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The influence of biofloc system on *Vibrio* composition, the growth and the gut microvilli performance of the Pacific white shrimp P*enaeus vannamei*

Pengaruh sistem bioflok pada komposisi *Vibrio*, performa pertumbuhan, dan mikrovili usus udang vaname *Penaeus vannamei*

Muhamad Gustilatov[1], Widanarni Widanarni[1*], Julie Ekasari[1], Pande Gde Sasmita Julyantoro[2], Diana Elizabeth Waturangi[3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, West Java 16680, Indonesia
[2]Department of Aquatic Resources Management, Faculty of Marine Science and Fisheries, University of Udayana, Denpasar, Bali, 80361, Indonesia
[3]Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jenderal Sudirman no. 51, Jakarta, 12930, Indonesia
*Corresponding author: widanarni@apps.ipb.ac.id

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ABSTRACT

Biofloc technology has been shown to have a positive impact on shrimp culture by controlling pathogenic *Vibrio*. This study aimed to analyze the effect of biofloc on the *Vibrio* composition in water and shrimp gut, as well as the growth performance and microvilli of Pacific white shrimp (*Penaeus vannamei*). Shrimp post-larvae measuring 0.66 ± 0.02 g were reared in a glass aquarium (working volume 22 L) of 6 units with a density of 150 shrimp m^{-3} for 28 days. The treatments included rearing in the biofloc system with C/N ratio 10, and without a biofloc system as a control. The results showed that *Vibrio* was highly prevalent in the control gut (4.13%) and biofloc water (3.77%), but only a few were found in the control water (0.16%) and biofloc-treated gut (0.11%). *V. hepatarius* (1.10%) and *V. nereis* (1.06%) were found to dominate the *Vibrio* bacterial community in the biofloc system maintenance media, while *Vibrio* sp. Hep-1b-8 (2.26%) and *V. parahaemolyticus* (0.80%) known as a pathogenic bacteria dominated the control shrimp gut. The biofloc system significantly increased the digestive enzyme activity, growth performance, and microvilli length in the shrimp gut. In conclusion, the application of a biofloc system in shrimp culture can affect the composition and abundance of bacterial communities in both the culture environment and the shrimp gut, and improve growth performance with higher digestive enzyme activity and longer microvilli in the gut.

Keywords: biocontrol, biofloc, microbiota, shrimp, *Vibrio*

ABSTRAK

Teknologi bioflok menunjukkan dampak positif pada kegiatan budidaya udang vaname dengan mengendalikan bakteri patogen salah satunya *Vibrio*. Penelitian ini bertujuan untuk menganalisis efek bioflok terhadap komposisi *Vibrio* pada air dan usus udang, serta kinerja pertumbuhan dan mikrovili udang vaname (*Penaeus vannamei*). Udang post-larva berukuran 0.66 ± 0.02 g dipelihara pada akuarium kaca (volume air 22 L) sebanyak 6 unit dengan kepadatan 150 ekor m^{-3} selama 28 hari. Perlakuan meliputi pemeliharaan udang pada sistem bioflok dengan rasio C/N 10, dan tanpa sistem bioflok sebagai perlakuan kontrol. Hasil pengamatan menunjukkan kelimpahan *Vibrio* yang cukup tinggi pada usus perlakuan kontrol (4.13%) dan air perlakuan bioflok (3.77%), tetapi sangat sedikit ditemukan pada air perlakuan kontrol dan usus perlakuan bioflok. Selanjutnya, *V. hepatarius* (2.26%) dan *V. nereis* (1.06%) terdeteksi mendominasi komunitas bakteri *Vibrio* di media pemeliharaan pada sistem bioflok, sedangkan *Vibrio* sp. Hep-1b-8 (2.26%) dan *V. parahaemolyticus* (0.80%) mendominasi usus udang vaname pada perlakuan kontrol. Sistem bioflok juga mampu meningkatkan aktivitas enzim pencernaan, performa pertumbuhan, dan panjang mikrovili usus secara signifikan ($P<0.05$). Kesimpulan dari penelitian ini adalah penerapan sistem bioflok pada budidaya udang vaname mampu memengaruhi komposisi dan kelimpahan komunitas bakteri *Vibrio* pada lingkungan budidaya maupun pada usus udang, serta meningkatkan performa pertumbuhan dengan aktivitas enzim pencernaan yang lebih baik dan mikrovilli usus yang lebih panjang.

Kata kunci: bioflok, biokontrol, microbiota, udang vaname, *Vibrio*

INTRODUCTION

Global aquaculture production increased by 4.5% between 2010 and 2020, with Pacific white shrimp (*Penaeus vannamei*) being one of its primary commodities, with a production of 5.81 million tons in 2020 (FAO, 2022). According to KKP (2020), Indonesia was one of the world's major shrimp producers, with 1.053.206 tons produced in 2019 and contributing more than $2 billion USD to worldwide exports (FAO, 2022). Shrimp production has the highest economic value in Indonesia among all aquaculture commodities, followed by tilapia (*Oreochromis* sp.) and catfish (*Clarias* sp.). The potential for expanding the white shrimp culture industry is still considerable, given the broad target market, which includes Japan, the United States, the United Kingdom, and other countries that import shrimp (FAO, 2020).

Despite a significant increase in production of almost 120% in 2020 compared to 2010, the intensification of white shrimp culture remains a challenge due to the decrease in water quality caused by excessive feed and shrimp metabolism waste. This leads to instability in the microbial composition of the water, which increases the threat of bacterial diseases caused by various *Vibrio*, such as *V. harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, and others, commonly known as vibriosis (Valente & Wan, 2021). *Vibrio* are naturally occurring as opportunistic pathogens in the marine and estuarine environment that can infect white shrimp under poor environmental and low shrimp immunity, causing various abnormalities such as gill necrosis, lethargy, loss of appetite, and mass mortality reaching up to 100% in acute conditions (Raja *et al*., 2017; Abdel-latif *et al*., 2022).

The dominance of *Vibrio* in the digestive tract of white shrimp is also suspected to be one of the agents causing white feces disease (WFD) (Kurniawinata *et al*., 2022; Subash *et al*., 2023). Apart from vibriosis, infections caused by *V. parahaemolyticus* strains containing the pirA and pirB toxins have been reported to cause acute hepatopancreatic necrosis disease (AHPND) or early mortality syndrome (EMS). These infections have gained global attention due to their widespread occurrence, starting with the first EMS outbreak in China in 2009 and rapidly spreading to other countries such as Vietnam (2010), Malaysia (2011), Thailand (2012), Mexico (2013), and others. AHPND has also resulted in significant losses for the aquaculture industry worldwide (Kumar *et al*., 2020).

Shinn *et al*. (2018) estimated the economic losses caused by AHPND to be approximately $4 billion USD in the Asian region between 2009 and 2018, with a total more than $44 billion USD worldwide (Tang & Bondad-reantaso, 2019). Besides probiotics as a solution to prevent vibriosis disease (Wang *et al*., 2018; Kewcharoen & Srisapoome, 2019; Torpee *et al*., 2021), the application of biofloc technology is also known to have a positive impact on shrimp culture activity. Biofloc technology converts nitrogen from feed and metabolic waste into microbe biomass by adding organic carbon sources, this process creates biofloc, which is an aggregate of heterotrophic bacterial biomass, phytoplankton, fungi, and other organisms (Avnimelech, 2009).

According to previous studies, biofloc has been shown to enhance the immune response of white shrimp (Ekasari *et al*., 2014; Panigrahi *et al*., 2018) and function as a biocontrol agent for pathogenic bacteria. Biofloc can produce extracellular compounds such as bromophenol, poly-β-hydroxybutyrate, carotenoids, chlorophyll, amino acids, and other compounds that can decrease the virulence factors of bacteria, modulate gut microbiota structure, and enhance the shrimp gut microvilli height (Crab *et al*., 2010; Silva *et al*., 2016; Qiao *et al*., 2020). Additionally, our previous research showed that biofloc can lower the density and bacterial biofilms activity, increase the immune response, and enhance the resistance of white shrimp against *V. parahaemolyticus* infection (Gustilatov *et al*., 2022). According to Tepaamorndech *et al*. (2020) and Schveitzer *et al*. (2020), biofloc can initiate a shift in bacterial dominance in the aquaculture environment and affect the diversity of microbiota in the shrimp gut.

The change in microbiota composition is related to the modulation of various functions, including the suppression and competition with populations of pathogenic bacteria and the enhancement of shrimp health with an appropriate environmental and gut microbiota composition (Xiong, 2018). Suitable microbiota conditions in the environment and gut, along with minimal pathogens, are essential in preventing disease outbreaks and maintaining good shrimp growth performance. Based on the research findings, it indicates the benefits of biofloc application as a biocontrol agent for pathogenic bacteria and modulation of the environmental microbiota and

gut microbiota composition of white shrimp. The role of biofloc in influencing the microbiota especially *Vibrio* composition as a major in shrimp in the environment and gut, and its relationship with growth performance and microvilli of white shrimp, needs further investigation. Therefore, this study aims to analyze the application of biofloc in influencing the *Vibrio* composition of water and gut microbiota, as well as the growth performance and microvilli of white shrimp.

MATERIALS AND METHODS

Experimental Design and Biofloc Preparation

In this study, a completely randomized design (CRD) with two treatments and three replications was used. The control treatment involved rearing shrimp without a biofloc system, while the biofloc treatment involved rearing shrimp with a biofloc system. The biofloc used for the initial shrimp maintenance (biofloc inoculants) came from a biofloc in shrimp culture container, where molasses was used as the source of organic carbon. During the shrimp culture process, molasses was added once a day, two hours after the morning feeding, with a C:N ratio of 10.

To determine the amount of carbon needed, the De Schryver *et al*. (2008) carbon requirements scheme was used, assuming a 40% protein content of the feed, 16% nitrogen in protein, around 85% nitrogen excretion, and approximately 38% carbon content in molasses. Based on these values, approximately 14.3 grams of molasses was required for every 10 grams of feed given. Biofloc inoculants were added to the biofloc treatment aquarium by adding 25% inoculant to 75% seawater.

Shrimp Maintenance

The experiment involved rearing post-larvae 10 white shrimp to an average weight of 0.66 g before moving the treated group to aquariums with an initial density of 150 individuals/m^3 (33 shrimp per aquarium). The shrimp were fed four times a day, at 7:00, 11:00, 15:00, and 19:00, at an 8% feeding rate. During maintenance, molasses was given to the shrimp aquarium once per day, two hours after the morning meal. The treatment had an estimated C:N ratio of 10, whereas the control group received no molasses. The white shrimp were grown for 28 days. Every three days, water quality measures such as dissolved oxygen, pH, and salinity were also monitored, while total ammonia nitrogen, nitrite, nitrate, and total suspended solids were observed at the beginning, middle, and end of the cultivation at the Environmental Laboratory, Department of Aquaculture, Bogor Agricultural University, following APHA (2005) standards.

Parameters of observation

Microbiota composition in water and shrimp gut

At the end of the maintenance period, a 1 L sterile sample bottle was used to collect water samples, which were then filtered using a 0.22 µm millipore filter paper (Merck, Germany) under a vacuum. The shrimp intestines were then dissected and aseptically collected. The filter paper and intestine samples were separately placed in 2 mL microtubes and treated with DNA Shield (Zymo, US) for DNA preservation. The samples were stored in a -80°C freezer for subsequent DNA extraction. Sequencing preparation included DNA extraction from the water and shrimp intestine samples, PCR amplification, quantification and mixing of PCR products, purification of PCR products, and library preparation.

NGS (Next Generation Sequencing) technology was used to analyze the genomic DNA (Kurniawinata *et al*., 2021). The PCR amplification of the 16S rRNA gene in the V3-V4 region was performed using the 515F-806R primers (5'-GTGCCAGCMGCCGC-GGTAA-3' and 5'-GGACTACHVGGGTWTCTAAT-3'), which were linked to barcodes. Equal amounts of PCR products from each sample were collected, end-repaired, A-tailed, and then ligated with Illumina adapters. The resulting library was sequenced on the Illumina paired-end platform to generate 250bp paired-end raw reads. The quantified library will be collected and sequenced on the Illumina platform, according to the effective library concentration and the required amount of data. The data obtained were analyzed using Microsoft Excel 2013 and tabulated using Origin 2022 software.

Shrimp growth performance

The shrimp biomass was measured at the beginning and end of the maintenance period. Sampling was done before feeding when the shrimp gut was empty. Specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) parameters were calculated using the equation according to Li *et al*. (2021):

$$SGR\ (\%/\ day) = \frac{\ln W_t - \ln W_0}{t} \times 100$$

$$FCR = FCI/(F_t - F_0 - F_d)$$

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Note:

W_t = Average weight of the shrimp at the end of the maintenance period (g)

W_0 = Average weight of the shrimp at the beginning of the maintenance period (g)

t = duration of the maintenance period (day)

FCI = Amount of feed consumed (g)

F_t = Final shrimp biomass (g)

F_0 = Initial shrimp biomass (g)

F_d = Observed dead biomass (g)

N_t = Number of shrimp at the end of the maintenance period

N_0 = Number of shrimp at the beginning of the maintenance period

Digestive enzyme

The measurement of enzyme activity in the digestive tract of shrimp includes the activity of amylase, protease, and lipase enzymes. Sampling of the shrimp digestive tract was done at the end of the treatment and in an empty gut condition. The shrimp digestive tract was collected using aseptic techniques and transferred to a sample bottle with 0.5 g. After that, the samples were stored in a deep freezer at -80°C for later analysis of digestive enzyme activity, which followed method by Muttharasi *et al*. (2021).

Shrimp gut histology and microvilli height

Histopathology of the gut was observed at the end of the rearing period. The gut tissue was aseptically collected, fixed in Bouin's solution for 24 hours, and then cut into 1×1 cm pieces with a thickness of 3-5 mm. The subsequent steps involved dehydration, clearing, embedding, blocking paraffin, sectioning, and staining (Munaeni *et al*., 2020). Microvilli length in the gut was then observed using a microscope (Olympus CX31) with IndomicroView software.

Statistical Analyses

The obtained data were tabulated using Microsoft Excel 2013 and Origin 2022 software. The data analysis of growth performance, digestive enzyme activity, and microvilli length in the gut was conducted using analysis of variance (ANOVA) with SPSS version 20. If a significant difference was found, a further test using the Tukey test with a 95% confidence interval was performed. The diversity analysis of microbiota in the culture media and shrimp digestive tract, and gut histology was done descriptively.

RESULTS AND DISCUSSION

Result

Water and shrimp gut bacterial composition and Vibrio abundance

The study investigated the impact of biofloc on the bacterial composition of rearing water and shrimp gut samples. A heatmap displaying the 25 most prevalent genera for each sample treatment was produced (Figure 1), with the color of the heatmap indicating the relative abundance of the genus (darker red indicates higher relative abundance). The findings indicated that the biofloc treatment resulted in differences in the dominant genus composition compared to the control. The control water was dominated by Flavilitoribacter, Phaeodactylibacter, Lewinella, Kriegella, and Rubinisphaera genera, with relative abundance of 7.31%, 6.39%, 4.72%, 2.13%, and 1.76% respectively.

On the other hand, the biofloc water was dominated by *Hoeflea*, *Vibrio*, *Aliiroseovarius*, *Ruegeria*, and *Novipirellula* genera, with respective relative abundance of 3.89%, 3.77%, 2.26%, 1.64%, and 1.61%. In the control gut treatment, the *Klebsiella, Vibrio, Kriegella, Streptomyces*, and *Maribacter* genera dominated with relative abundance of 7.88%, 4.13%, 2.61%, 2.24%, 1.72% respectively. Finally, the biofloc gut sample was dominated by *Sedimentitalea, Aliiroseovarius, Ruegeria, Roseovarius*, and *Sulfitobacter* genera, with respective relative abundance of 7.55%, 5.45%, 4.61%, 3.11%, and 2.63%. The *Vibrio* was highly prevalent in the control gut (4.13%) and biofloc water (3.77%), while the control water and biofloc gut samples had low relative abundance, 0.16% and 0.11% respectively (Figure 2).

Water and shrimp gut Vibrio composition and relative abundance

The analysis of *Vibrio* relative abundance in the shrimp gut and shrimp rearing water revealed that *Vibrio* was prevalent in the biofloc water and control gut, but not in the control water and biofloc gut samples (Figure 3). Moreover, the dominant *Vibrio* species in the biofloc water sample were *V. hepatarius* (1.10%) and *V. nereis* (1.06%), whereas in the control gut the dominant species were *Vibrio* sp. Hep-1b-8 (2.26%) and *V. parahaemolyticus* (0.80%)

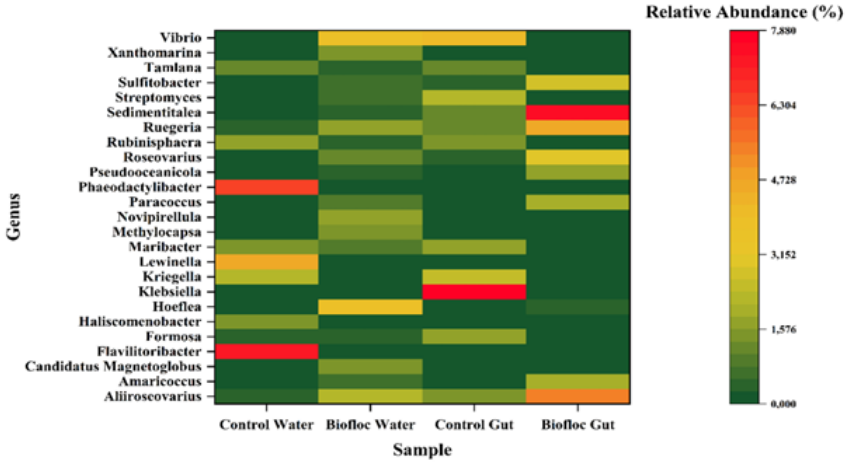

Figure 1. Relative abundance heat-map of the top 25 genera in rearing water and shrimp gut.

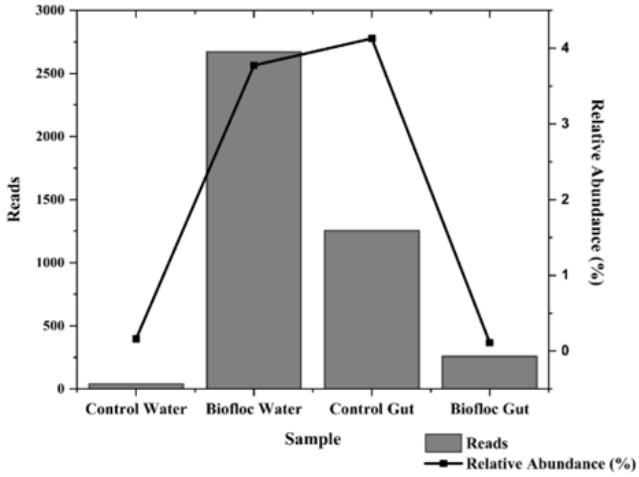

Figure 2. The *Vibrio* relative abundance in rearing water and shrimp gut.

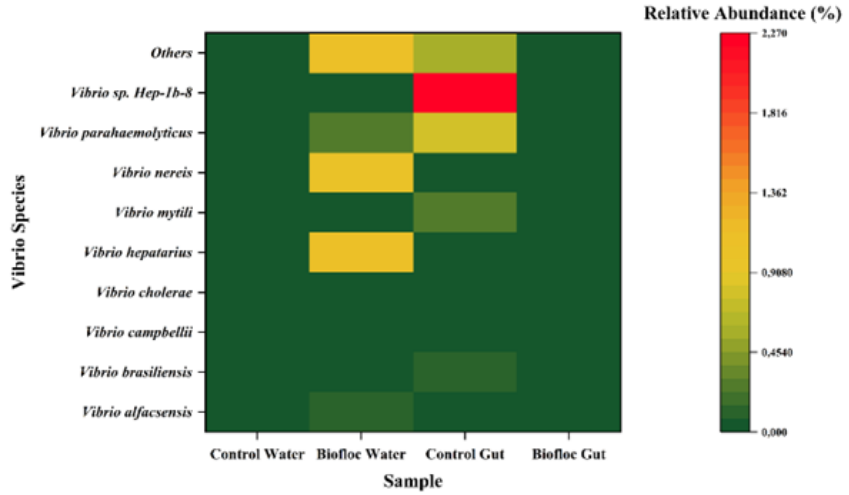

Figure 3. *Vibrio* composition and relative abundance heat-map in rearing water and shrimp gut.

Shrimp digestive enzyme activity and growth performance

The results of the examination on the shrimp digestive enzymes activity following 28 days of maintenance in the biofloc system revealed a significant increase in the activity of lipase and protease, compared to the control treatment (P<0.05) (Table 1). Although there was no significant difference compared to the control treatment, amylase activity exhibited a similar pattern. Furthermore, growth performance indicators, as shown by the SGR and FCR revealed significantly better results in the biofloc system for shrimp rearing compared to the control treatment (P<0.05). Finally, no significant difference was found in the SR parameter.

Shrimp gut histology and microvilli height

The biofloc system is able to elongate the microvilli of the shrimp gut, thereby increasing its surface area. This is demonstrated in the histological image of the shrimp gut in Figure 4, which reveals denser and longer microvilli. Additionally, Figure 5 displays data indicating that the length of the microvilli was significantly higher in the biofloc treatment at 76.47 µm compared to the control treatment, which measured 29.76 µm (P<0.05).

Table 1. Digestive enzyme activity and growth performance of shrimp reared in the biofloc system.

Parameters	Control	Biofloc
Amilase (IU/mL)	5.96 ± 0.38	8.60 ± 0.78
Lipase (IU/mL)	0.11 ± 0.00	0.21 ± 0.00*
Protease (IU/mL)	0.17 ± 0.00	0.42 ± 0.02*
SGR (%)	3.80 ± 0.10	5.15 ± 0.25*
FCR	1.78 ± 0.07*	1.20 ± 0.01
SR (%)	91.75 ± 0.85	94.45 ± 5.55

Note: *within the same column indicate significantly higher value (P< 0.05). Each treatment has three replications. SGR: specific growth rate, FCR: feed conversion ratio, SR: survival rate.

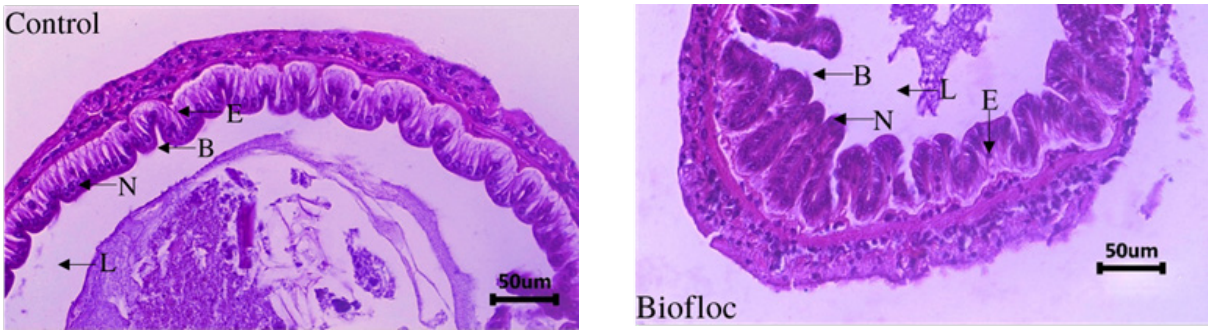

Figure 4. Shrimp gut histology from control (left) and biofloc (right) treatment. L: lumen, B: brush border, N: nuclei, E: epithelium.

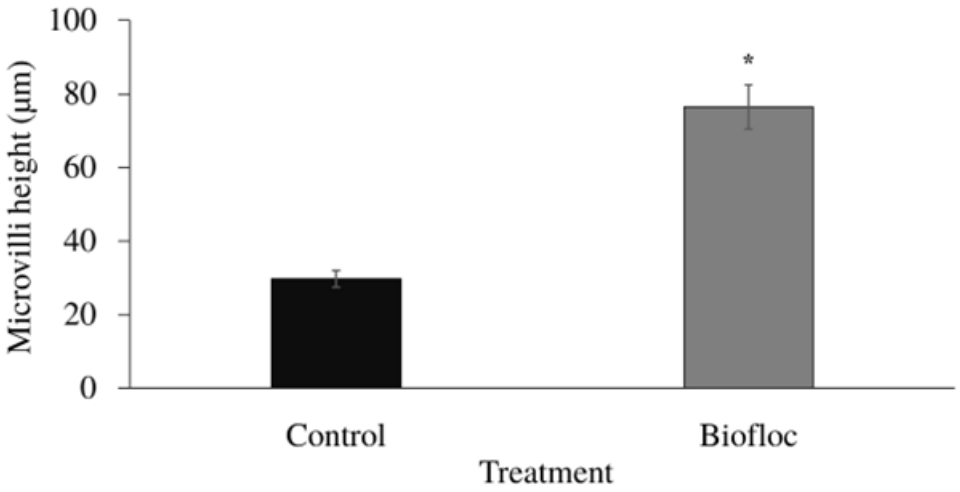

Figure 5. Shrimp microvilli height. Sign * indicate significantly higher value (P< 0.05).

Water quality parameter

The water quality observations are presented in Table 2. At day 14 of the rearing period, the biofloc treatment exhibited significantly lower levels of nitrite compared to the control treatment ($P < 0.05$). Additionally, on day 28, the nitrate parameter of the biofloc treatment also showed significantly lower levels. Though not significantly different, a similar trend was observed in the TAN parameter overall.

Discussion

This study was conducted to analyze the diversity of microbiota composition, particularly the composition and relative abundance of *Vibrio*, in both maintenance media and white shrimp gut reared in a biofloc system using high-throughput/ next-generation sequencing (NGS) technology. NGS is a type of DNA sequencing technology that allows for the analysis of the whole genome, all exons within known genes (whole exome), or only selected genes' exons (target panel) on a large scale (Alfaro *et al*., 2019), thus it can be used to identify and analyze the abundance of microbiota in maintenance media and shrimp gut. One approach in aquaculture activities to convert nitrogen waste into microbial biomass is through the application of biofloc system. The controlled addition of carbohydrates with the appropriate C:N ratio will promote the growth of heterotrophic bacteria, and the production of bacterial protein will consume nitrogen (Avnimelech, 2009; Padeniya *et al*., 2022). According to Tepaamorndech *et al*. (2020), the biofloc system is capable of initiating changes in bacterial dominance in both the culture media and the gut microbiota of shrimp.

The report from Wei *et al*. (2016), also showed that differences in carbon sources in the biofloc system can produce different diversity and abundance of microbiota, and ultimately exhibit different responses to the presence of pathogenic bacteria, especially *Vibrio*, in the aquaculture environment. Before bacteria can thrive and dominate the aquaculture environment, they may have been normal microflora bacteria and also come from the cultivated shrimp. Subsequently, bacteria must be able to compete for nutrients and sites to grow and reproduce with other microorganisms (Ferreira *et al*., 2020). The observation of the microbiota composition in the shrimp culture media in this study revealed differences in composition between the biofloc system and the control treatment. In the biofloc treatment, *Vibrio* also showed dominance and became one of the bacteria composing the floc, which is consistent with Tepaamorndech *et al*. (2020), showing the dominance of *Vibrio* in biofloc.

Furthermore, the bacteria that participated in dominating the biofloc were *Hoflea, Aliiroseovarius, Ruegeria,* and *Novipirellula*. *Hoflea* is a marine bacteria that functions as a neutrophilic iron-oxidizing bacteria (Sorokina *et al*., 2012), while *Ruegeria* is known to have the ability to degrade organophosphate-containing substances using phosphotriesterase enzymes and acrylate degradation (Hussaan *et al*., 2021; Bullock *et al*., 2017). In contrast to the control treatment, the bacteria that dominated the microbiota in the shrimp culture media were from the genus *Flavilitoribacter*, *Phaeodactylibacter, Lewinella, Kriegella*, and *Rubinisphaera*. Biofloc can also influence the composition of the microbiota in the digestive tract of shrimp. Based on the observations, the abundance of *Vibrio* in the biofloc treatment was lower compared to the control treatment, while the genera that dominated in the biofloc treatment were *Sedimentitalea*, *Aliiroseovarius, Ruegeria, Roseovarius*, and *Sulfitobacter*.

Sedimentitalea is known as a type of bacteria found in the digestive tract of Japanese flying squid (Kim *et al*., 2016) and also in marine sediment (Sun *et al*., 2010). This may be correlated to the biofloc treatment, where the water sample showed higher abundance values of *Sedimentitalea* compared to the control treatment. The same trend was also

Table 2. Water quality in the white shrimp rearing medium.

Observation time	Treatment	TAN (mg /L)	Nitrite (mg/L)	Nitrate (mg/L)	TSS (mg/L)
Day 14	Control	0.447 ± 0.064	0.087 ± 0.008	0.928 ± 0.150	35 ± 6.5*
	Biofloc	0.294 ± 0.050	0.002 ± 0.001*	0.837 ± 0.059	216.5 ± 21.2
Day 28	Control	0.540 ± 0.265	0.078 ± 0.004	3.218 ± 0.074	81.7 ± 4.4*
	Biofloc	0.248 ± 0.042	0.090 ± 0.007*	1.474 ± 0.032*	316.3 ± 11.5

Note: *within the same column indicate significantly lower value ($P<0.05$).

shown by *Aliiroseovarius* and *Ruegeria*, where both types of bacteria dominated the water and shrimp gut, indicating that bacteria that dominate the biofloc culture can also dominate the shrimp digestive tract. Furthermore, *Sulfitobacter* was also found to dominate the shrimp digestive tract in the biofloc treatment.

This bacterium is known to have several functions in the marine environment, including organic sulfur cycling, production of sodium-channel blocking toxins, host chemical defense, and marine oil biodegradation. Consequently, secondary metabolites of *Sulfitobacter* may play important roles in marine ecosystems (Long *et al*., 2011). In contrast to the biofloc treatment, the shrimp gut in the control treatment was dominated by *Klebsiella, Vibrio, Kriegella, Streptomyces*, and *Maribacter* bacteria, where *Klebsiella* and *Vibrio* are known to exhibit pathogenic characteristics in aquatic animals (Vaneci-Silva *et al*., 2022; Valente & Wan, 2021). *Vibrio* dominance in the shrimp gut also correlates with diseases such as Vibriosis and WFD, further leading to decreased survival rates, growth performance, and increased feed conversion ratio (Kurniawinata *et al*., 2022).

This was confirmed by the relative abundance of *Vibrio* (Figure 2), which was lower in the gut of shrimp in the biofloc treatment compared to the control treatment. This proves that the biofloc system is capable of influencing the dominance and abundance of microbiota compositions both in the water and in the shrimp gut, as well as reducing the potential dominance of pathogenic bacteria especially *Vibrio*. According to Asplund *et al*. (2011), most *Vibrio* strains are harmless, although some species have the ability to cause diseases, such as *V. parahaemolyticus*, which can cause acute hepatopancreatic necrosis disease (AHPND) in white shrimp (Han *et al*., 2015). In the control treatment, *Vibrio* sp. Hep-1b-8 and *V. parahaemolyticus* were found to dominate the shrimp intestine (Figure 3), and these are suspected to be potentially pathogenic bacteria.

On the other hand, *Vibrio* species that dominate the biofloc media are *V. hepatarius* and *V. nereis*. Based on research, *V. hepatarius* does not exhibit pathogenic activity and has even been reported to have probiotic action in white shrimp against *V. parahaemolyticus* infection (Ramirez *et al*., 2022). Therefore, it appears that biofloc has the ability to minimize the dominance of pathogenic bacteria in the environment and the digestive tract of shrimp. Biofloc has a sufficiently complete nutrient composition including protein, carbon, ash content, fatty acids, minerals, and other nutrients, making it useful as a natural feed that is always available in the culture media (Rajkumar *et al*., 2015, Toledo *et al*., 2016). In addition, biofloc can enhance digestive enzyme activity, leading to better utilization of feed for growth support (Wang *et al*., 2016).

This is supported by the biofloc aggregate components, which consist of microbes that produce extracellular products that act as a supplement to endogenous digestive enzymes to break down proteins and carbohydrates in the animal's intestinal tract. This can facilitate digestion, absorption, and utilization of feed (Najdegerami *et al*., 2016; Zafar *et al*., 2022). This can be seen in the higher overall digestive enzyme activity observed in the biofloc treatment compared to the control. With a higher nutrient intake and better digestive enzyme activity, shrimp growth is improved and the feed conversion ratio (FCR) is lower in the biofloc treatment. One of the extracellular products of biofloc is poly-β-hydroxybutyrate (PHB), which can reach a concentration of 50-200 mg g^{-1} suspended solids in biofloc (Ruan *et al*., 2011; Klanian *et al*., 2020).

This compound has various functions, such as controlling *Vibrio* bacteria (Hoseinifar *et al*., 2016; Liu *et al*., 2010), serving as a carbon source and influencing the microbiota community (Qiao *et al*., 2020), and providing energy for cells in the intestinal tissue (Duan *et al*., 2017). According to Ringø *et al*. (2016), PHB particles are partially degraded into β-hydroxybutyrate in the shrimp gut, and the release of this fatty acid provides the shrimp with energy, resulting in an improved gut epithelium. This energy further supports longer and denser microvilli growth, increasing the surface area and optimizing nutrient absorption. This is also supported by the research of Duan *et al*. (2017) and Sahin *et al*. (2021), which demonstrate the effect of PHB on increasing the length and density of fish intestinal microvilli.

Finally, due to the nitrogen uptake for bacterial growth, the concentration of ammonia in water decreases rapidly (Padeniya *et al*., 2022). This is evident from the observation of water quality at the end of maintenance which showed significantly lower nitrite and nitrate values in the biofloc treatment compared to the control treatment. This is largely influenced by the ability of the microorganisms that compose the biofloc to reduce the levels of nitrite and nitrate.

CONCLUSION

The application of the biofloc system in the white shrimp culture has the ability to influence the composition and relative abundance of bacterial communities in both the water environment and the shrimp's gut. The use of biofloc system leads to a lower relative abundance of *Vibrio* in the shrimp's gut compared to the control group. *V. hepatarius* and *V. nereis* are the dominant bacterial species in the *Vibrio* community in the maintenance media of the biofloc traetment, while *Vibrio* sp. Hep-1b-8 and *V. parahaemolyticus* are dominant in the shrimp gut in the control group. The biofloc system can improve growth performance with higher digestive enzyme activity and longer intestinal microvilli.

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REFERENCES

Abdel-Latif HM, Yilmaz E, Dawood MA, Ringø E, Ahmadifar E, Yilmaz S. 2022. Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. Aquaculture 551: 737951.

Alfaro MP, Sepulveda JL, Lyon E. 2019. Molecular testing for targeted therapies and pharmacogenomics. In Accurate Results in the Clinical Laboratory 349–363.

[APHA] American Public Health Associatio. 2005. Standard Methods for the Examination of Water and Wastewater (21st ed., 1220 p). Washington DC: American Public Health Association.

Asplund ME, Rehnstam-Holm AS, Atnur V, Raghunath P, Saravanan V, Härnström K, Godhe A. 2011. Water column dynamics of Vibrio in relation to phytoplankton community composition and environmental conditions in a tropical coastal area. Environmental Microbiology 13: 2738–2751.

Avnimelech Y. 2009. Biofloc Technology. A Practical Guide Book. Baton Rouge (US): The World Aquaculture Society.

Bullock HA, Luo H, Whitman WB. 2017. Evolution of dimethylsulfoniopropionate metabolism in marine phytoplankton and bacteria. Frontiers in Microbiology 8: 637.

Crab R, Lambert A, Defoirdt T, Bossier P, Verstraete W. 2010. The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. Journal of Applied Microbiology 109: 1643–1649.

De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. 2008. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 277:125–137.

Duan Y, Zhang Y, Dong H, Zheng X, Wang Y, Li H, Liu Q, Zhang, J. 2017. Effect of dietary poly-β-hydroxybutyrate (PHB) on growth performance, intestinal health status and body composition of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Fish and shellfish immunology 60: 520–528.

Ekasari J, Azhar MH, Surawidjaja EH, Nuryati S, Schryver, Bossier. 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon source. Fish and Shellfish Immunology 41: 332–339.

[FAO] Food and Agriculture Organization of the United Nation. 2020. Fisheries and Aquaculture Information and Statistics Branch. http://www.fao.org/fishery/statistics. [29 December 2020]

[FAO] Food and Agricultural Organization of the United Nation. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome: FAO.

Ferreira GS, Santos D, Schmachtl F, Machado C, Fernandes V, Bögner M, Vieira FN. 2020. Heterotrophic, chemoautotrophic and mature approaches in biofloc system for Pacific white shrimp. Aquaculture 533: 1–12.

Gustilatov M, Widanarni, Ekasari J, Pande GSJ. 2022. Protective effects of the biofloc system in Pacific white shrimp *(Penaeus vannamei*) culture against pathogenic *Vibrio parahaemolyticus* infection. Fish and Shellfish Immunology 124: 66–73.

Han JE, Tang KF, Tran LH, Lightner DV. 2015. Photorhabdus insect-related (Pir) toxin-like genes in a plasmid of *Vibrio parahaemolyticus*,

the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp. Diseases of aquatic organisms 113: 33–40.

Hoseinifar SH, Sun YZ, Caipang CM. 2017. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquaculture Research 48: 1380–1391.

Hussaan M, Javed MT, Akram MS, Ali S. 2021. Physiological and molecular basis of bioremediation of micropollutants. In Handbook of Bioremediation (pp. 447-464). Academic Press.

Kewcharoen W, Srisapoome P. 2019. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). Fish and Shellfish Immunology 94: 175–189.

Kim HS, Hyun DW, Lee JY, Kim PS, Whon TW, Kang W, Bae JW. 2016. *Sedimentitalea todarodis* sp. nov., isolated from the intestinal tract of a Japanese flying squid. International Journal of Systematic and Evolutionary Microbiology 66: 3293–3298.

[KKP] Kementerian Kelautan dan Perikanan. 2020. Laporan Tahunan Kementerian Kelautan dan Perikanan. http://www.kkp.go.id. [30 March 2023].

Klanian MG, Díaz MD, Solís MJS, Aranda J, Moral PM. 2020. Effect of the content of microbial proteins and the poly-β-hydroxybutyric acid in biofloc on the performance and health of Nile tilapia (*Oreochromis niloticus*) fingerlings fed on a protein-restricted diet. Aquaculture 519: 734872.

Kumar R, Ng TH, Wang HC. 2020. Acute hepatopancreatic necrosis disease in penaeid shrimp. Reviews in Aquaculture 12: 1867–1880.

Kurniawinata MI, Sukenda S, Wahjuningrum D, Widanarni W. 2022. Bacterial diversity and community composition in the gut and rearing water of Pacific White shrimp *Penaeus vannamei* during an outbreak of white feces disease. Aquaculture 559: 738431.

Kurniawinata MI, Sukenda S, Wahjuningrum D, Widanarni W, Hidayatullah D. 2021. White faeces disease and abundance of bacteria and phytoplankton in intensive pacific white shrimp farming. Aquaculture Research 52(: 5730–5738.

Li Y, Yuan W, Zhang Y, Liu H, Dai X. 2021. Single or combined effects of dietary arabinoxylan-oligosaccharide and inulin on growth performance, gut microbiota, and immune response in Pacific white shrimp *Litopenaeus vannamei*. Journal of Oceanology and Limnology 39: 741–754.

Liu Y, De Schryver P, Van Delsen B, Maignien L, Boon N, Sorgeloos P, Verstraete W, Bossier P, Defoirdt T. 2010. PHB-degrading bacteria isolated from the gastrointestinal tract of aquatic animals as protective actors against luminescent vibriosis. FEMS microbiology ecology 74: 196–204.

Long C, Lu XL, Gao Y, Jiao BH, Liu XY. 2011. Description of a Sulfitobacter strain and its extracellular cyclodipeptides. Evidence-Based Complementary and Alternative Medicine 2011: 393752.

Munaeni W, Widanarni, Yuhana M, Setiawati M, Wahyudi AT. 2020. Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. Powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. Fish and Shellfish Immunology 102: 218–227.

Muttharasi C, Gayathri V, Muralisankar T, Mohan K, Uthayakumar V, Radhakrishnan S, Kumar P, Palanisamy M. 2021. Growth performance, digestive enzymes and antioxidants activities in the shrimp *Litopenaeus vannamei* fed with *Amphiroa fragilissima* crude polysaccharides encapsulated *Artemia nauplii*. Aquaculture 545: 737263.

Najdegerami EH, Bakhshi F, Lakani FB. 2016. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish physiology and biochemistry 42: 457–465.

Padeniya U, Davis DA, Wells DE, Bruce TJ. 2022. Microbial interactions, growth, and health of aquatic species in biofloc systems. Water 14: 4019.

Panigrahi A, Saranya C, Sundaram M, Kannan SRV, Das RR, Kumar RS, Rajesh P, Otta SK. 2018. Carbon: Nitrogen (C:N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. Fish and Shellfish Immunology 81: 329–337.

Qiao G, Chen P, Sun Q, Zhang M, Zhang J, Li Z, Li Q. 2020. Poly-β-hydroxybutyrate (PHB) in bioflocs alters intestinal microbial community structure, immune-related gene expression and early *Cyprinid herpesvirus* 2 replication in gibel carp (*Carassius auratus* gibelio). Fish and Shellfish Immunology 97: 72–82.

Raja RA, Sridhar R, Balachandran C, Palanisammi A, Ramesh S, Nagarajan K. 2017. Pathogenicity profile of *Vibrio parahaemolyticus* in farmed Pacific white shrimp, *Penaeus vannamei*. Fish and Shellfish Immunology 67: 368–381.

Rajkumar M, Pandey PK, Aravind R, Vennila A, Bharti V, Purushothaman CS. 2015. Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). Aquaculture Research 2015: 1–13.

Ramirez M, Domínguez-Borbor C, Salazar L, Debut A, Vizuete K, Sonnenholzner S, Alexis F, Rodríguez J. 2022. The probiotics *Vibrio diabolicus* (Ili), *Vibrio hepatarius* (P62), and *Bacillus cereus* sensu stricto (P64) colonize internal and external surfaces of *Penaeus vannamei* shrimp larvae and protect it against *Vibrio parahaemolyticus*. Aquaculture 549: 737826.

Ringø EZZV, Zhou Z, Vecino JG, Wadsworth S, Romero J, Krogdahl Å, Merrifield DL. 2016. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story?. Aquaculture nutrition 22: 219–282.

Ruan YJ, Zhu L, Xu XY. 2011. Study on the flocs poly-β-hydroxybutyrate production and process optimization in the bio-flocs technology system. Bioresource Technology 102: 7599–7602.

Şahin T, Yılmaz S, Yazıcı İS, Berber S. 2021. The effects of dietary poly-β-hydroxybutyrate on growth parameters, intestinal microflora, and histopathology of rainbow trout, *Oncorhynchus mykiss*, fingerlings. Journal of the World Aquaculture Society 52: 73–87.

Schveitzer R, Fonseca G, Orteney N, Menezes FCT, Thompson FL, Thompson CC, Gregoracci GB. 2020. The role of sedimentation in the structuring of microbial communities in biofloc-dominated aquaculture tanks. Aquaculture 514: 734493.

Shinn AP, Pratoomyot J, Griffiths D, Trong TQ, Vu NT, Jiravanichpaisal P, Briggs M. 2018. Asian shrimp production and the economic costs of disease. Asian Fisheries Science 31: 29–58.

Silva JLSD, Cavalcante DDH, Carvalho FCTD, Vieira RHSDF, Sá MVDC, Sousa OVD. 2016. Aquatic microbiota diversity in the culture of Nile tilapia (*Oreochromis niloticus*) using bioflocs or periphyton: virulence factors and biofilm formation. Acta Scientiarum Animal Sciences 38: 233–241.

Sorokina AY, Chernousova EY, Dubinina GA. 2012. *Hoeflea siderophila* sp. nov., a new neutrophilic iron-oxidizing bacterium. Microbiology 81: 59–66.

Subash P, Chrisolite B, Sivasankar P, George MR, Amirtharaj KV, Padmavathy P, Mageshkumar, P. 2023. White feces syndrome in *Penaeus vannamei* is potentially an *Enterocytozoon hepatopenaei* (EHP) associated pathobiome origin of *Vibrio* spp. Journal of Invertebrate Pathology 198: 107932.

Sun F, Wang B, Liu X, Lai Q, Du Y, Li G, Luo Z, Shao Z. 2010. *Leisingera nanhaiensis* sp. nov., isolated from marine sediment. International journal of systematic and evolutionary microbiology 60: 275–280.

Tang KFJ, Bondad-Reantaso MG. 2019. Impacts of acute hepatopancreatic necrosis disease on commercial shrimp aquaculture. Revue Scientifique et Technique - Office International des Epizooties 38: 477–490.

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

Toledo TM, Silva BC, Vieira FDN, Mouriño JLP, Seiffert WQ. 2016. Effects of different dietary lipid levels and fatty acids profile in the culture of white shrimp *Litopenaeus vannamei* (Boone) in biofloc technology: water quality, biofloc composition, growth and health. Aquaculture Research 47: 1841–1851.

Torpee S, Kantachote D, Rattanachuay P, Chiayvareesajja S, Tantirungkij M. 2021. Dietary supplementation with probiotic *Rhodobacter sphaeroides* SS15 extract to control acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio parahaemolyticus* in cultivated white shrimp. Journal Invertebrate Pathology 186: 107585.

Valente CDS, Wan AH. 2021. Vibrio and major commercially important vibriosis diseases in decapod crustaceans. Journal of Invertebrate Pathology 181: 107527.

Vaneci-Silva D, Assane IM, de Oliveira Alves L,

Gomes FC, Moro EB, Kotzent S Pitondo-Silva A, Pilarski F. 2022. *Klebsiella pneumoniae* causing mass mortality in juvenile Nile tilapia in Brazil: Isolation, characterization, pathogenicity and phylogenetic relationship with other environmental and pathogenic strains from livestock and human sources. Aquaculture 546: 737376.

Wang H, Wang C, Tang Y, Sun B, Huang J, Song X. 2018. *Pseudoalteromonas* probiotics as potential biocontrol agents improve the survival of *Penaeus vannamei* challenged with acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio parahaemolyticus*. Aquaculture 494: 30–36.

Wang C, Pan L, Zhang K, Xu W, Zhao D, Mei L. 2016. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. Aquaculture research 47: 3307–3318.

Wei Y, Liao SA, Wang AL. 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture 465: 88–93.

Xiong J. 2018. Progress in the gut microbiota in exploring shrimp disease pathogenesis and incidence. Applied Microbiology and Biotechnology 102: 7343–7350.

Zafar MA, Talha MA, Rana MM. 2022. Effect of biofloc technology on growth performance, digestive enzyme activity, proximate composition, and hematological parameters of Asian stinging catfish (*Heteropneustes fossilis*). Journal of Applied Aquaculture 34: 755–773.