

Biofloc system enhances growth, immunity, and stress tolerance in high-density cultured Pacific White Shrimp

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Abstract. The main challenge in Pacific white shrimp farming is disease. One of the technologies applied to address this issue is the biofloc system, which enhances growth performance, survival rate (SR), feed conversion ratio (FCR), water quality, suppresses pathogen activity, and boosts immune response. This study aimed to evaluate the effects of the biofloc system on growth performance, health status, and stress response of high-density cultured vannamei shrimp at an intermediate scale. Postlarvae (PL-10) were reared for 56 days in biofloc and control systems at a density of 500 shrimp m⁻³ using Intermediate Bulk Containers (IBC) with a volume of 1000 L (1.2 × 1.0 × 1.15 m³). A completely randomized design (CRD) was used with two treatments: control (C) and biofloc (BF) with three replicates. Parameters observed included growth rate, weight gain, biomass gain, FCR, SR, bacterial abundance, total hemocyte count (THC), respiratory burst (RB), phenoloxidase (PO) activity, and water quality. Results showed that the BF treatment yielded superior outcomes, indicated by SR (81.24±0.02%), FCR (1.67±0.08), reduced *Vibrio* sp. and *V. parahaemolyticus* abundance, and improved immune and stress responses.

1 Introduction

The Pacific white shrimp (*Litopenaeus vannamei*) remains one of Indonesia's principal aquaculture species. Despite its strong contribution to national aquaculture output, overall productivity has not yet achieved levels required to compete effectively in global markets. In 2023, national production reached approximately 764 thousand tons—a modest 4.5% increase from 2022 yet still slightly below the 2021 output of 768 thousand tons [1]. To enhance yields, farmers have widely adopted intensification strategies; however, higher stocking densities frequently lead to greater susceptibility to infectious diseases. Major health problems commonly encountered in shrimp farming include acute hepatopancreatic necrosis disease (AHPND), infectious myonecrosis, white feces disease (WFD), white spot disease (WSD), and yellow head disease (YHD) [2].

One of the key approaches implemented to mitigate disease risks is the use of biofloc technology. This system promotes the proliferation of beneficial microbial communities—comprising bacteria, microalgae, protozoa, and suspended organic aggregates—through the addition of carbon sources. Biofloc-based culture has been reported to improve survival rate (SR), feed conversion ratio (FCR), and growth performance. These improvements arise because microbes within the system recycle organic residues into nutritionally valuable flocs

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containing microbial biomass, amino acids, vitamins, and essential fatty acids that serve as natural supplemental feed for shrimp [3].

Beyond nutritional contributions, biofloc technology offers health-related benefits. Microbial interactions within biofloc systems can suppress pathogen activity, partly through quorum sensing (QS) interference. QS allows bacteria to regulate gene expression based on cell density, including virulence factors. Findings by Widanarni et al. (2024) [4] demonstrated that biofloc systems can downregulate QS-associated mechanisms in *Vibrio parahaemolyticus*, reducing the expression of *pirA* and *pirB*—key toxin genes responsible for AHPND. Consequently, toxin production and overall virulence can be diminished. Biofloc-derived microbial compounds have also been shown to enhance innate immune responses in *L. vannamei* [3].

Although biofloc technology has been widely studied, the performance of this system under high-density culture at an intermediate operational scale remains insufficiently explored. An intermediate-scale assessment is essential as a transitional step before implementation at commercial farm levels. Therefore, this study evaluates the growth, health condition, and stress responses of *L. vannamei* reared in a high-density biofloc system compared with a conventional control system.

2 Materials and methods

2.1 Experimental design

This study employed a completely randomized design (CRD) consisting of two treatments: a control treatment (without the biofloc system) and a biofloc treatment (with the biofloc system). Each treatment was carried out with three replicates. The stocking density of Pacific white shrimp was 500 individuals m³ across all treatments. The culture was conducted indoors at PT. Suri Tani Pemuka, Purwakarta, West Java, for 56 days.

2.2 Biofloc preparation

The biofloc inoculum was sourced from an existing *L. vannamei* biofloc culture maintained in a 1000 L intermediate bulk container (IBC). Molasses served as the primary carbon source and was added once daily to maintain an estimated C/N ratio of 10. The application occurred approximately two hours after the morning feeding. The amount of molasses was calculated using the carbon supplementation formula described by based on assumptions of 36% feed protein, 16% nitrogen content in protein, 85% uneaten feed, and 38% carbon content in molasses. For instance, an estimated daily ration of 50 g required 64.42 g of molasses to achieve the desired C/N ratio. Prior to stocking, the prepared biofloc inoculum was added to the culture tanks at a 4:1 ratio (seawater : inoculum) and allowed to stabilize for 24 hours.

2.3 Shrimp maintenance

The test animals used in this study were Pacific white shrimp (*Litopenaeus vannamei*) postlarvae (PL10) with a total of 2,250 individuals obtained from the hatchery of PT. Suri Tani Pemuka, Indramayu, West Java. The shrimp postlarvae were first acclimatized in plastic container boxes for 24 hours. The acclimatization process was conducted to assess the health condition of the shrimp postlarvae to ensure their suitability for the experiment. The monitoring indicators included responsiveness to disturbance and good physical condition.

After acclimatization, the postlarvae were stocked into treatment tanks consisting of six Intermediate Bulk Containers (IBC) measuring 1.2 × 1.0 × 1.15 m³, each with a capacity of

1,000 L and filled with 750 L of water. Feeding was carried out using the blind feeding method for the first 30 days (DOC 1–30) and the demand feeding method for the following 26 days (DOC 31–56). Blind feeding followed standard pond operation guidelines at a rate of 2 kg per 100,000 shrimp, while demand feeding was adjusted according to the feeding rate (FR). Feed was provided four times daily at 08:00, 11:00, 14:00, and 17:00 WIB.

Water quality monitoring was conducted daily and weekly. Daily measurements included dissolved oxygen (DO), temperature, pH, and salinity, while weekly measurements included total ammonia nitrogen (TAN), nitrite, nitrate, and alkalinity. In the control treatment (K), water exchange was performed when water quality parameters (TAN and nitrite) reached maximum threshold levels.

2.4 Parameters of observation

2.4.1 Growth performance

The parameters measured in this study included survival rate (SR), average daily growth (ADG), specific growth rate (SGR), weight gain (ΔW), biomass gain (ΔB), and feed conversion ratio (FCR). ADG was calculated as $(W_t - W_0) / t$, while SGR was calculated as $100 \times [(\ln W_t - \ln W_0) / t]$, where W_t represents the final shrimp weight, W_0 the initial shrimp weight, and t the duration of the culture period in days.

2.4.2 Bacterial abundance

Bacterial enumeration followed the total plate count (TPC) approach. Samples from rearing water, hepatopancreas, and intestine were plated on three types of agar media: thiosulfate citrate bile salt sucrose (TCBS) agar for total *Vibrio* count (TVC), seawater complete (SWC) agar for total bacterial count (TBC), and HiChrome agar for quantifying *Vibrio parahaemolyticus* (TVP). Approximately 0.1 g of organ tissue or 0.1 mL of water sample was homogenized in phosphate-buffered saline (PBS), followed by serial dilutions. A 50 μ L aliquot of each dilution was then evenly spread across the respective media. All plates were incubated at 27°C for 24 hours, after which visible colonies were enumerated using standard TPC calculations.

$$\text{Total bacterial count} = \text{bacterial colonies} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{sample volume}}$$

2.4.3 Immune response

To quantify total hemocyte count (THC), hemolymph was mixed with anticoagulant at a 1:3 ratio and examined using a hemacytometer under a light microscope (100 \times). Respiratory burst (RB) activity was assessed spectrophotometrically at 630 nm using nitroblue tetrazolium (NBT) reduction as an indicator. Phenoloxidase (PO) activity was measured by converting L-DOPA into dopachrome, with absorbance read at 490 nm. Results were expressed as dopachrome formation per 100 μ L of hemolymph.

2.4.4 Water quality

Daily monitoring included dissolved oxygen (DO), pH, temperature, and salinity using standard portable meters, while weekly analyses involved total ammonia nitrogen (TAN), nitrite, nitrate, and alkalinity. Biofloc volume was measured using an Imhoff cone. When

TAN and nitrite levels in the control treatment exceeded threshold limits, partial water exchange was performed.

2.4.5. Statistical analysis

The data obtained were analyzed using Microsoft Excel 2016 and IBM SPSS Statistics version 26. Differences between the two treatments were assessed using an Independent Sample T-Test with a significance level of 0.05 ($\alpha = 5\%$).

3 Result

3.2 Growth performance

Based on the observations, the growth performance of Pacific white shrimp cultured in the biofloc system and the control over 56 days is presented in Table 1. Survival rate (SR) and feed conversion ratio (FCR) showed significant differences ($p < 0.05$) between the two treatments. In contrast, average daily growth (ADG), specific growth rate (SGR), weight gain (Wt, ΔW), and biomass gain (Bt, ΔB) of the shrimp did not differ significantly between the treatments according to the statistical analysis.

Table 1. Growth performance of Pacific white shrimp cultured in the biofloc system and control.

Parameter	K	BF
W ₀ (g)	0,01±0,00 ^a	0,01±0,00 ^a
W _t (g)	3,46±0,12 ^a	3,27±0,42 ^a
ΔW (g)	3,45±0,12 ^a	3,26±0,42 ^a
ADG (g day ⁻¹)	0,06±0,00 ^a	0,06±0,01 ^a
SGR (% day ⁻¹)	10,44±0,06 ^a	0,33±0,22 ^a
B ₀ (g)	3,75±0,00 ^a	3,75±0,00 ^a
B _t (g)	868±8 ^a	997±142 ^a
ΔB (g)	865±8 ^a	994±142 ^a
FCR	1,67±0,08 ^a	1,48±0,15 ^b
SR (%)	67,02±0,02 ^a	81,24±0,02 ^b

Different superscript letters within the same row indicate significant differences ($p < 0.05$). K = Pacific white shrimp cultured without the biofloc system (control); BF = Pacific white shrimp cultured with the biofloc system.

3.3 Bacterial abundance

On day 56, total bacterial count (TBC) showed a significant decrease ($p < 0.05$) in the hepatopancreas and intestine samples, with the biofloc (BF) treatment exhibiting better results, ranging from 10^5 to 10^6 CFU mL⁻¹ (Fig. 1). In contrast, no significant differences were observed in the water samples on day 56.

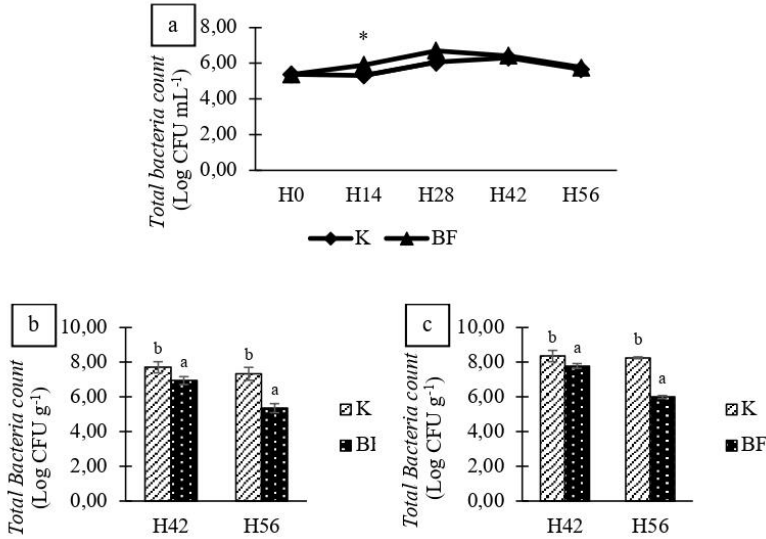


Fig. 1. Total bacterial count in the rearing water (a), hepatopancreas (b), and intestine (c) of Pacific white shrimp cultured in the biofloc system and control over 56 days. Different letters between treatments or * indicate significant differences ($p < 0.05$). There were two treatments with three replicates. K = control; BF = biofloc.

At the end of the culture period, presumptive *Vibrio* count (PVC) showed significant differences ($p < 0.05$) in the water, hepatopancreas, and intestine samples, with the biofloc (BF) treatment exhibiting a decrease, resulting in better outcomes ranging from 10^3 to 10^4 CFU mL⁻¹ (Fig. 2). In contrast, the control (K) treatment tended to increase, ranging from 10^4 to 10^7 CFU mL⁻¹.

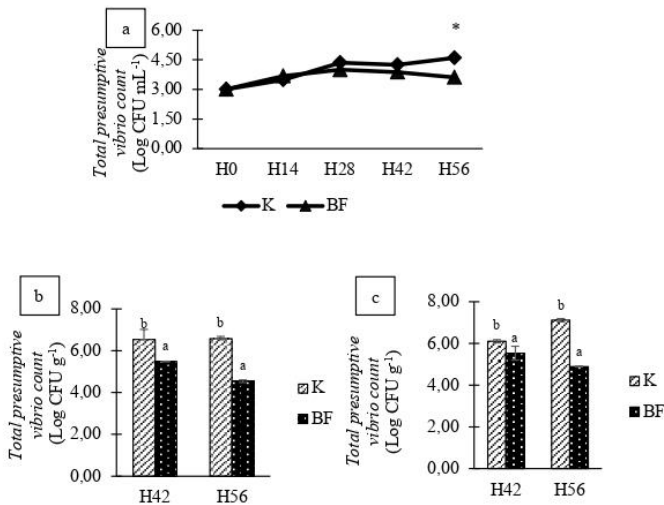


Fig. 2. Presumptive *Vibrio* count in the rearing water (a), hepatopancreas (b), and intestine (c) of Pacific white shrimp cultured in the biofloc system and control over 56 days. Different letters between treatments or * indicate significant differences ($p < 0.05$). There were two treatments with three replicates. K = control; BF = biofloc.

At the end of the culture period, total *Vibrio parahaemolyticus* (TVP) showed significant differences ($p < 0.05$) in the water, hepatopancreas, and intestine samples. The biofloc (BF) treatment exhibited a decrease, resulting in better outcomes of approximately 10^3 CFU mL⁻¹ (Fig. 3), whereas the control (K) treatment tended to increase, ranging from 10^4 to 10^6 CFU mL⁻¹.

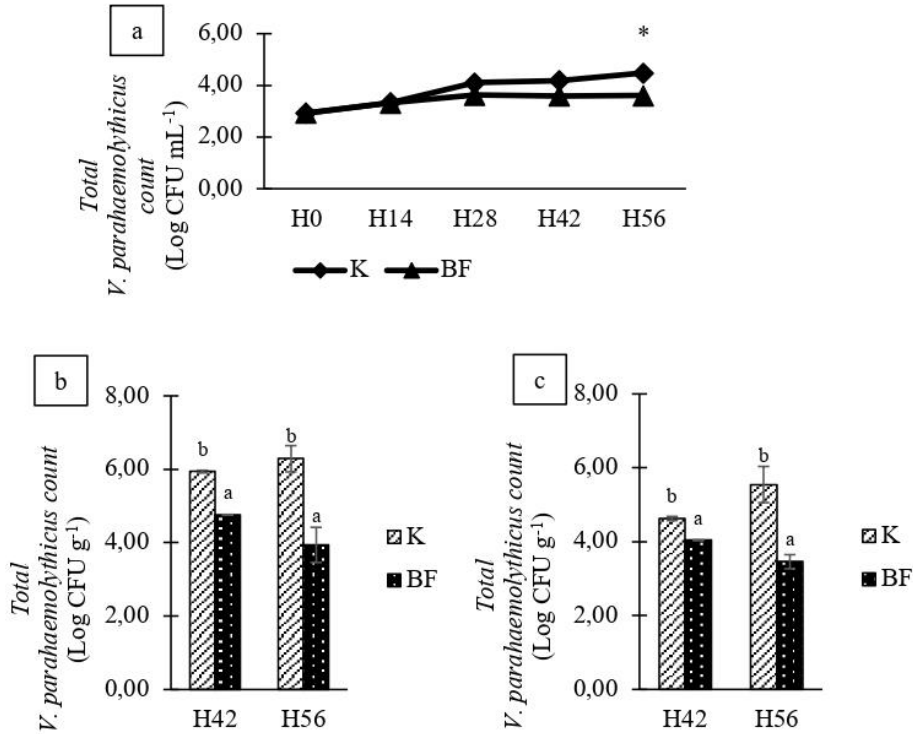


Fig. 3. Total *Vibrio parahaemolyticus* abundance in the rearing water (a), hepatopancreas (b), and intestine (c) of Pacific white shrimp cultured in the biofloc system and control over 56 days. Sampling was performed at day 0 (H0), day 14 (H14), day 28 (H28), day 42 (H42), and day 56 (H56) of the rearing period. Different letters between treatments or * indicate significant differences ($p < 0.05$). There were two treatments with three replicates each. K = control; BF = biofloc.

3.4 Immune response

Total hemocyte count (THC) on days 14, 28, and 42 showed an increase, with the control (K) treatment exhibiting significant differences ($p < 0.05$), followed by a decrease on day 56. However, on day 56, the biofloc (BF) treatment showed a significant difference ($p < 0.05$), with a higher value of $4.50 \pm 0.35 \times 10^6$ cells mL⁻¹ (Fig. 4). Respiratory burst (RB) activity in the BF treatment increased on day 14, then decreased until day 42, with a slight increase on day 56 (Fig. 5). On day 56, BF showed a significant difference ($p < 0.05$), with a higher value of 0.08 ± 0.00 per 10 μ L hemolymph. In contrast, phenoloxidase (PO) activity tended to increase until day 56 (Fig. 6); however, no significant differences were observed between the two treatments.

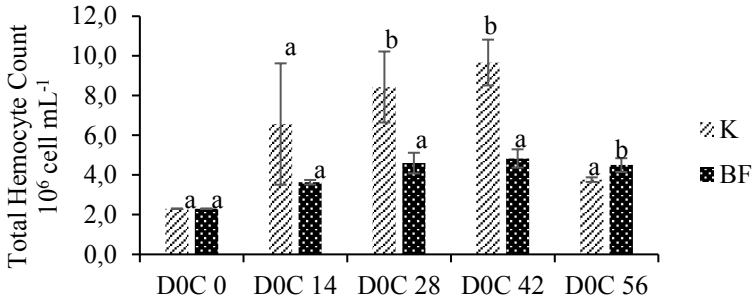


Fig. 4. Total hemocyte count of Pacific white shrimp cultured in the biofloc system and control over 56 days. Sampling was conducted at different culture periods, namely day 0 (DOC 0), day 14 (DOC 14), day 28 (DOC 28), day 42 (DOC 42), and day 56 (DOC 56), where DOC refers to *days of culture*. Different letters between treatments indicate significant differences ($p < 0.05$). There were two treatments with three replicates each. K = control; BF = biofloc.

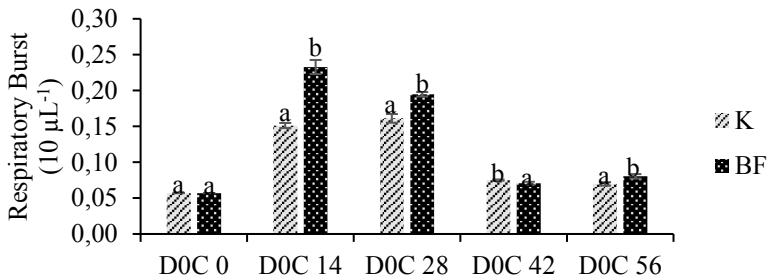


Fig. 5. Respiratory burst of Pacific white shrimp cultured in the biofloc system and control over 56 days. Sampling was conducted at different culture periods, namely day 0 (DOC 0), day 14 (DOC 14), day 28 (DOC 28), day 42 (DOC 42), and day 56 (DOC 56), where DOC refers to *days of culture*. Different letters between treatments indicate significant differences ($p < 0.05$). There were two treatments with three replicates each. K = control; BF = biofloc.

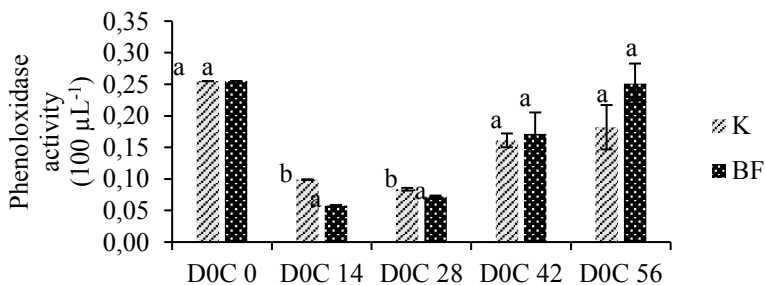


Fig. 6. Phenoloxidase activity of Pacific white shrimp cultured in the biofloc system and control over 56 days. Sampling was conducted at different culture periods, namely day 0 (DOC 0), day 14 (DOC 14), day 28 (DOC 28), day 42 (DOC 42), and day 56 (DOC 56), where DOC refers to *days of culture*. Different letters between treatments indicate significant differences ($p < 0.05$). There were two treatments with three replicates each. K = control; BF = biofloc.

3.5 Water quality

Daily water quality parameters, including dissolved oxygen (DO), temperature, pH, and salinity, remained within the optimal ranges according to the Indonesian National Standard (*Standar Nasional Indonesia*, SNI 9267-5:2024) for Pacific white shrimp (*Litopenaeus vannamei*) culture. Based on this standard, the optimal ranges are as follows: DO ≥ 3.5 mg L⁻¹, temperature 25–32 °C, pH 7.5–8.5, and salinity 15–35 g L⁻¹. In the present study, daily water quality values for the control (K) and biofloc (BF) treatments were: DO = 3.58–6.93 mg L⁻¹, temperature = 25.50–28.00 °C, pH = 6.83–8.28, and salinity = 29.20–36.00 g L⁻¹, which were generally within or close to the optimal ranges. Weekly parameters, including total ammonia nitrogen (TAN), nitrite (NO₂⁻), nitrate (NO₃⁻), alkalinity, and biofloc volume, were also monitored (Fig. 7). These parameters generally increased over the 56-day culture period, except for alkalinity in the control treatment, which decreased. Significant differences ($p < 0.05$) were observed between the two treatments on day 56 for TAN, nitrite, nitrate, and alkalinity, with the BF treatment showing better values compared to K. In addition, biofloc volume increased throughout the culture period (Fig. 8), with significant increases observed from DOC 28 to DOC 56. The highest biofloc volume was recorded on day 56, reaching 50.25 mL L⁻¹.

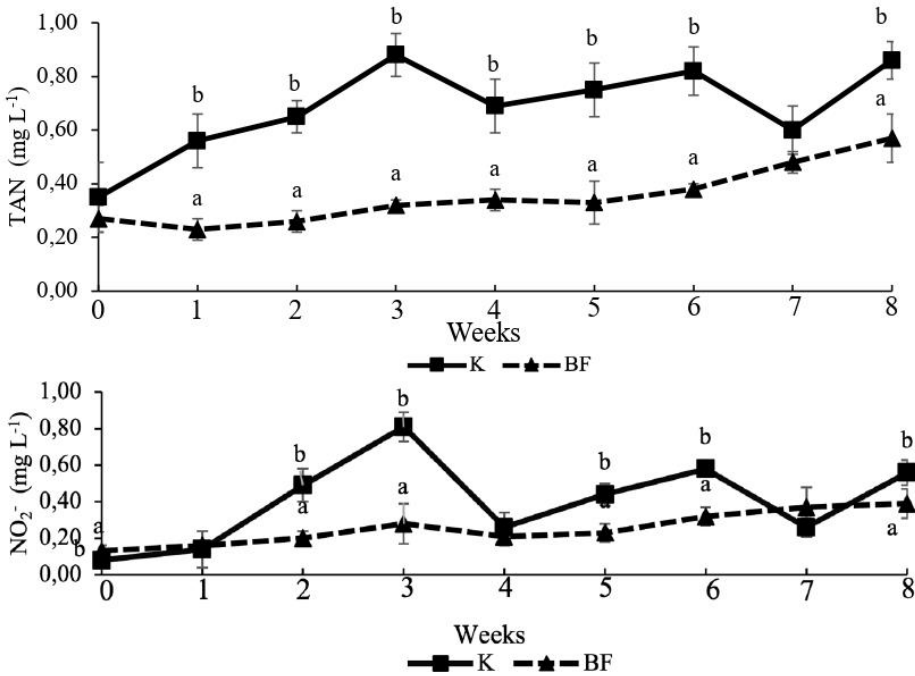


Fig. 7. Water quality parameters (TAN, nitrite, nitrate, and alkalinity) of Pacific white shrimp reared in the biofloc and control systems for 56 days. Different superscript letters between treatments indicate significant differences ($p < 0.05$). Two treatments were conducted with three replicates each. K = control, BF = biofloc.

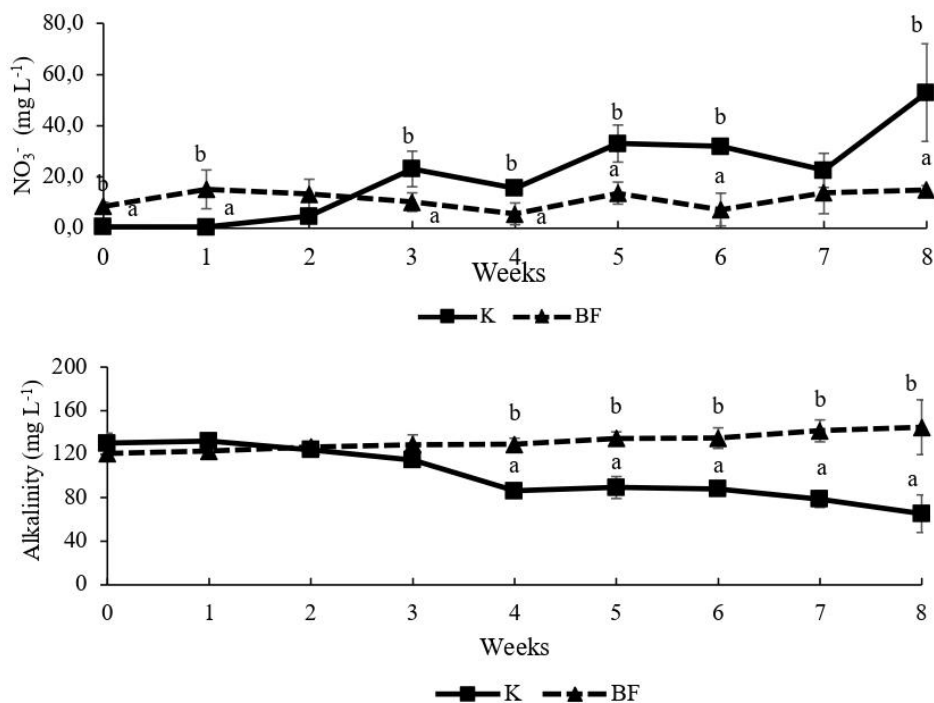


Fig. 7 (continued).

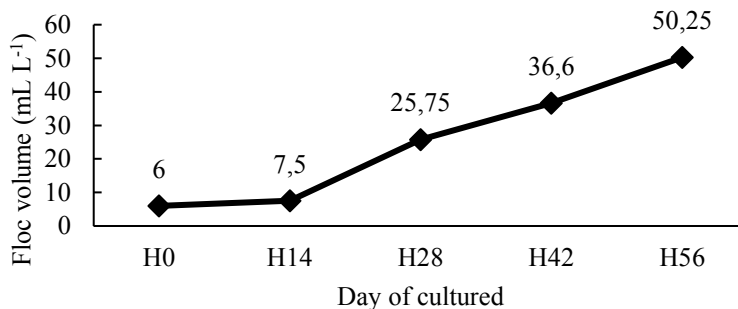


Fig. 8. Floc volume of Pacific white shrimp reared in the biofloc and control systems for 56 days. Different superscript letters between treatments indicate significant differences ($p < 0.05$). Two treatments were conducted with three replicates each. K = control, BF = biofloc.

4 Discussion

Survival rate (SR) of *L. vannamei* after 56 days differed significantly between treatments, with the biofloc (BF) system yielding notably higher SR than the control (K). These findings agree with reports by El-Sayed (2021) [5], stating that biofloc systems enhance shrimp survival. The improved SR in BF was likely driven by more stable water quality, particularly lower TAN concentrations resulting from active heterotrophic bacterial assimilation. Additional pathogen-suppressing mechanisms within biofloc including extracellular

enzymatic degradation of autoinducers, production of antimicrobial metabolites such as bromophenols, and phenotypic interference in *V. parahaemolyticus* further contributed to better survival outcomes [6]. Improvements in FCR under biofloc conditions were attributed to the availability of microbial biomass rich in nutrients such as proteins, lipids, minerals, and functional compounds (e.g., carotenoids and chlorophenols) that enhance digestion and nutrient uptake [6].

Despite improvements in health-related parameters, growth performance metrics such as ADG, SGR, final weight, and biomass gain showed no significant differences between treatments. High stocking density likely constrained these variables by intensifying competition for feed, reducing space availability, and increasing susceptibility to oxidative stress factors previously recognized as limiting growth under crowded conditions.

Fluctuations in bacterial abundance were observed throughout the culture period. Mid-cycle increases in TBC and presumptive *Vibrio* counts were consistent with elevated nutrient availability from carbon supplementation, feed residues, and organic detritus, which favor rapid proliferation of heterotrophic bacteria and *Vibrio* spp. [7]. Although PVC levels temporarily approached 10^4 CFU mL⁻¹ the upper threshold considered safe the counts remained within non-pathogenic limits. Elevated TVP levels detected during days 28–56 were still below concentrations known to initiate infections, typically 10^5 – 10^7 CFU mL⁻¹ within 24–48 hours. Importantly, only certain *V. parahaemolyticus* strains harboring virulence plasmids induce AHPND. Competition for nutrients and QS suppression within the biofloc environment likely played major roles in reducing pathogenicity and TVP abundance [4].

Within the digestive tract, biofloc exposure influenced microbiota composition and immune activation. Although some *Vibrio* strains proliferate in biofloc systems, most are non-pathogenic and may stimulate immune responses without causing disease. Previous studies have shown that biofloc enhances phagocytic activity and suppresses virulence gene expression in pathogenic *Vibrio* spp. [8], consistent with the lower bacterial loads found in BF shrimp organs.

Shrimp under high-density conditions often experience suppressed immune functions, including reduced THC, RB, and PO activities, due to increased metabolic demands and stress [9]. Decreases in THC may also indicate active hemocyte mobilization to infected tissues [10]. RB, representing the production of reactive oxygen intermediates during phagocytosis, is sensitive to environmental stressors such as poor water quality and crowding [9, 11]. Higher RB values indicate robust immune defense, enabling more efficient elimination of pathogens. PO activity, a key component of the crustacean melanization response, was enhanced in the presence of biofloc, likely due to continuous exposure to microbial-associated molecular patterns such as β -glucans and peptidoglycans [12].

Water quality stability is essential under intensive culture. Increasing floc volume in BF systems supports microbial nutrient recycling but must remain below the recommended threshold of 50 mL L⁻¹ to avoid oxygen depletion. TAN reductions in BF were attributed to the activities of ammonia-oxidizing and nitrite-oxidizing bacteria involved in nitrification and denitrification pathways [13-14]. Alkalinity trends further indicated active nitrogen cycling, with BF systems maintaining levels above the recommended 100 mg L⁻¹, preventing pH declines that could inhibit nitrification [15].

5 Conclusion

The cultivation of Pacific white shrimp using the biofloc system at high density on an intermediate scale was proven to increase total hemocyte count (THC), improve feed conversion ratio (FCR), enhance immune responses, and reduce the abundance of *Vibrio* sp. and *Vibrio parahaemolyticus*. Furthermore, the high-density biofloc system is more

recommended for application in the nursery phase. The addition of commercial probiotics is also suggested for further evaluation to determine their effects on the productivity of Pacific white shrimp in the biofloc system at the field scale.

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