrating commercial feed with BFT systems can lead to improved feed conversion efficiency (Kaya *et al.* 2020; Prates *et al.* 2023). A comparative analysis of feeding rates shows that our study's FCR values (1.08, 1.31 and 1.80 for 2%, 4% and 6% body weight feeding levels respectively) were notably lower than those reported by Kaya *et al.* (2020), who found FCR values ranging from 1.38 to 2.87 for feeding rates between 3% and 12% body weight.

The higher FCRs observed in BFT systems can be attributed to the high nutritional value of the microbial flocs that shrimp consume. In contrast, the higher FCR values in CW6 group appear to correlate with reduced survival and weight gain in this group, suggesting that feeding level of 6% body weight may be less effective in clear water system compared to BFT system. Research has consistently shown that the presence of biofloc communities leads to improved FCR in shrimp (Kumar *et al.* 2018; Khanjani *et al.* 2025a), likely due to enhanced digestive processes and feed absorption facilitated by beneficial bacteria colonising their digestive systems (Gustilatov *et al.* 2024). The growth performance data indicates that juvenile green tiger shrimp in the BFT4 treatment achieved comparable results to both BFT6 and CW6 treatments, suggesting that a 4% body weight feeding level in a BFT system represents an optimal balance for enhancing both growth and survival performance.

4.3 Body and biofloc composition

The experimental findings revealed that different feeding levels significantly impacted both the biochemical composition of green tiger shrimp and the resulting biofloc. Notably, feeding rates of 4% and 6% body weight in the BFT system yielded superior body quality, characterised by enhanced protein and lipid content. This improvement in protein content can be attributed to more efficient utilisation and retention of dietary protein (Ghosh *et al.* 2018). Conversely BFT0 treatment showed significant decreases in both protein and body lipid content, likely due to restricted access to commercial feed. Multiple studies have consistently demonstrated that biofloc supplementation positively influences the biochemical quality of shrimp (Vungarala *et al.* 2021; Irani *et al.* 2023; Vinasyiam *et al.* 2023).

The elevated ash content observed in BFT0, BFT4, and BFT6 treatments can be explained by increased access to biofloc, which serves as a rich source of essential minerals for the shrimp. This relationship between biofloc consumption and ash content is supported by previous research showing that the mineral content of biofloc directly contributes to increased ash levels in body composition (Ju *et al.* 2008).

The biochemical composition values obtained in this study demonstrate remarkable consistency with previously published research. For example, Vinasyiam *et al.* (2023) documented similar ranges for Pacific white shrimp, with protein content varying between 72.70% and 75.2%, lipid levels ranging from 2.6% to 3.7% and ash content spanning 14.30% to 18.0% of dry weight. Similarly, Kaya *et al.* (2020) reported comparable measurements for green tiger shrimp, with protein content between 15.95% and 21.05%, lipid content ranging from 0.7% to 1.26%, and ash content varying between 2.67% and 3.84% of wet weight.

The variations in the biochemical composition of green tiger shrimp can be attributed to several key factors, including the quantity of biofloc present, the associated microbial communities and their diversity and abundance within the cultivation tanks. The enhanced proximate body composition achieved through BFT systems represents a significant advantage, as these systems provide essential fatty acids and amino acids while offering a cost-effective solution for producing high-quality feed suitable for shrimp consumption (Uawisetwathana *et al.* 2021).

Biofloc demonstrates a notably favourable nutritional profile, with protein content ranging from 20% to 45%, lipid content between 1% and 8% and ash content spanning 15% to 60% (El-Sayed 2021). Recent studies have reported similar protein content ranges, with Hosain *et al.* (2021) and Xavier *et al.* (2022) documenting levels of 15.9% to 32.6% and 19.38% to 23.53% respectively. Our research revealed protein levels between 20.66% and 27.80% across different treatments, with the lowest concentrations observed in BFT0 and BFT2 treatments. This variation can be attributed to two primary factors: the reduced input of commercial feed (which contains 38% protein) and alterations in the biofloc microbial communities.

The lipid content of biofloc was found to be relatively low, falling within a narrow range of 0.95% to 1.37%. The BFT0 treatment showed the lowest lipid levels, which can be attributed to the absence of commercial feed in these culture tanks. Research has shown that biofloc lipid composition is significantly influenced by two key factors: the type of carbon source used and the specific microbial populations present in the system (Khanjani *et al.* 2025a). The choice of carbon sources, such as glucose, molasses, or starch, can lead to variations in microbial metabolism, promoting the growth of specific microbial communities that affect the types and proportions of lipids produced.

For instance, some carbon sources may enhance the production of polyunsaturated fatty acids (PUFAs), while others may result in higher levels of saturated fatty acids. The type of carbon source also leads to different compositions of microorganisms, including certain bacteria, protozoa, and algae, which play a crucial role in lipid synthesis (Wei *et al.* 2016; Khanjani *et al.* 2025b). Bacteria, algae, and protozoa contribute to the overall lipid profile, with certain algae known to produce high levels of omega-3 fatty acids, thereby enriching the lipid composition of biofloc. Notably, protozoa present in the BFT system contain steroids in their chemical composition, a significant portion of which is converted into cholesterol and other forms of lipid (Loureiro *et al.* 2012). Furthermore, the interaction between carbon sources and microbial populations can create a synergistic effect on lipid production, where readily available carbon sources stimulate microbial growth and lipid accumulation, while limited carbon availability may induce stress responses in microbes, altering lipid composition as they adapt to their environment (Sánchez-Muros *et al.* 2020; Hosain *et al.* 2021).

Our analysis revealed that biofloc collected from the green tiger shrimp BFT system exhibited notably high ash content. This elevated ash content can be attributed to several factors, including the salt content in the BFT culture system, as documented by Khatoon *et al.* (2016) and Xavier *et al.* (2022). The progressive increase in ash content from BFT2 to BFT6 treatments appears to correlate with variations in TSS among the different treatments. Research has established that high ash content in biofloc is primarily associated with the presence of acid-insoluble oxides and mixed silicates (Binalshikh-Abubkr *et al.* 2021).

4.4 Hepatopancreas biochemical parameters

The assessment of oxidative stress in crustaceans, particularly shrimp, is fundamentally linked to monitoring changes in metabolic and antioxidant enzyme activities (Wang *et al.* 2006; Sharifinia *et al.* 2024). These enzymes are crucial indicators of overall health and tissue integrity in aquatic organisms. Research indicates that elevated levels of specific enzymes, such as ALP, AST, and ALT, can signify damage to the hepatopancreas, a vital organ in shrimp (Yu *et al.* 2016). Notably, ALT and AST are integral to amino acid metabolism and are widely recognized as diagnostic markers for liver damage. Our study revealed significant variations in enzyme levels across different treatments, with particularly higher AST and ALT levels in the BFT0 treatment, suggesting that the absence of commercial feed resulted in tissue damage and compromised shrimp health. Conversely, treatments with 4% and 6% body weight exhibited lower AST and ALT levels, indicating improved health outcomes.

Supporting these findings, Hussain *et al.* (2021) reported decreased ALT levels in the hemolymph of various shrimp species raised in BFT systems, suggesting enhanced liver function in biofloc-cultured shrimp. Furthermore, ALP functions as a primary detoxification enzyme in aquatic organisms, playing essential roles in calcium absorption, chitin secretion, and lysosomal enzyme activity (Qiu *et al.* 2016). This enzyme also serves as a key indicator of tissue damage in aquatic organisms exposed to environmental stressors or xenobiotics, providing valuable insights into their health and immune status (Gyan *et al.* 2020). Our research demonstrated higher ALP levels in BFT4 and BFT6 treatments, indicating that the presence of biofloc enhances both health and immune function in shrimp.

The antioxidant defense system in shrimp is a complex interplay of enzymes, including SOD, CAT and GPx, which collectively protect against oxidative damage. This defence mechanism operates through a sequential process where SOD converts superoxide anions into hydrogen peroxide, which is subsequently processed by CAT into water and oxygen, serving as a primary line of defense against reactive oxygen species (Panigrahi *et al.* 2020). Our research demonstrated enhanced antioxidant enzyme activity across all BFT treatments (BFT2, BFT4, BFT6), with significant improvements compared to the control group. This finding aligns with previous research by Xu and Pan (2013), who observed elevated SOD activity in *P. vannamei* and other shrimp species cultured in BFT systems, indicating strengthened antioxidative defense and immune responses. Multiple studies have consistently shown increased SOD activity in biofloc-cultured shrimp (Zhao *et al.* 2016; Tepaamorndech *et al.* 2020; Vungarala *et al.* 2021), suggesting that the natural microorganisms and bioactive compounds present in BFT systems positively influence both shrimp physiological health and antioxidant enzyme activity.

Moreover, it is essential to recognise that highly unsaturated fatty acids are particularly vulnerable to oxidation and deterioration. Therefore, ensuring that the fish oil used in the study was not exposed to air and did not have a rancid smell is crucial to confirm that fatty acids were not subjected to auto-oxidation, which can escalate as the reaction progresses. This aspect is vital to verify in the context of our recent study, as free radical damage and the multi-level defence mechanisms of cells, including the roles of SOD, CAT and GPx, are critical for maintaining the integrity of lipid profiles in shrimp.

5 | CONCLUSIONS

This research provides valuable insights into the growth performance of green tiger shrimp in BFT systems, indicating that feeding at 4% body weight can yield optimal results comparable to 6% feeding levels. While the findings suggest improvements in water quality parameters and overall shrimp health, it is essential to acknowledge certain limitations. The BFT system appears to offer advantages over traditional methods, with shrimp exhibiting enhanced growth rates, improved survival, and better body composition compared to the control group. However, claims regarding the superiority of the 4% feeding level must consider potential confounding factors, such as variations in water exchange rates and the unquantified contributions of biofloc. The elevated activity levels of ALP and antioxidant enzymes (SOD, CAT and GPX) in BFT treatments indicate a positive health status and improved resilience to environmental stressors. Nonetheless, it is crucial to clarify the practical significance of these enzyme activity changes in relation to shrimp health and production outcomes. Including these caveats would provide a more balanced perspective and better inform aquaculture applications. To strengthen the conclusions, future research should validate these findings under commercial pond conditions, focusing on a comprehensive cost-benefit analysis of feed reduction in BFT systems. This approach will enhance the robustness of the conclusions and support the development of sustainable aquaculture practices.

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ETHICAL APPROVAL
All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST
The author declares no conflict of interest.

AUTHORS' CONTRIBUTION
Mohammad Hossein Khanjani: Conceptualisation, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft, review & editing. Moslem Sharifinia: Conceptualisation, Validation, Visualization, Writing - review & editing.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on a reasonable request from the corresponding author.

REFERENCES
Addo FG, Zhang S, Manirakiza B, Ma Y, Yuan S, ... Abakari G (2023) Brown sugar addition enhanced nutrient removal rates, growth performance, and bacterial community in a rice straw-based biofloc shrimp culture system. Aquaculture 567: 739274.

Al-Maslamani I, Le Vay L, Kennedy H, Jones D (2007) Feeding ecology of the grooved tiger shrimp *Penaeus semisulcatus* De Haan (Decapoda: Penaeidae) in inshore waters of Qatar, Arabian Gulf. Marine Biology 150: 627–637.

Anand PS, Kohli M, Kumar S, Sundaray J, Roy SD, ... Pailan G (2014) Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 418: 108–115.

APHA (2012) Standard methods for the examination of water and wastewater, 22nd edition. American Public Health Association, Washington DC, USA.

Avnimelech Y (2012) Biofloc technology – a practice guide book, 2nd edition. The World Aquaculture Society, Baton Rouge.

Barzamini M, Harsij M, Adineh H, Jafaryan H (2021) The effect of biofloc-supplemented diets on the Pacific white shrimp (*Litopenaeus vannamei*): analysis of water quality, growth performance, and biochemical composition. Sustainable Aquaculture and Health Management Journal 7(2): 30–43.

Binalshikh-Abubkr T, Hanafiah MM, Das SK (2021) Proximate chemical composition of dried shrimp and tilapia waste bioflocs produced by two drying methods. Journal of Marine Science and Engineering 9(2): 193.

Burtis CA, Ashwood E, Bruns DE (2007) Tietz fundamentals of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences.

Cavalcanti NR, Costa CB, Rodrigues F, Soares R, Bezerra RS, Peixoto S (2019) Effect of feeding frequency on growth and digestive enzyme activity in Litopenaeus vannamei during the grow-out phase in biofloc system. Aquaculture Nutrition 25(3): 577–584.

de Oliveira Alves GF, Fernandes AFA, de Alvarenga ÉR, Turra EM, de Sousa AB, de Alencar Teixeira E (2017) Effect of the transfer at different moments of juvenile Nile tilapia (*Oreochromis niloticus*) to the biofloc system in formation. Aquaculture 479: 564–570.

Effendy I, Al Deen S, Chithambaran S (2016) Semi intensive and semi biofloc methods for the culture of Indian white prawn, *Fenneropenaeus indicus* in high-density polyethylene liner ponds. HAYATI Journal of Biosciences 23(3): 106–110.

El-Sayed AFM (2021) Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. Reviews in Aquaculture 13(1): 676–705.

Emerenciano MGC, Khanjani MH, Sharifinia M, Miranda-Baeza A (2025) Could biofloc technology (BFT) pave the way toward a more sustainable aquaculture in line with the circular economy? Aquaculture Research 2025(1): 1020045.

FAO (2022) In brief to the state of world fisheries and aquaculture 2022. Towards blue transformation.

FAO, Rome.

FAO (2024) In brief to the state of world fisheries and aquaculture 2024. Towards blue transformation. FAO, Rome.

Fernandes MR, Pedroso AR (2017) Animal experimentation: a look into ethics, welfare and alternative methods. Revista da Associacao Medica Brasileira 63: 923–928.

Garlock T, Asche F, Anderson J, Bjørndal T, Kumar G, ... Tveterås R (2020) A global blue revolution: aquaculture growth across regions, species, and countries. Reviews in Fisheries Science & Aquaculture 28(1): 107–116.

Ghosh AK (2018) Effect of feeding level on growth, body composition, fatty acid profile, and nutrient accumulation in shrimp (*Litopenaeus vannamei*). Aquaculture International 26: 405–417.

Gustilatov M, Widanarni W, Ekasari J, Julyantoro PGS, Waturangi DE (2024) The influence of biofloc system on Vibrio composition, the growth and the gut microvilli performance of the Pacific white shrimp *Penaeus vannamei*. Jurnal Akuakultur Indonesia 23(1): 12–23.

Gyan WR, Yang Q, Tan B, Jan SS, Jiang L, ... Shuang Z (2020) Effects of antimicrobial peptides on growth, feed utilization, serum biochemical indices and disease resistance of juvenile shrimp, *Litopenaeus vannamei*. Aquaculture Research 51(3): 1222–1231.

Haque MM, Mahmud MN (2025) Potential role of aquaculture in advancing sustainable development goals (SDGs) in Bangladesh. Aquaculture Research 2025: 6035730.

Hosain ME, Amin SN, Arshad A, Kamarudin MS, Karim M (2021) Effects of carbon sources on the culture of giant river prawn in biofloc system during nursery phase. Aquaculture Reports 19: 100607.

Huang HH, Li CY, Lei YJ, Zhou BL, Kuang WQ, ... Yang PH (2023) Effects of Bacillus strain added as initial indigenous species into the biofloc system rearing *Litopenaeus vannamei* juveniles on biofloc preformation, water quality and shrimp growth. Aquaculture 569: 739375.

Hussain AS, Mohammad DA, Sallam WS, Shoukry NM, Davis DA (2021) Effects of culturing the Pacific white shrimp *Penaeus vannamei* in biofloc vs synbiotic systems on the growth and immune system. Aquaculture 542: 736905.

Irani M, Islami HR, Bahabadi MN, Shekarabi SPH (2023) Production of Pacific white shrimp under different stocking density in a zero-water exchange biofloc system: effects on water quality, zootechnical performance, and body composition. Aquacultural Engineering 100: 102313.

Ju Z, 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.

Kaya D (2025) Improvement of brown shrimp (*Penaeus aztecus*) culture parameters through dietary enriched synbiotic in a biofloc system. Aquaculture International 33: 232

Kaya D, Genc E, Genc MA, Aktas M, Eroldogan OT, Guroy D (2020) Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: effects of different feeding rates and stocking densities. Aquaculture 528: 735526.

Kaya D, Genc MA, Aktas M, Yavuzcan H, Ozmen O, Genc E (2019) Effect of biofoc technology on growth of speckled shrimp, *Metapenaeus monoceros* (Fabricus) in diferent feeding regimes. Aquaculture Research 50(10): 2760–2768.

Keshavarzifard M, Vazirzadeh A, Sharifinia M (2020) Implications of anthropogenic effects on the coastal environment of Northern Persian Gulf, using jinga shrimp (*Metapenaeus affinis*) as indicator. Marine Pollution Bulletin 159: 111463.

Khanjani MH (2025) Evaluating the performance of Pacific white shrimp (*Penaeus vannamei* Boone, 1931) reared under different stocking densities: a study of their biochemical, immune, metabolic and antioxidant responses in a biofloc aquaculture system. Annals of Animal Science. DOI 10.2478/aoas-2025-0087

Khanjani MH, Alizadeh M (2024) Effects of different salinity levels on performance of Nile tilapia fingerlings in a biofloc culture system. Annals of Animal Science 24(1): 235–245.

Khanjani MH, Eslami J, Emerenciano MGC (2025a) Wheat flour as carbon source on water quality, growth performance, hemolymph biochemical and immune parameters of Pacific white shrimp (*Penaeus vannamei*) juveniles in biofloc technology (BFT). Aquaculture Reports 40: 102623.

Khanjani MH, Mohammadi A, Emerenciano MGC (2024b) Water quality in biofloc technology (BFT): an applied review for an evolving aquaculture. Aquaculture International 32(7): 9321–9374.

Khanjani MH, Sharifinia M (2022) Biofloc as a food source for banana shrimp *Fenneropenaeus merguiensis postlarvae*. North American Journal of Aquaculture 84(4): 469–479.

Khanjani MH, Sharifinia M (2024) Feeding Nile tilapia with varying levels of biofloc: effect on growth performance, survival rate, digestive and liver enzyme activities, and mucus immunity. Aquaculture International 32: 8171–8194.

Khanjani MH, Sharifinia M, Akhavan-Bahabadi M, Emerenciano MGC (2024a) Probiotics and phytobiotics as dietary and water supplements in biofloc aquaculture systems. Aquaculture Nutrition 2024(1): 3089887.

Khanjani MH, Sharifinia M, Emerenciano MGC (2023) A detailed look at the impacts of biofloc on immunological and hematological parameters and improving resistance to diseases. Fish & Shellfish Immunology 137: 108796.

Khanjani MH, Zahedi S, Sharifinia M, Hajirezaee S, Singh SK (2025b) Biological removal of nitrogenous waste compounds in the biofloc aquaculture system: a review. Annals of Animal Science 1: 3–21.

Khatoon H, Banerjee S, Yuan GTG, Haris N, Ikhwanuddin M, ... Endut A (2016) Biofloc as a potential natural feed for shrimp postlarvae. International Biodeterioration & Biodegradation 113: 304–309.

Kumar VS, Pandey PK, Anand T, Bhuvaneswari GR, Dhinakaran A, Kumar S (2018) Biofloc improves water, effluent quality and growth parameters of Penaeus vannamei in an intensive culture system. Journal of Environmental Management 215: 206–215.

Loureiro CK, Wasielesky W, Abreu PC (2012) The use of protozoan, rotifers and nematodes as live food for shrimp raised in BFT system. Atlantica Rio Grande 34(1): 5–12.

Luedeman R, Lightner DV (1992) Development of an in vitro primary cell culture system from the penaeid shrimp, *Penaeus stylirostris* and *Penaeus vannamei*. Aquaculture 101(3–4): 205–211.

Martins GB, da Rosa CE, Tarouco FM, Robaldo RB (2019) Growth, water quality and oxidative stress of Nile tilapia *Oreochromis niloticus* (L.) in biofloc technology system at different pH. Aquaculture Research 50(4): 1030–1039.

Megahed ME (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.

Miao S, Sun L, Bu H, Zhu J, Chen G (2017) Effect of molasses addition at C:N ratio of 20:1 on the water quality and growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture International 25: 1409–1425.

Mohammad Moradi S, Safaei M, Saraji F (2023) Feeding habits of green tiger prawn, *Penaeus semisulcatus* (De Hann, 1848) in the coastal waters of the Persian Gulf (Hormozgan Province). Iranian Scientific Fisheries Journal 32(1): 73–83.

Panigrahi A, Sivakumar M, Sundaram M, Saravanan A, Das R, ... Gopikrishna G (2020) Comparative study on phenoloxidase activity of biofloc-reared pacific white shrimp *Penaeus vannamei* and Indian white shrimp *Penaeus indicus* on graded protein diet. Aquaculture 518: 734654.

Panigrahi A, Sundaram M, Chakrapani S, Rajasekar S, Syama Dayal J, Chavali G (2019) Effect of carbon and nitrogen ratio (C:N) manipulation on the production performance and immunity of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. Aquaculture Research 50(1): 29–41.

Prates E, Holanda M, Pedrosa VF, Monserrat JM, Wasielesky W (2023) Compensatory growth and energy reserves changes in the Pacific white shrimp (*Litopenaeus vannamei*) reared in different temperatures and under feed restriction in biofloc technology system (BFT). Aquaculture 562: 738821.

Qiu M, Wang Y, Wang X, Sun L, Ye R, ... Yao Y (2016) Effects of T-2 toxin on growth, immune function and hepatopancreas microstructure of shrimp (*Litopenaeus vannamei*). Aquaculture 462: 35–39.

Rather MA, Ahmad I, Shah A, Hajam YA, Amin A, ... Rasool S (2024) Exploring opportunities of artificial intelligence in aquaculture to meet increasing food demand. Food Chemistry: X 22: 101309.

Samocha TM (2019) Sustainable biofloc systems for marine shrimp. Elsevier, Academic Press, USA.

Sánchez-Muros MJ, Renteria P, Vizcaino A, Barroso FG (2020) Innovative protein sources in shrimp (*Litopenaeus vannamei*) feeding. Reviews in Aquaculture 12(1): 186–203.

Schveitzer R, Baccarat RFC, Gaona CAP, Wasielesky W, Arantes R (2024) Concentration of suspended solids in superintensive culture of the Pacific white shrimp *Litopenaeus vannamei* with biofloc technology (BFT): A review. Reviews in Aquaculture 16(2): 785–795.

Sharifinia M (2025) From nutrient bioavailability to disease resistance: The comprehensive benefits of chelated minerals in aquaculture. Fish & Shellfish Immunology 160: 110218.

Sharifinia M, Bahmanbeigloo ZA, Keshavarzifard M, Khanjani MH, Daliri M, ... Jasour MS (2023a) The effects of replacing fishmeal by mealworm (*Tenebrio molitor*) on digestive enzymes activity and hepatopancreatic biochemical indices of Litopenaeus vannamei. Annals of Animal Science. DOI 10.2478/aoas-2022-0098.

Sharifinia M, Bahmanbeigloo ZA, Keshavarzifard M, Khanjani MH, Daliri M, ... Jasour MS (2023b) Fishmeal replacement by mealworm (*Tenebrio molitor*) in diet of farmed Pacific white shrimp (*Litopenaeus vannamei*): effects on growth performance, serum biochemistry, and immune response. Aquatic Living Resources 36: 19.

Sharifinia M, Dashtiannasab A, Keshavarzifard M, Pazir M (2025) Optimizing shrimp growth and yield production: a comparative study of Pacific white shrimp farms along the Persian Gulf coastline. Iranian Journal of Fisheries Sciences 24(3): 619–631.

Sharifinia M, Tayemeh MB, Dashtiannasab A, Kaviani A, Faghih S, ... Nazaran MH (2024) Enhancement of growth performance, meat quality, digestive system, and immunity of *Litopenaeus vannamei* fed with advanced chelate compounds-based minerals: a new perspective on chelation technology. Annals of Animal Science 25(2): 619–631.

Tepaamorndech S, Nookaew I, Higdon SM, Santiyanont P, Phromson M, ... Visessanguan W (2020) Metagenomics in bioflocs and their effects on gut microbiome and immune responses in Pacific white shrimp. Fish & Shellfish Immunology 106: 733–741.

Uawisetwathana U, Situmorang ML, Arayamethakorn S, Haniswita Suantika G, Panya A, Karoonuthaisiri N, Rungrassamee W (2021) Supplementation of ex-situ biofloc to improve growth performance and enhance nutritional values of the Pacific white shrimp rearing at low salinity conditions. Applied Sciences 11(10): 4598.

Ullman C, Rhodes M, Hanson T, Cline D, Davis DA (2017) A new paradigm for managing shrimp feeding. World Aquaculture 2017: 31.

Vinasyiam A, Kokou F, Ekasari J, Schrama JW, Verdegem MC (2023) Effects of high wheat bran input on the performance of a biofloc system for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Reports 33: 101853.

Vungarala H, Sudhagar A, Reddy AK, Rani Asanaru Majeedkutty B (2021) Growth, body composition and antioxidant status of *Litopenaeus vannamei* juveniles reared at different stocking densities in the biofloc system using inland saline groundwater. Aquaculture Research 52(12): 6299–6307.

Wang D, Wang Y, Liu L, Chen Y, Wang C, ... Zhang T (2022) Niche differentiation and symbiotic association among ammonia/nitrite oxidizers in a full-scale rotating biological contactor. Water Research 225: 119137.

Wang YJ, Chien YH, Pan CH (2006) Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. Aquaculture 261(2): 641–648.

Wei YF, Liao SA, Wang AL (2016) The efect of diferent carbon sources on the nutritional composition, microbial community and structure of biofocs. Aquaculture 465: 88–93.

Xavier M, Wasielesky W, Hostins B, Bequé E, Krummenauer D (2022) The use of a flocculant additive and its effect on biofloc formation, nitrification, and zootechnical performance during the culture of Pacific white shrimp *Penaeus vannamei* (Boone, 1931) in a BFT system. Latin American Journal of Aquatic Research 50(2): 181–196.

Xu WJ, Pan LQ (2013) Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture 412: 117–124.

Yang SP, Wu ZH, Jian JC, Zhang XZ (2010) Effect of marine red yeast *Rhodosporidium paludigenum* on growth and antioxidant competence of *Litopenaeus vannamei*. Aquaculture 309(1–4): 62–65.

Yu YB, Choi JH, Lee JH, Jo AH, Lee JW, ... Lee KM (2024) The use, application and efficacy of biofloc technology (BFT) in shrimp aquaculture industry: a review. Environmental Technology & Innovation 33: 103345.

Yu YY, Chen WD, Liu YJ, Niu J, Chen M, Tian LX (2016) Effect of different dietary levels of *Gracilaria lemaneiformis* dry power on growth performance, hematological parameters and intestinal structure of juvenile Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 450: 356–362.

Zhao D, Pan L, Huang F, Wang C, Xu W (2016) Effects of different carbon sources on bioactive compound production of biofloc, immune response, antioxidant level, and growth performance of *Litopenaeus vannamei* in zero-water exchange culture tanks. Journal of the World Aquaculture Society 47(4): 566–576.

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