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Research Article

EFFICACY OF THE BIOFLOC SYSTEM WITH THE ADDITION OF CHITOSAN ON TIGER PRAWN (*Penaeus monodon*) PRODUCTIVITY

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HIGHLIGHTS

- Biofloc technology with chitosan addition improved the growth performance of *Penaeus monodon*, increased floc volume and heterotrophic bacteria, and reduced *Vibrio*-like bacteria.
- Biofloc technology with chitosan addition enhanced water quality and microbial community, supporting *P. monodon* growth.
- *P. monodon* in a biofloc system with chitosan had higher weight gain, growth rate, and survival rate.
- The study provides insights for sustainable intensive shrimp farming using biofloc technology.
- Findings contribute to improving productivity and maintaining environmental conditions in shrimp aquaculture.

Keyword: Biofloc Technology; Chitosan; Shrimp Aquaculture; *Vibrio*-like Bacteria; Heterotrophic bacteria

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ABSTRACT

Penaeus monodon (tiger prawn) farming failures often occur in the post-larval initial stocking phase until harvesting. High mortality rates of *P. monodon* post-larvae are often observed after stocking. Disease attack in the second month of the rearing period eventually causes failure in extensive ponds. The primary objective of this study was to investigate the impact of chitosan addition on *P. monodon* yield in a biofloc technology (BFT) culture system. The experiment was performed in triplicate using three treatments, i.e., AL (a control pond without biofloc), ABF1 (a biofloc pond without chitosan addition), and ABF2 (a biofloc pond with 100 mg/L chitosan). The post-larval *P. monodon* (PL-10), which was visually healthy and disease-free, was obtained from a hatchery. The stocking density used in this study was 40 individuals per 100 L. The prawns were fed a commercial diet four times daily for 40 days. A statistically significant difference was observed ($P < 0.05$) in weight gain (0.213 - 0.299 g), average daily growth (0.0054 - 0.0074 g/day), specific growth rate (13.4 - 14.3% per day), survival rate (62.5 - 68.33%), food conversion ratio (1.14 - 1.79), and protein efficiency ratio (2.41 - 3.33) of prawn among all of the treatments. Biofloc treatment with the addition of chitosan showed better prawn growth than that in the control and ABF1 treatments, with a weight gain of 0.30 g, average daily growth of 0.00075 g/day, specific growth rate of 14.3%, survival rate of 68.33%, food conversion ratio of 1.14, and protein efficiency ratio of 3.33. The inclusion of chitosan in the biofloc system led to a prominent increase in floc volume and heterotrophic bacterial populations while simultaneously reducing the number of *Vibrio*-like bacteria (VLB) colonies. No notable variations were noted in the food conversion ratio (FCR), total length, survival rate, total nitrite-N, nitrate-N, and total ammonia nitrogen levels. Biofloc technology supplemented with chitosan was found to boost the growth rate of *P. monodon* by enhancing both the water quality and the microorganisms in the ponds. The findings of this study provide a fundamental contribution to sustainable intensive shrimp farming practices through the implementation of biofloc technology, thereby enhancing productivity and maintaining environmental conditions in aquaculture.

Keywords: floc, penaidae, probiotic bacteria, *Vibrio*

INTRODUCTION

Global shrimp farming production reached 8.9% of the total production in 2021. Indonesia accounts for approximately 7% of the total shrimp production globally and is ranked the fifth largest producer of cultivated shrimp worldwide (FAO 2020). Tiger prawn (*Penaeus monodon*) was the prima donna of fisheries commodities from 1990 to 1995. At that time, North Kalimantan was one of the areas that produced large quantities of tiger prawns. To date, tiger prawns have been extensively studied. Currently, the farming of *P. monodon* has been experiencing significant degradation in Indonesia, notably in North Kalimantan; from a total pond area of 140 thousand ha the annual production obtained is only 10 thousand tonnes (Ministry of Marine Affairs and Fisheries 2021). The failures of *P. monodon* farming are often found from the post-larvae's initial stocking phase until harvesting, with a common problem being the death of *P. monodon* post-larvae after stocking. Disease attack in the second month of the rearing period eventually causes failure in the extensive ponds. Several factors contribute to the decline in productivity, including a decrease in the carrying capacity of land and the emergence of various shrimp diseases that are difficult to cope with (Rukisah *et al.* 2019).

Various preventive efforts have been made, such as the use of antibiotics and herbal extracts, such as *Allium sativum* (Rahmani *et al.* 2023), *Centella asiatica* (Rukisah *et al.* 2019), *A. paniculatum, Cinnamomum kanehirae, Citrus aurantifolia, Psidium guajava,* and *A. sativum* (Citarasu *et al.* 2022). However, these efforts have not shown significant impacts on increasing production. Failure to address this issue can lead to significant economic losses for hatcheries and pond farmers. Therefore, a more effective cultivation system is required to maintain tiger prawn production. Studies have demonstrated the effectiveness of implementing biofloc systems in shrimp farming. This practice is becoming more widely used in shrimp aquaculture because it has demonstrated improvements in the quality of water, a stronger immune response, and enhanced disease resistance (Martínez-Córdova *et al.* 2015; Anand *et al.* 2017).

The biofloc system functions are based on the simultaneous cultivation of heterotrophic bacteria and algae in a confined environment, which results in the formation of flocs. This system fosters the development of heterotrophic bacteria that utilize organic carbon, thereby decreasing nitrogen levels. The equilibrium between carbon and nitrogen yields microbial proteins, which provide a dietary supply for various organisms. Biofloc particles are beneficial bacteria, including *Bacillus* sp. and *Lactobacillus* sp. (Naiel *et al.* 2021), as well as bioactive compounds such as carotenoids (Xu *et al.* 2013). The addition of biofloc can also result in a greater quantity of hemocytes and antioxidant status in shrimp (Khanjani *et al.* 2023). Microbial populations in biofloc treatment systems (BFT) safeguard water quality by facilitating the oxidation of organic matter (Sun *et al.* 2024), improve shrimp farming biosecurity by reducing the risk of various diseases (Avnimelech *et al.* 2013), and help prevent disease outbreaks, such as EMS, RMS, and EHP, through the use of a minimal water exchange system. In addition, the use of biofloc systems resulted in a reduction in the feed conversion ratio, increased final yield, increased enzyme activity in the hepatopancreas, and increased immune response (Bernardi *et al.* 2018).

Biofloc-based hatchery systems have shown positive outcomes in boosting survival rates, producing robust juveniles, lowering nitrogen metabolite levels, and decreasing production costs by reducing feed conversion ratios (Dauda *et al.* 2018). The biofloc system for *P. vannamei* shrimp has been widely used and has shown good results with increased productivity. However, more information on the implementation of biofloc for tiger prawn farming is still needed. The initial implementation of a biofloc system for tiger prawn farming in Bangladesh yielded remarkable results in increasing prawn growth and survival, improving water quality, increasing heterotrophic bacterial growth, and decreasing *Vibrio* bacterial concentrations (AftabUddin *et al.* 2020). Therefore, it is necessary to research biofloc technology for tiger prawns and determine appropriate procedures and treatments to apply this technology in North Borneo.

A major problem in biofloc system is the floc formation. Flocs that are formed tend to be unstable and less aggregated, coupled with the influence of water stirring by aerators causing flocs to not form properly. The addition of chitosan is expected to increase the aggregation of particles and microorganisms, forming flocs that can be consumed by shrimp. Chitosan is known to have the ability to reduce nitrite and BOD concentrations, control the population of *Vibrio* bacteria, and enhance shrimp immune system activity (Ruswahyuni *et al.* 2010; Liang *et al.* 2020). Chitosan is known to act as a coagulant (Iber *et al.* 2023). The biofloc system can be implemented for the cultivation of tiger prawns by incorporating chitosan into the water. Our hypothesis is that the addition of chitosan enhances floc formation, potentially leading to reduced feed requirements. This study aimed to assess the use of chitosan in tiger prawn farming using a biofloc system.

MATERIALS AND METHOD

Experimental Setup

The experiment followed a Completely Randomized Design (CRD), implementing three treatments with three replications. The treatments were: 1) AL (a control pond without biofloc); 2) ABF1 (a biofloc pond without chitosan); and 3) ABF2 (a biofloc pond with 100 mg/L chitosan). In accordance with the protocol of AftabUddin *et al.* (2020), each pond was subsequently stocked with 40 post-larvae (PL) of tiger prawn per 100 L.

Biofloc Pond Construction

Nine circular PVC ponds, each measuring 0.6 m in diameter and height, with a 100 L water volume, were used in this study. Aeration was set by using an L-shaped PVC pipe (1-inch diameter) positioned at the bottom of each pond, functioning as a lift aeration system. Each pipe was connected to an air hose (10 cm diameter) and a central blower (0.042 MPa) that supplied air throughout the experiment. Following AftabUddin *et al.* (2020), the ponds were disinfected before use by washing each pond with 100 mg/L chlorine and detergent, followed by filling up each pond with sterilized seawater to a depth of 10 cm. After 24 hours, the seawater was drained, and the ponds were rinsed. Aeration was maintained throughout the disinfection process, which was consistent with the methods described by Kumar *et al.* (2019).

Culture of Probiotics

All equipment used for probiotic culture was sterilized. Glassware were autoclaved, while fiber tubes, plastic buckets, and other non-glass items were disinfected with a 500 ppm chlorine solution. The culture medium was prepared using 3 kg of bran, 100 g of fish meal, 200 mL of molasses, 50 g of non-iodized salt, and 10 L of sterilized seawater. Seawater was first sterilized with 50 mg/L chlorine and left to stand overnight. After 24 hours, the solution was neutralized with 25 mg/L sodium thiosulfate and aerated for 24 hours. The chlorine levels were then checked to ensure complete neutralization. Sterilized seawater, bran, and fish meal (10 L) were boiled for 30 minutes for probiotic culture. Molasses and salt were added, and the mixture was poured into a culture vessel. Once the water temperature was below 35 °C, the 67 g of probiotic inoculant was added.

Biofloc Production

Materials such as dolomite ($Ca_2Mg(CO_3)_2$) (2 g/m^3), non-iodized salt (1.5 kg/m^3), commercial probiotics 5 mL/m3 (containing *Bacillus megaterium, Bacillus polymyxa, Lactobacillus plantarum, Nitrobacter winogradskyi, Nitrosomonas europaea*), molasses (250 mL/m^3), and wheat flour (250 g/m^3) were added to the maintenance pond containing 90 L sterilized seawater (salinity of 16 ppt) and aerated for 7 days. During the floc formation, probiotics and molasses were added to the pond daily, and at the same time chitosan (100 mg/L) was added to the ABF2 pond. Ponds can be used once a floc has formed, with a floc volume exceeding 5 mL/L (BPPP 2021).

Management and Stocking of Tiger Prawn

Post-larvae of tiger prawn (*P. monodon*) with a size of PL-10 (0.001 g) were obtained from a nearby hatchery and stocked at a density of 40 larvae per 100 L in each rearing pond. The prawn were fed commercial feed four times a day, at 07.00, 11.00, 19.00, and 23.00 hours, and made up 3% of their body weight. Probiotics were administered to each pond at a rate of 5 mg/m^3 at a 3-day interval. The water was replaced at a rate of 10% every two weeks or whenever there was a shift in the salinity level. The weight and length as growth parameters of the prawn were measured on the Day of Culture (DOC)-0 and at DOC-40.

Observation of the water's condition across the experimental duration

Water quality parameters, such as temperature, dissolved oxygen, and pH, were monitored daily throughout the experiment using a water testing device supplied by WTW. Water salinity was measured using a hand-held refractometer (Atago). Water quality parameters, such as nitrate (NO_3-N), nitrite (NO_2-N), and Total Ammonia Nitrogen (TAN), were tested every three nights using the spectrophotometric techniques outlined in the APHA guidelines (2005). The volume of floc was measured with a 1,000 mL Imhoff cone manufactured by Plastic Brand.

Counting Procedure of Bacteria

Total heterotrophic bacteria (THB) and *Vibrio*-like bacteria (VLB) counts were carried out biweekly throughout the entire duration of the experimental period. Random water samples were collected from the experimental and control ponds in sterile glass containers and then refrigerated at 4 °C until further analysis. Bacteriological analyses were conducted according to the methods outlined in APHA (2005) guidelines. To summarize, the water samples were diluted in a concentration range of 10^{-1} to 10^{-6} with a sterilized saline solution consisting of 2.5% NaCl (Merck). Samples (0.5 mL) of the respective dilutions were plated onto Zobell Marine Agar (ZMA, HiMedia) for THB, and onto thiosulfate-citrate bile salts-sucrose (TCBS, HiMedia) agar plates for VLB. Each analysis was performed in triplicate using the spread plate method. The inoculated plates were then incubated at 35 ^{0}C for 48 hours. Colonies were counted using a digital colony counter, and the results were expressed as colony-forming units (cfu/mL).

Data Analysis

The data were analyzed using SPSS 21 software. To identify variations in water quality, prawn growth, and bacterial levels between the treatments, a series of one-way ANOVA tests was conducted. If there was a significant difference between the treatments, Duncan's test was used to determine the best treatment. Prior to the analysis, all proportional data were transformed using the arcsine square root technique. The findings are presented as the mean values ± standard errors. Statistical significance was set at a threshold of $P < 0.05$.

RESULTS AND DISCUSSION

Growth Parameters

Notable variances ($P < 0.05$) were evident in the average body weight, average daily growth, specific growth rate, and protein efficiency ratio (Table 1). ABF2 (Biofloc with the addition of chitosan) treatment demonstrated notably higher body weight, daily growth, specific growth rate, and protein efficiency ratio than those in ABF1 (Biofloc without chitosan) and the control (AL) treatments ($P < 0.05$). Furthermore, ABF2 exhibited a significantly higher average length and a higher survival rate, specifically 68.33 ± 15.7%, surpassing ABF1 treatments and the control (AL) (Table 2). The feed conversion ratio in ABF2 was significantly lower ($P < 0.05$) than that in the control (AL) and biofloc treatments without chitosan (ABF1) (Table 1).

ABF2 demonstrated significantly higher growth and survival rates than those of other treatments, indicating that the combination of chitosan and probiotic supplementation positively influenced the growth performance and survival rate of *P. monodon*. These results are likely attributable to the synergistic effects of enhanced water quality and an increased population of heterotrophic bacteria. Previous studies have consistently shown that cultivating Penaeid shrimp in biofloc systems leads to higher survival and growth rates than those

Table 1 Growth performance of *P. monodon* comparison among treatments

Parameter	Treatment		
	AL	ABF1	ABF2
Average body weight (g)	0.24 ± 0.02^{a}	0.22 ± 0.06^{a}	0.30 ± 0.02^{b}
Average daily growth (ADG) (g/day)	0.00059 ± 0.001^{a}	0.00054 ± 0.000^{a}	0.00075 ± 0.001^{b}
Specific growth rate (SGR) (%/day)	13.6 ± 0.22^{a}	13.4 ± 0.10^{a}	14.3 ± 0.17^{b}
Survival rate (SR) (%)	65.83 ± 35^{a}	62.50 ± 9.1^{a}	68.33 ± 15.9^{a}
Food conversion ratio (FCR)	1.79 ± 0.9^{a}	1.69 ± 0.2^{a}	1.14 ± 0.2^{a}
Protein efficiency ratio (PER)	2.63^{a}	2.41^{a}	3.33^{b}

Notes: Data represent the mean values (± standard error) of three replicates. Mean values within the same row are marked with different superscripts indicate statistically significant differences ($P < 0.05$); AL = control; ABF1 = without chitosan; ABF2 = added with chitosan.

in traditional water-based systems (AftabUddin *et al*. 2020; Brito *et al*. 2013; Kumar *et al*. 2019). In the present study, the average daily growth and survival rates of *P. monodon* were slightly lower than those documented in similar biofloc systems (AftabUddin *et al*. 2020; Brito *et al*. 2013; Kumar *et al*. 2019). However, the feed conversion ratio (FCR) of *P. monodon* (1.14 - 1.69) in our biofloc system was better than the FCR range (1.42 - 2.16) reported by AftabUddin *et al*. (2020). Notably, the FCR from our study also outperformed the range (1.8 - 3.6) observed by Kumar *et al*. (2015) for *P. monodon* under varying protein levels and carbon sources.

In this study, the growth of tiger shrimp (*P. monodon*) was relatively slow, with an average daily growth rate of 0.54 - 0.75 mg/day (Table 1). This value is significantly lower (approximately 80% lower) than that in the study by AftabUddin *et al*. (2020), who reported a daily growth rate of 0.11 - 0.14 g/day. This difference is likely due to differences in the age and initial weight of the post-larvae (PL). This study used 10-day-old PL with an average weight of 0.002 g, whereas AftabUddin *et al*. (2020) used PL with an average weight of 0.18 g. In addition, *P. monodon* post-larvae are suspected to have not received adequate nutrition for weight gain. Tiger shrimp in the PL phase generally consume phytoplankton of *Skeletonema costatum* and *Chaetoceros* species, and as they aged, they consume zooplankton. In this study, *P. monodon* consumed artificial feed and flocs that formed. Although artificial feed was provided, it was only administered at 3% of the shrimp body weight. Another possibility is that plankton that grew in the biofloc media were not preferred or did not significantly influence shrimp growth, even though the floc volume formed was > 10 mL/L.

Rearing time can also affect shrimp growth, and shrimp rearing in this study lasted for 40 days. The growth of tiger shrimp during the first 40 days of DOC tended to be slower. Based on the growth curve published by the WHO, the daily growth of tiger shrimp is relatively varied; in the first 40 days of rearing, the growth of tiger shrimp ranges from 0 to 2 g, and accelerated growth occurred after 60 days of rearing. This study utilized commercial feed administered at 3% shrimp body weight, which contained 40% protein, 7% fat, 3% fiber, 13% ash, and 10% water throughout the rearing period. Effective feed utilization typically results in an FCR ranging from one to two. As the FCR value decreased, the efficiency increased. The biofloc system exhibited a reduced feed conversion ratio compared with the control treatment. The ANOVA test results showed a substantial difference in feed conversion ratio between treatments, with a P value less than 0.05. The ABF2 treatment had a notable impact on the feed conversion ratio. Overall, the FCR in this study was within a reasonable range. Although the FCR value indicates the efficiency of feed utilization, it cannot be used to calculate the quantity and quality of nutrients consumed by shrimp.

The direct effects of chitosan addition to biofloc media on the growth of tiger shrimp (*Penaeus monodon*) remain unclear. However, several studies have shown that chitosan can act as a coagulant and particle binder, thereby facilitating the formation of more stable microbial flocs and improving the water quality (Zaki *et al*. 2023). Improvements in the water quality can also improve shrimp growth. Niu *et al*. (2013) found that chitosan supplementation improved growth performance, survival, and immune function of tiger shrimp. Although this study focused on feed supplementation, the results demonstrated the potential of chitosan as a beneficial additive in tiger shrimp cultivation. In addition, Hermawan *et al*. (2019) reported that the addition of chitosan to feed increased the total number of haemocytes and resistance to salinity stress in white-leg shrimp (*Litopenaeus vannamei*). Although the species studied were different, these findings reveal a positive effect of chitosan on the shrimp's immune system. Although no research has specifically examined the effects of adding chitosan to biofloc media on the growth of tiger shrimp, the use of chitosan in the maintenance media is thought to improve water quality and shrimp health. Chitosan helps to form more stable bioflocs and improves water quality, which ultimately supports the growth and survival of tiger shrimp.

Water Quality Parameters

Water quality parameters of the treatments did not show statistically significant differences in salinity, water temperature, and total ammonia nitrogen (TAN), nitrate-N (NO_3-), and nitrite-N (NO2) concentrations ($P > 0.05$) (Table 2). The culture medium of *P. monodon* was consistent with established experimental parameters. Variations in pH, dissolved oxygen (DO), and floc volume levels were noted between the control (AL) and the biofloc treatments.

The average concentration of DO in the AL

Table 2 Influence of biofloc on the physical and chemical properties of culture water throughout the experiment

Parameters	Range of water quality			Average of water quality		
	AL	ABF1	ABF2	AL	ABF1	ABF2
Temperature (^{o}C)	27 - 30	27 - 30	27 - 30	28.59 ± 0.02^{a}	28.63 ± 0.1^{a}	28.58 ± 0.03^{a}
Salinity (ppt)	16 - 18	16 - 18	16 - 18	16.35 ± 0.26^{a}	16.17 ± 0.51^{a}	16.49 ± 0.51^{a}
pH	7.53 - 8.40	7.67 - 8.40	7.67 - 8.40	7.99 ± 0.03^{b}	7.93 ± 0.02^{a}	7.92 ± 0.02^{a}
TAN (mg/L)	0.08 - 1.50	0.08 - 1.33	0.08 - 1.67	1.05 ± 0.27^{a}	0.92 ± 0.10^{a}	1.02 ± 0.18^{a}
DO (mg/L)	2.8 - 5.5	3.3 - 6.1	3.3 - 6.3	4.54 ± 0.09^{a}	4.78 ± 0.05^{b}	4.95 ± 0.07^{c}
Nitrite (mg/L)	0 - 0.42	0 - 0.08	0 - 0.17	0.04 ± 0.01^{a}	0.03 ± 0.02^{a}	0.03 ± 0.00^{a}
Nitrate (mg/L)	0 - 6.67	0 - 3.33	0 - 3.33	1.33 ± 0.63^{a}	0.83 ± 0.40^{a}	0.80 ± 0.14^{a}
Floc (mg/L)	0 - 4	1.33 - 25	0.57 - 17	3.09 ± 0.33^{a}	16.60 ± 5.86^{b}	12.80 ± 3.75^{c}

Notes: Data represent the mean values (± standard error); Values within the same row marked with different superscripts indicate statistically significant differences ($P < 0.05$); AL = control; ABF1 = biofloc without chitosan; ABF2 = biofloc with the addition of chitosan.

treatment (4.54 ± 0.09) was lower than that in ABF1 (4.78 ± 0.05 mg/L) and ABF2 (4.95 ± 0.07 mg/L). In the biofloc tanks, the volume of flocs increased substantially, which was notably higher than that observed in the control. This escalation in floc volume continued throughout the experiment, with the floc color progressively deepening to a brownish hue. In ABF1, the largest floc volume achieved was 25 mL/L, with an average volume of 16.60 ± 5.86 mL/L.

The temperature, salinity, dissolved oxygen, and pH levels of the water in all treatments (control and biofloc) met the required parameters for *P. monodon*, which is consistent with earlier research (Santhana Kumar *et al.* 2018; AftabUddin *et al.* 2020). This study found that the oxygen concentration was lower in the AL (control) treatment than in the biofloc treatments. The biofloc system can increase the dissolved oxygen more effectively than the control tanks, even though all treatments use aeration. This is because of the presence of beneficial microorganisms in the biofloc system.

Microorganisms, such as heterotrophic bacteria, can consume organic matter and excess nutrients in water and convert them into biomass, which can be used as a supplementary food source for shrimp. This process aids in preserving water quality and boosts oxygen levels in the water. In addition, the biofloc system fosters phytoplankton growth, which can generate oxygen via photosynthesis. Despite employing aeration in all treatment methods, the biofloc system can be more effective at enhancing the oxygen levels in the water, mainly because of the role of bacteria in reducing organic matter, the balance of the microbial ecosystem that maintains the stability of water quality, and the ability of flocs to act as oxygen buffers (Avnimelech 2015; De Schryver *et al.* 2018; Crab *et al.* 2007). The dissolved oxygen level in all treatments was adequate for the survival rate and growth of *P. monodon*. Although the oxygen level did not reach 5 mg/L, the DO value in the study was still sufficient to support the survival and growth of *P. monodon* according to McGraw *et al.* (2001) and Panigrahi *et al.* (2020) which stated that DO concentration between 4 - 5 mg/L was still adequate for *P. monodon* as long as the aeration system is still available in the maintenance media. Continuous aeration in the biofloc tank is important for maintaining adequate DO levels in all treatments throughout the experimental period (Maulianawati *et al.* 2024).

The pH levels in ABF1 and ABF2 (biofloc treatments), which range from 7.67 to 8.40, were lower than the pH of the control treatment (7.99). The lower pH concentration in ABF1

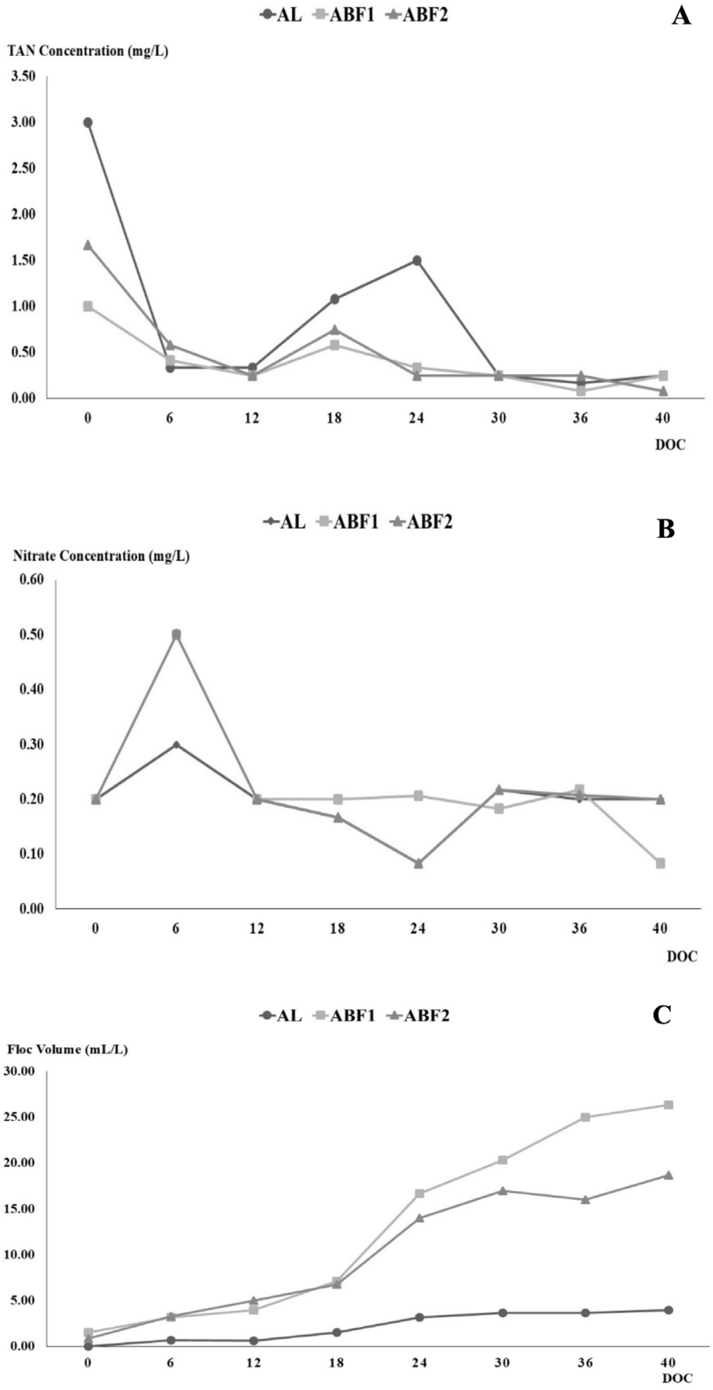

Figure 1 The fluctuation of water quality parameters
Notes: (a) TAN; (b) nitrate-N; and (c) floc volume were measured over a specified time period; AL = control; ABF1 = biofloc without chitosan (ABF1); ABF2 = biofloc with the addition of chitosan.

and ABF2 (biofloc treatments) compared to the control ponds is typically due to the activity of heterotrophic bacteria (HB) in the biofloc system. In water, heterotrophic bacteria consume nutrients and organic matter, resulting in the production of carbon dioxide compound (CO_2) as a waste product. Following the dissolution of carbon dioxide in water, it comes into contact with water molecules (H_2O) and reacts to produce carbonic acid (H_2CO_3), a reaction that can decrease the pH of water (Ahmed Alkhamis *et al.* 2023).

In this study, TAN concentrations (0.08 - 1.67 mg/L) were somewhat higher than those reported in earlier biofloc system studies on *P. monodon*. This finding has significant implications for our understanding of aquatic systems and their potential impacts on aquatic life. Ammonia, the main nitrogenous waste product of aquatic animals, is toxic if it is not adequately removed from the environment. Elevated ammonia levels in water impair the ability of organisms to excrete ammonia, leading to a proportional increase in the ammonia levels in the blood of fish and other aquatic species. Although the exact mechanism of ammonia toxicity in fish remains unclear, several physiological and histological effects have been linked to high ammonia concentrations in blood and tissues. These effects include increased blood pH, disruption of enzyme systems and membrane integrity, increased water intake, elevated oxygen consumption, gill damage, and histological changes in various internal organs (Zhao *et al.* 2020).

Meanwhile, a low concentration of nitrite (Table 1) and variation in the concentration of nitrate (Fig. 1b) were observed in our study. Among the harmful nitrogenous compounds used in aquaculture, nitrite toxicity is primarily responsible for hypoxia. The effects of nitrites are linked to its disruption of oxygen transport, resulting from the formation of meta-hemocyanin when combined with hemocyanin, a compound incapable of effectively transporting oxygen to tissues, leading to increased mortality (Wasielesky *et al.* 2017). In biofloc technology (BFT) systems, excessive nitrite accumulation may lead to the suppression of specific enzymes, notably carbonic anhydrase, which is a crucial metalloenzyme involved in ion transport within fish and shrimp tissues and organs (Abakari *et al.* 2021).

On the other hand, nitrate poses a relatively lower toxicity threat to fish and shrimp, except at very high concentrations exceeding 100 mg/L and when there is a combined action of nitrates and other nitrogenous substances, which can produce synergistic effects (Wasielesky *et al.* 2017). In biofloc systems, which are designed as zero-exchange systems, nitrate toxicity is a critical concern because of the potential for nitrate accumulation to reach harmful or lethal levels. Although instances of nitrate toxicity are uncommon, research has shown that nitrates can become problematic in tilapia farming, particularly at concentrations between 600 mg/L and 700 mg/L. At these levels, nitrates primarily affect the fish feed intake (Prates *et al.* 2024).

In this study, the nitrite and nitrate levels remained within the safe range for the growth and survival of *P. monodon*. Biofloc Technology (BFT) systems rely on the breakdown and recycling of nitrogenous compounds derived from uneaten feed and metabolic waste produced by tilapia and shrimp. These compounds are processed by diverse microbial communities, including algae, autotrophic bacteria, and heterotrophic bacteria, each contributing uniquely to nitrogen transformation (Emerenciano *et al.* 2017). The biofloc system contains various nitrogen forms, such as ammonia (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), total ammonia nitrogen (TAN), and total Kjeldahl nitrogen (TKN), which are metabolized by microorganisms according to their specific metabolic pathways (Abakari *et al.* 2021). Under aerobic conditions, nitrifying bacteria, primarily autotrophic bacteria, play a critical role in converting toxic ammonia into less harmful nitrates. This nitrification process is carried out in two stages: first, ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) and archaea (AOA), with hydroxylamine as an intermediate product (Martinez-Cordova *et al.* 2015). The key bacterial genera involved in this process include *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus,* and *Nitrospira*, all of which have been classified into specific taxonomic groups (Kumar *et al.* 2020). These microorganisms ensure efficient conversion of nitrogenous waste, maintain water quality, and support the health of aquatic organisms in biofloc systems.

Schveitzer *et al.* (2013), Panigrahi *et al.* (2020), and Khanjani *et al.* (2023) highlighted that shrimp cultivation in biofloc systems enhances the water quality through the presence of beneficial microorganisms. Khanjani *et al.* (2023) further noted a marked rise in nitrogen metabolites, including pH, ammonia, nitrite, and nitrate, in biofloc-based nurseries used for rearing *P. vannamei*. Notably, the total ammonia nitrogen (TAN) levels were significantly reduced in the biofloc treatments supplemented with probiotics compared to those in the control group. In such systems, heterotrophic bacteria play a key role in assimilating the majority of ammonia, whereas nitrate (NO_3-N) in the biofloc system supports the proliferation of phytoplankton.

Total Counts of Heterotrophic and *Vibrio*-like Bacteria

The concentration of *Vibrio*-like bacteria in the control tank was substantially greater (8.1 ± 5.0 (×10^3 cfu/mL)), compared with the levels found in ABF1 (3.4 ± 0.09 (×10^2 cfu/mL)) and ABF2 (0.5 ± 0.4 (×10^3 cfu/mL)) treatments (Table 3). In the ABF2 sample, THB (Total Heterotrophic Bacteria) counts were notably higher between DOC 10 and DOC 30, with a concentration of 1.6 ± 0.23 (×10^5 cfu/mL), whereas the ABF1 sample yielded 5.7 ± 1.4 (×10^4 cfu/mL) and the AL sample had 2.7 ± 2.3 (×10^4 cfu/mL) at the final sampling. The concentration of *Vibrio*-like bacteria (VLB) in the control tank (AL) was significantly higher (8.1 ± 5.0 (×10^3 cfu/mL)) than that in ABF1 3.4 ± 0.09 (×10^2 cfu/mL) and ABF2 0.5 ± 0.4 (×10^3 cfu/mL) treatments (Table 3). Considerable variation in THB levels was observed across the biofloc treatments, with a statistically significant difference observed at a P value of 0.05. The VLB concentrations were substantially lower in ABF1 than those in other biofloc treatments, and this variation was evident across multiple sampling days, with statistical significance at $P < 0.05$.

Heterotrophic bacteria use complex organic compounds as carbon sources for growth and metabolic processes, resulting in a specific number of bacteria. Heterotrophic bacteria require organic compounds such as glucose and other complex substances as energy and carbon sources, which are used to construct their cell structures. Bacteria that obtain nutrients from external sources are crucial for the breakdown of organic substances, reusing vital elements such as carbon, nitrogen, and phosphorus, as well as numerous other ecological

Table 3 Total heterotrophic bacteria and total *Vibrio*-like bacteria counts

Treatment	Total Heterotrophic Bacteria (×10^4 cfu/mL)			Total *Vibrio*-like Bacteria (×10^3 cfu/mL)		
	DOC 10	DOC 20	DOC 30	DOC 10	DOC 20	DOC 30
AL	0.9 ± 06^{a}	2.6 ± 1.1^{b}	2.7 ± 2.3^{a}	2.1 ± 1.5^{b}	1.1 ± 1.2^{a}	8.1 ± 5.0^{b}
ABF1	1.3 ± 1.8^{a}	0.7 ± 0.6^{a}	5.7 ± 1.4^{b}	0.053 ± 0.007^{a}	1.4 ± 1.2^{a}	0.34 ± 0.958^{a}
ABF2	2.6 ± 1.5^{b}	15 ± 4.1^{c}	16 ± 2.3^{c}	3.0 ± 0.3^{c}	2.2 ± 1.4^{b}	0.5 ± 0.4^{a}

Notes: Data represent the mean values (± standard error); Values within the same column marked with different superscripts indicate statistically significant differences ($P < 0.05$); AL = control; ABF1 = biofloc without chitosan; ABF2 = biofloc with the addition of chitosan.

processes (Wang *et al*. 2021; Maulianawati *et al*. 2021; Purnomo *et al*. 2019).

Heterotrophic bacteria play a crucial role in biofloc systems. Cultivation operated by minimizing water exchange, such as in biofloc systems, produces many organic compounds. If not managed, these organic compounds can lead to a decline in water quality. Heterotrophic bacteria degrade organic compounds (Ferreira *et al*. 2021; Khanjani *et al*. 2022).

Results of our study indicated that the concentration of THB in ABF2 was greater than that in the control, suggesting that the combined addition of chitosan and probiotics boosted the growth of heterotrophic bacteria. Compared to the control, the levels of ABF1 THB were lower in the DOC 20. Several factors, including the dynamics of the bacterial population in the culture medium, likely caused the low THB levels in ABF1. The presence of chitosan may have influenced bacterial regeneration in the culture medium. Chitosan is a polysaccharide derived from chitin and is known for its antimicrobial properties and ability to interact with microorganisms in various ways (Asiri *et al*. 2022). Some studies have shown that chitosan can function as a prebiotic, providing benefits for probiotic mobilization. Moreover, chitosan has also been reported to increase the enzymatic activity of probiotics and help improve the microenvironment that supports probiotic growth in the digestive system or in biofloc media (Guo *et al*. 2021; Shitu *et al*. 2022).

The concentration of THB in the biofloc system with the addition of chitosan (ABF2) was higher than that of the control. However, the average concentration of ABF1 was close to the THB concentration in a previous study of AftabUddin *et al*. (2020). Meanwhile, the THB concentrations in AL and ABF2 were higher than those recorded in the study of AftabUddin *et al*. (2020). The THB concentration in this study was lower than that reported by Kumar *et al*. (2019), where additional carbon sources were provided in the treatments. Adding chitosan to the biofloc media did not significantly affect the increase in the THB concentration ($P > 0.05$), but provided better results than other treatments, in which the THB value in the ABF2 treatment tended to increase significantly.

CONCLUSION

Chitosan addition to the biofloc system resulted in a substantial increase in floc volume and a decrease in VLB populations, while significantly lowering THB. There were substantial differences in the weight, average daily gain, and slaughter growth rates among the treatment groups. No notable discrepancies were observed for food conversion ratio, length, survival rate as well as for total ammonia nitrogen, nitrite, and nitrate concentrations. Biofloc technology combined with chitosan supplementation improves the water quality and microbial community, ultimately enhancing the growth performance of *P. monodon*.

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