



Wheat flour as carbon source on water quality, growth performance, hemolymph biochemical and immune parameters of Pacific white shrimp (*Penaeus vannamei*) juveniles in biofloc technology (BFT)

Mohammad Hossein Khanjani^{a,*}, Jamshid Eslami^b, Maurício Gustavo Coelho Emerenciano^c

^a Department of Fisheries Sciences and Engineering, Faculty of Natural Resources, University of Jiroft, Jiroft, Kerman, Iran

^b Persian Gulf Aquatic Resources Reproduction and Reconstruction Center, Bandar Kolahy, Minab, Hormozgan, Iran

^c Commonwealth Scientific and Industrial Research Organization (CSIRO), CSIRO Agriculture and Food, Livestock and Aquaculture Program, Aquaculture Systems Team, Bribie Island Research Centre, Woorim, Australia

ARTICLE INFO

Keywords:

P. vannamei
Biofloc
Carbon source
Growth performance
Immunity

ABSTRACT

This study examined how adding wheat flour (relative to daily feed) affected water quality, growth performance, body composition, hemolymph biochemical and immunity activities of *Penaeus vannamei*. In 5 experimental enclosures, pacific white shrimp juveniles weighing an average of 2.80 g were reared for 49 days at a density of 1 g/L. The experimental groups include a control group (without wheat flour, 35–50 %/day water exchange) and four biofloc treatments containing wheat flour in proportions of 25 % (WF25), 50 % (WF50), 75 % (WF75) and 100 % (WF100) of daily feed added in the water. The method of feeding and the type of feed was the same in all treatments. In the control group, the highest amounts of total ammonium nitrogen (TAN) (1.14 mg/L), nitrite (8.15 mg/L), and nitrate (3.60 mg/L) were observed ($P < 0.05$). In biofloc WF50 and WF75 treatments, the highest body weight (9.09–9.18 g), feed efficiency (74.11–74.68 %) and survival rate (93.20–93.83 %) were obtained ($P < 0.05$). Adding wheat flour to the rearing system improved the biochemical composition of shrimp bodies, so that the control group had the lowest level of protein (75.31 % DW), lipid (6.81 % DW), and ash (11.48 % DW) ($P < 0.05$). With wheat flour added to the rearing system, shrimp hemolymph glucose and triglyceride levels increased, and the highest levels of glucose were obtained in treatments WF50 (41.33 mg/dL) and WF75 (42.0 mg/dL). Adding wheat flour to the rearing system increased shrimp hemolymph immune activity. The control group exhibited the lowest levels of lysozyme (19 u/mL/min) and phenoloxidase (0.41 u/mL). The results demonstrated that by adding wheat flour to the biofloc cultivation system (under the conditions of this study) in the amount of 50–75 % of the daily feed ration, the water quality, growth performance, feed efficiency, survival rate, hemolymph biochemical, and immunity activities of *P. vannamei* improved.

1. Introduction

In response to the growing population and food scarcity, and decline of wild fisheries, aquaculture has shown great potential as an alternative food source (FAO, 2024). Among all aquaculture species, the Pacific white shrimp (*Penaeus vannamei*) is considered a key commodity (Dai et al., 2023). *P. vannamei* is the most important aquaculture species in many developing Asian countries, especially in Iran (Abbaszadeh et al., 2019). The top species produced in 2020 was *P. vannamei*, with 5.8 million tonnes produced in global, which had doubled since 2010 (FAO, 2022). The expansion is expected to continue, driven by a steady increase in global demand. Traditional shrimp farming expansion is

possible, but may exacerbate climate change and biodiversity loss, and compromise other ecosystem services (Sun et al., 2024). There are numerous environmental challenges associated with aquaculture's rapid global expansion, including water pollution, ecosystem degradation, and disease outbreaks (Koyama et al., 2020; Emerenciano et al., 2025). Shrimp aquaculture needs to use new systems that can achieve sustainable goals. As a sustainable aquaculture approach based on waste nutrient recycling, biofloc technology (BFT) has attracted attention (Khanjani et al., 2023a). With limited water exchange, BFT, in its various forms, has been found to be an efficient way to utilize nutrient input (Khanjani et al., 2024a). By manipulating the carbon/nitrogen (C/N) ratio in the culture water by adding an external carbon source,

* Corresponding author.

E-mail addresses: m.h.khanjani@ujiroft.ac.ir, m.h.khanjani@gmail.com (M.H. Khanjani).

<https://doi.org/10.1016/j.aqrep.2025.102623>

Received 11 October 2024; Received in revised form 2 January 2025; Accepted 4 January 2025

2352-5134/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

microbial biomass is enhanced (Nguyen et al., 2024). BFT has several advantages, such as a biosecure system, improved water quality via self-generated bioremediation, and improved shrimp growth and immunity via heterotrophic bacterial systems (Abbaszadeh et al., 2019; Mansour et al., 2022; Raza et al., 2024). Over the past two decades, BFT shrimp farming has attracted more attention and has been shown to allow for high stocking loads of Pacific white shrimp (Papadopoulos et al., 2024). Many studies have demonstrated that the effects of BFT on water quality and shrimp growth are related to the addition of carbon sources enabling key microbial communities to thrive (Addo et al., 2023; Sun et al., 2024). The study by Sun et al. (2024) showed higher shrimp yields and lower environmental impact with the addition of carbohydrates. As a result of adding a carbon source to the biofloc system, water quality (Khanjani et al., 2024b), growth performance (Rind et al., 2023), microbial and planktonic communities (Addo et al., 2023; Maciel de Lima et al., 2022), body compositions (Khanjani and Sharifinia, 2024), antioxidant activities (Miao et al., 2020) and immunity (Panigrahi et al., 2018; Khanjani et al., 2024c) are all affected. Carbon and nitrogen have different effects depending on the type of carbon source and ratio (Xu and Pan, 2013; Addo et al., 2023). The carbon sources used in the BFT system are typically by-products from the animal or human food industries and act as fertilizers to increase the C/N ratio of over 10:1, promoting the conversion of inorganic nitrogen into microbial biomass and providing a source of feed for shrimp (Wei et al., 2016; Khanjani et al., 2024a). Common carbon sources for biofloc production include corn flour, tapioca flour, cassava flour, granulated sugar, wheat flour, starch, and molasses (Putra et al., 2020; Khanjani et al., 2021). BFT uses simple carbon sources since they are easily absorbed and consumed by bacteria, allowing them to proliferate more rapidly (Addo et al., 2023). These sources must meet several criteria, including cost, local availability, biodegradability, and bacterial assimilation efficiency.

Although carbon sources can boost vibrio populations, it can also promote positive *Bacillus* sp. that may outcompete harmful vibrio if the system is properly managed (de Souza et al., 2012; Monroy-Dosta, et al., 2013). Wheat flour is a globally important commodity and is used as a carbon source in BFT shrimp culture. This carbon source has been investigated previously (Pamanna et al., 2017; Kim et al., 2021; Vinasiam et al., 2024) but there is no literature related to wheat flour relative to daily feed provision. In this sense, the present study investigated the effect of wheat flour addition on (i) water quality, (ii) growth performance, (iii) body and biofloc compositions, and (iiii) hemolymph biochemical and immunity activities of Pacific white shrimp (*P. vannamei*) cultivated in BFT system.

2. Materials and methods

2.1. Experimental setup

The experiment was conducted at Kolahi marine shrimp hatchery in Hormozgan province, Iran. Juvenile Pacific white shrimp *P. vannamei* were obtained from the same facility with an average initial weight of 2.80 ± 0.25 g. A 49-day experiment was conducted in an indoor experimental room with natural photoperiod. In this study, five experimental groups were considered with three replicates per treatment. A control group (without wheat flour addition, clear water) and four biofloc treatments with wheat flour addition were evaluated in the experiment. Wheat flour was added to the rearing tank water as a percentage of daily feed in the biofloc treatments (25 %, 50 %, 75 %, and 100 %) (Table 1). A total of 15 tanks with a volume of 150 L, salinity of 32 g/L and stocking density of 1 g/L (i.e., 54 shrimps per experimental unit) were utilized. In BFT treatments, to promote bioflocs 0.5 mL/L of mature biofloc water from an existing shrimp culture were added to the experimental units.

Wheat flour (90 % dry matter, 78.5 % carbohydrate) was passed through a sieve with mesh number 270 (allowing particles smaller than 60 microns). Daily the carbon source was weighed (tank daily

Table 1

Features of treatments based on adding wheat flour (relative to daily feed) in 3 repetitions for 49 days.

Treatments	Amounts of WF used relative to daily feed intake	No.	WE (daily)
Clear water (Control)	Without adding WF	CW	35–50 % WE
Biofloc 1	WF, 25 % of daily feed	WF25	0.5–1 % WE
Biofloc 2	WF, 50 % of daily feed	WF50	0.5–1 % WE
Biofloc 3	WF, 75 % of daily feed	WF75	0.5–1 % WE
Biofloc 4	WF, 100 % of daily feed	WF100	0.5–1 % WE

Abbreviation: wheat flour (WF), number (No), water exchange (WE)

requirement), fully mixed using a 500 mL beaker and relevant tank water and uniformly distributed over the tank's surface directly after second feed application (14:00 pm).

Before feeding, 35–50 % of the water in the rearing tank was replaced with fresh water with the same salinity in the control group, while 0.5–1 % of the water was replaced in biofloc treatments due to siphoning. During the rearing period, the amount of feeding in each treatment was reduced according to the increase in growth, starting at 6 % of body weight at the beginning of the experiment up to 4 % at the end. The same feeding ratio (i.e. grams of feed per experimental unit per day) was kept in all treatments. Shrimp was fed using a commercial diet (38 % protein, 9 % lipid, and 14 % ash) three times a day (8:00 am, 14:00 pm., 20:00 pm), adjusted every ten days.

2.2. Water quality monitoring

Temperature (digital thermometer), pH (Lutron 208, pH meter), dissolved oxygen (DO) (Lutron 5510 Oxygen meter) and salinity (salinity refractometer) was monitored once a day from 9:00–11:00 am. Water transparency was measured using a sechi disk at 10:00 am. To determine the amount of biofloc volume (BV), one liter of tank water was collected weekly and poured into an Imhoff cone and kept for 20 minutes for settling (Effendy et al., 2016). The total suspended solids (TSS) were measured weekly by filtering 100 mL of tank water with whatman No. 42 filter paper and drying it for 3 hours at 105°C (APHA, 2012). Inorganic nitrogenous compounds, e.g. total ammonia nitrogen (TAN), nitrite (NO₂-N), and nitrate (NO₃-N) were monitored weekly using pre-filtered water (whatman filter) as per APHA (2012).

2.3. Growth performance

Shrimp weight measurements were performed at the beginning of the experiment and every ten days during the rearing period. At the end, the following parameters were evaluated using respective formulas:

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

Body weight index (BWI) (%) = [(final weight – initial weight)/initial weight] × 100

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

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

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

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

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

Feed efficiency (FE) = live weight gain (wet weight)/ feed consumed (dry weight) × 100

2.4. Proximate composition of biofloc

Water from each (biofloc) treatment was filtered through 20 µm mesh size. The samples were dried in an oven (at 105°C for 24 h) then stored at -18°C. Dry weight, crude protein, crude lipid, and ash were measured according to AOAC's method (AOAC, 2012).

2.5. Proximate composition of shrimp

At the end of the experimental period, 15 shrimps from each replicate treatment were collected for proximate composition. After removing the exoskeleton and head, samples were washed and homogenized with a meat grinder, then stored at -18°C until analysis. AOAC (2012) method was used to determine dry weight, crude protein, lipid and ash content. Samples were dried (for 24 h at 105°C) in porcelain to a constant weight, and difference in weight were used to estimate moisture content. Crude protein content was calculated by multiplying the nitrogen percentage by 6.25 (Krul, 2019). Soxhlet extraction with ether was used to determine crude lipid. Ash content was determined by incineration at 600°C for 3 h in a muffle furnace (Mabroke et al., 2021).

2.6. Biochemical analysis of hemolymph

The hemolymph of shrimp from different treatments was sampled at the end of the experiment. Ten shrimps were randomly selected from each tank and placed in a container containing ice at a temperature of 4°C for 15 minutes (to prevent stress and reduce shrimp mobility). To collect the hemolymph, a sterile one milliliter insulin syringe with a needle head number G26, was filled with 0.4 mL of Alzor anticoagulant solution (sodium citrate 27 mmol, sodium chloride 336 mmol, EDTA 9 mmol, glucose 115 mmol) to prevent coagulation (Jones et al., 2010).

Hemolymph (0.3 mL) was collected from the ventral sinus region (first and second swimming legs next to the ventral nerve cord) (Xu and Pan, 2013) by slowly dipping the needle tip of the syringe under the cortical layer of the shrimp shell at a 45-degree angle. Once collected, syringes were shaken to allow hemolymph and anticoagulant mixing. Hemolymph samples were stored in separate microtubes in a freezer at -80°C for further analysis.

Once transferred from the -80°C freezer to the laboratory at room temperature (27°C), samples were defrosted, homogenized using a vortex device for 30 seconds (Liu et al., 2019; Xu et al., 2019) and centrifuged at 12000 rpm at 4°C for 15 minutes to separate the plasma. Supernatant was then collected and commercial diagnostic kits (Pars Azmun Co., Tehran, Iran) were used to measure glucose, cholesterol, and triglyceride using a spectrophotometer by colorimetric method. Using *Micrococcus lysodeikticus* bacteria as substrate and egg white lysozyme as standard, lysozyme activity was measured by turbidity method (Ellis, 1990; Nayak et al., 2008). After oxidation of L-DOPA (Sigma) substrate, phenoloxidase activity was determined using the turbidity method (Söderhäll and Hall, 1984).

2.7. Statistical analysis

Kolmogorov-Smirnov was used to determine normality of dataset. Once assumptions were met, one-way ANOVA was performed. Significant differences among treatments were detected by Duncan's multiple range test at 95 % confidence level. Graphs were drawn using microsoft office software (word and excel, version 2013). Results are presented as

mean + standard deviation. Data was analyzed using SPSS software version 23 (Chicago, Illinois, USA).

3. Results

3.1. Water quality

The values of water quality parameters in pacific white shrimp rearing tanks are presented in Table 2 after adding different amounts of wheat flour (relative to daily feed). It was found that the DO and pH values of biofloc treatments containing wheat flour were lower than those in the control group, and the difference was significant ($P < 0.05$). WF100 treatment (adding wheat flour to 100 % of daily feed) showed the lowest levels of DO (5.42 mg/L). Biofloc treatments with wheat flour added showed lower salinity than the control group, which was significant ($P < 0.05$). As shown in Fig. 1, the control group had the highest transparency, the lowest amount of BV, and the lowest amount of TSS ($P < 0.05$).

With more wheat flour added to the rearing tanks, the transparency of the water decreased, and BV and TSS increased. Fig. 2 illustrates the changes in TAN, NO₂, and NO₃. The control group showed the highest levels of TAN (1.14 mg/L) and NO₂ (8.15 mg/L), and the lowest level nitrate (3.60 mg/L). Adding more wheat flour to rearing tanks results in a decrease in TAN and NO₂ and an increase in NO₃.

3.2. Growth performance and feed efficiency

The results of the growth performance and feed efficiency of Pacific white shrimp are presented in Table 3. Based on the results, it is evident that in the WF75 (adding wheat flour to 75 % of daily feed) and WF50 (adding wheat flour to 50 % of daily feed) treatments, the greatest increase in body weight (6.38 g) and (6.29 g) and the highest specific growth rate (2.42 %/day) and (2.40 %/day) respectively was observed, which showed a significant difference compared to the other treatments ($P < 0.05$).

The control group (without adding wheat flour) had the lowest final weight (8.02 g), growth rate (0.106 g/day), and biomass (243.6 g). The control group had the highest feed conversion ratio (1.77), as well as the lowest feed efficiency (56.65 %). The highest feed efficiency was obtained with wheat flour addition (75 % and 100 % of daily feed), which showed a significant difference with other treatments ($P < 0.05$). In Table 3, the survival rate for shrimp is shown to improve with the addition of wheat flour to the rearing tanks. In WF50 and WF75 treatments, the highest survival rates were 93.83 % and 93.20 %, respectively.

3.3. Shrimp body composition and collected bioflocs

The biochemical compositions of shrimp bodies and bioflocs produced in shrimp rearing tanks are presented in Table 4. The results show that adding wheat flour to the rearing tanks changes the shrimp's biochemical composition. Wheat flour increases the protein, lipid, and ash content of shrimp bodies when added to rearing tanks. Control group had the lowest protein (75.31 % DW), lipid (6.81 % DW) and ash (11.48 % DW) levels.

Biochemical compositions of the collected bioflocs showed that wheat flour added in different amounts affects their biochemical quality. It was found that the lowest amount of biofloc protein was 23.46 (% DW) in the WF25 treatment, which showed a significant difference from all other treatments ($P < 0.05$). The amount of biofloc lipid in all treatments was low in the range of 1.25–1.34. WF25 and WF50 were observed to have the lowest levels of biofloc ash (Table 4).

3.4. Haemolymph biochemical and immunity parameters

The results of the biochemical composition (glucose, triglyceride,

Table 2

Water quality parameters in cultivation tanks water of pacific white shrimp under the influence of adding wheat flour (relative to daily feed), for 49 days.

Parameters	CW	WF25	WF50	WF75	WF100
T (°C)	30.38 ± 0.39 ^a	30.40 ± 0.51 ^a	30.44 ± 0.37 ^a	30.41 ± 0.41 ^a	30.45 ± 0.44 ^a
DO (mg/L)	6.36 ± 0.37 ^a	6.10 ± 0.30 ^{ab}	5.84 ± 0.25 ^b	5.70 ± 0.26 ^b	5.42 ± 0.24 ^c
pH	8.12 ± 0.07 ^a	8.08 ± 0.09 ^{ab}	8.03 ± 0.07 ^b	7.91 ± 0.05 ^c	7.88 ± 0.06 ^c
Salinity (ppt)	32.76 ± 0.54 ^b	33.74 ± 1.00 ^a	33.75 ± 1.07 ^a	33.66 ± 0.95 ^a	33.78 ± 0.90 ^a
WT (cm)	39.91 ± 4.14 ^a	22.26 ± 10.97 ^b	20.80 ± 10.73 ^{bc}	19.62 ± 11.06 ^{bc}	17.51 ± 11.70 ^c
BV (mg/L)	1.07 ± 0.52 ^c	7.35 ± 3.67 ^b	8.77 ± 4.37 ^{ab}	9.7 ± 4.98 ^{ab}	11.36 ± 6.0 ^a
TSS (mg/L)	79.75 ± 35.52 ^c	194.12 ± 89.81 ^b	226.50 ± 104.19 ^{ab}	240.75 ± 115.66 ^{ab}	281.87 ± 135.58 ^a
TAN (mg/L)	1.14 ± 0.53 ^a	0.67 ± 0.36 ^b	0.44 ± 0.26 ^c	0.41 ± 0.30 ^c	0.35 ± 0.27 ^c
NO ₂ (mg/L)	8.15 ± 3.58 ^a	6.09 ± 2.81 ^b	5.64 ± 2.61 ^{bc}	4.51 ± 1.93 ^c	4.10 ± 1.82 ^c
NO ₃ (mg/L)	3.60 ± 1.68 ^d	6.08 ± 2.21 ^c	8.01 ± 3.41 ^b	9.06 ± 4.13 ^{ab}	10.02 ± 4.62 ^a

Abbreviation: temperature (T), dissolved oxygen (DO), water Transparency (WT), biofloc volume (BV), total suspended solids (TSS), total ammonium nitrogen (TAN), nitrite (NO₂), nitrate (NO₃)

Values are expressed as mean±SD. values in the same row with different letters are significantly different (P < 0.05).

Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

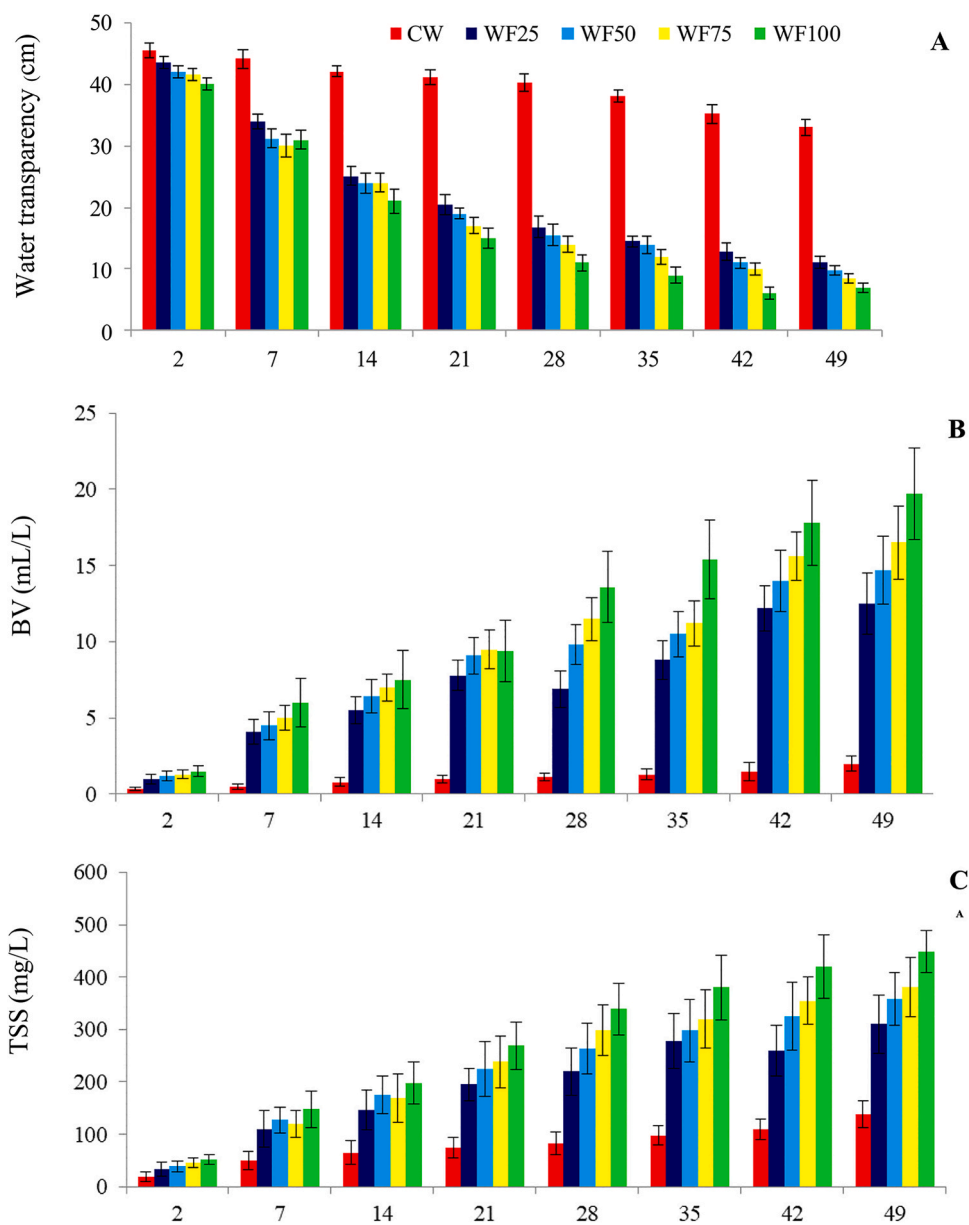


Fig. 1. Water quality parameters including water transparency (A), bifloc volume (BV; B), and total suspended solids (TSS; C) in cultivation tanks of *P. vannamei* in different treatments, for 49 days. Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

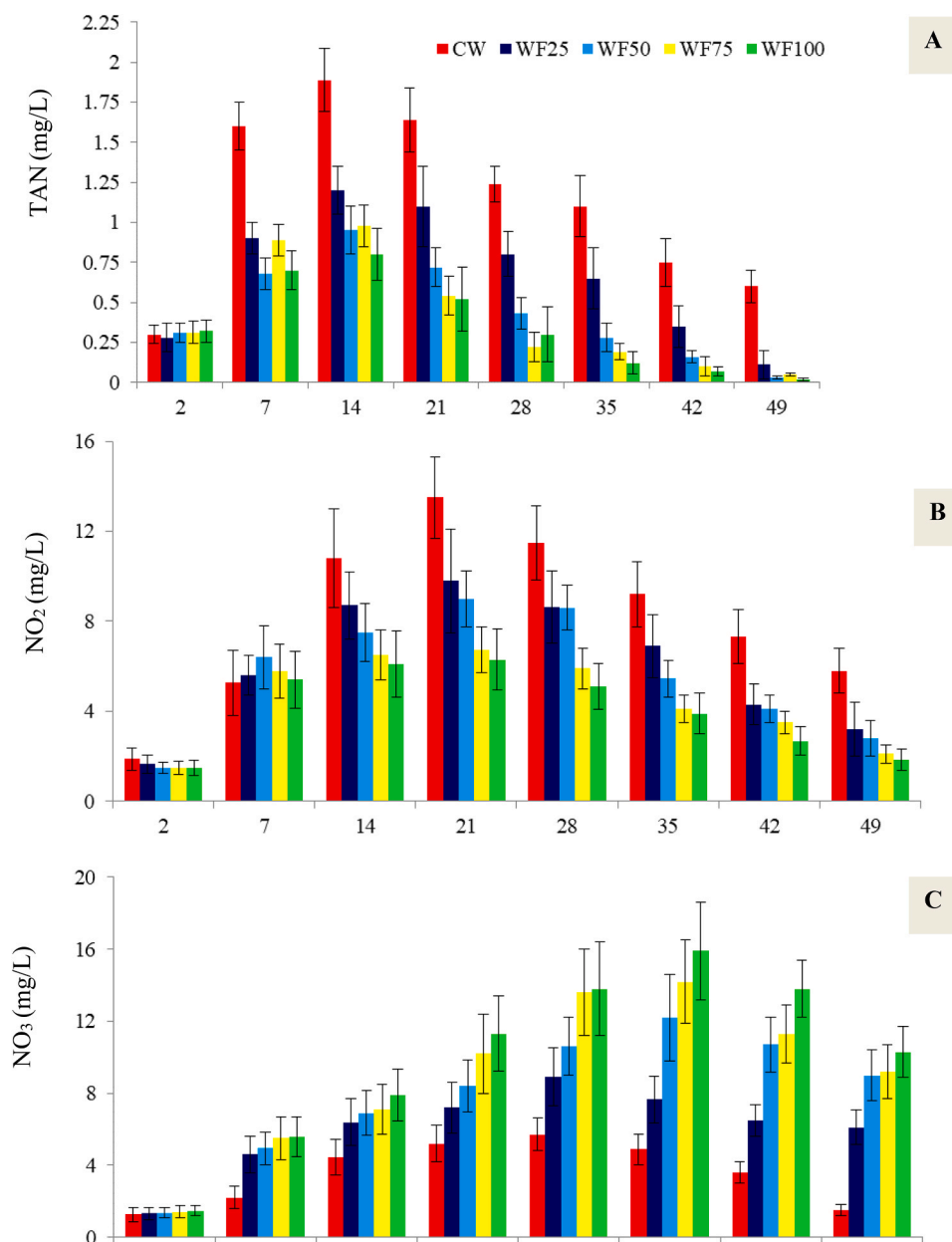


Fig. 2. Water quality parameters including total ammonia nitrogen (TAN; A); B) nitrite (NO₂; B); and C) nitrate (NO₃; C) in cultivation tanks of *P. vannamei* in different treatments, for 49 days. Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

and cholesterol) of the hemolymph of pacific white shrimp under the influence of different amounts of wheat flour addition (compared to the daily feed) are shown in Fig. 3. The results show that the lowest amount of glucose (32 mg/dL) and triglyceride (137.66 mg/dL) was observed in the control group which showed a significant difference with other treatments ($P < 0.05$).

The highest amount of glucose 41.33 and 42 mg/dL was obtained in WF50 and WF75 treatment, respectively, which showed a significant difference with other treatments.

No significant difference was observed in triglyceride values among biofloc treatments. The amount of shrimp hemolymph cholesterol was observed in the range of 90–94 mg/dL, and no significant difference was observed among the treatments ($P > 0.05$).

The results of immune factors (lysozyme, phenoloxidase (PO) are presented in Fig. 4. The results show that the lowest activity of lysozyme (19 u/mL/min) and PO (0.41 u/mL) is observed in the control group.

The amount of lysozyme activity in biofloc treatments with the addition of wheat flour was observed in the range of 22.33–25 u/mL/min, the lowest of which was related to the WF25 treatment. The highest amount of PO activity was 0.77 in WF50 treatment and 0.78 u/mL in WF75 treatment, which showed a significant difference with other treatments ($P < 0.05$).

4. Discussion

4.1. Water quality

Limited water change, carbon source addition, evaporation, and salt accumulation from uneaten feeds are key factors influencing water quality parameters dynamics in BFT (Alves et al., 2017). In our study, biofloc treatments with limited water exchange showed higher salinity values than control treatment. In addition, wheat flour added to biofloc

Table 3

Growth performance, feed utilization, and survival rate of pacific white shrimp (IBW: 2.80 ± 0.25 g) under the influence of adding wheat flour (relative to daily feed), for 49 days.

Parameters	CW	WF25	WF50	WF75	WF100
FW (g)	8.02 $\pm 0.34^c$	8.52 $\pm 0.32^b$	9.09 $\pm 0.27^a$	9.18 $\pm 0.32^a$	8.64 $\pm 0.30^b$
BWG (g)	5.22 $\pm 0.34^c$	5.72 $\pm 0.32^b$	6.29 $\pm 0.27^a$	6.38 $\pm 0.32^a$	5.84 $\pm 0.30^b$
BWI (%)	186.42 $\pm 12.13^c$	204.28 $\pm 11.40^b$	224.64 $\pm 9.88^a$	227.85 $\pm 11.39^a$	208.57 $\pm 10.80^b$
GR (g/day)	0.106 $\pm 0.007^c$	0.116 $\pm 0.006^b$	0.128 $\pm 0.005^a$	0.130 $\pm 0.006^a$	0.119 $\pm 0.006^b$
BG (g)	243.6 $\pm 15.85^c$	276.46 $\pm 15.42^b$	318.69 $\pm 14.02^a$	321.12 $\pm 16.07^a$	284.21 $\pm 14.72^b$
SGR (%/day)	2.14 $\pm 0.08^c$	2.27 $\pm 0.07^b$	2.40 $\pm 0.06^a$	2.42 $\pm 0.07^a$	2.30 $\pm 0.07^b$
FCR	1.77 $\pm 0.11^a$	1.56 $\pm 0.09^b$	1.35 $\pm 0.06^c$	1.34 $\pm 0.06^c$	1.51 $\pm 0.08^b$
FE (%)	56.65 $\pm 3.68^c$	64.29 $\pm 3.58^b$	74.11 $\pm 3.26^a$	74.68 $\pm 3.73^a$	66.09 $\pm 3.42^b$
SR (%)	86.42 $\pm 1.88^c$	89.50 $\pm 2.14^b$	93.83 $\pm 2.83^a$	93.20 $\pm 1.07^a$	90.12 $\pm 1.07^b$

Abbreviation: initial body weight (IBW), final weight (FW), body weight gain (BWG), body weight index (BWI), growth rate (GR), biomass gain (BG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), survival rate (SR).

Values are expressed as mean \pm SD. Values in the same row with different letters are significantly different ($P < 0.05$).

Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

Table 4

The amount of the approximate composition (% dry weight) of pacific white shrimp' body and biofloc obtained under the influence of adding wheat flour (relative to daily feed), at the end of the experiment (49 days).

treatment	Dry matter (%)	Crude protein(% DW)	Crude lipid (% DW)	Ash (% DW)
Shrimp				
CW	25.21 $\pm 0.18^a$	75.31 $\pm 0.10^c$	6.81 $\pm 0.09^b$	11.48 $\pm 0.12^c$
WF25	25.17 $\pm 0.11^a$	75.47 $\pm 0.07^b$	7.04 $\pm 0.12^a$	11.77 $\pm 0.07^b$
WF50	24.86 $\pm 0.12^b$	75.66 $\pm 0.08^a$	7.06 $\pm 0.06^a$	11.80 $\pm 0.10^b$
WF75	24.82 $\pm 0.08^b$	75.68 $\pm 0.09^a$	7.09 $\pm 0.08^a$	11.82 $\pm 0.09^b$
WF100	24.77 $\pm 0.21^b$	75.72 $\pm 0.13^a$	7.09 $\pm 0.11^a$	12.00 $\pm 0.05^a$
Biofloc				
WF25	21.32 $\pm 0.20^a$	23.46 $\pm 0.25^b$	1.26 $\pm 0.08^a$	33.43 $\pm 0.15^b$
WF50	21.04 $\pm 0.08^b$	23.86 $\pm 0.15^a$	1.25 $\pm 0.10^a$	33.48 $\pm 0.12^b$
WF75	20.97 $\pm 0.07^b$	23.88 $\pm 0.24^a$	1.28 $\pm 0.08^a$	33.81 $\pm 0.17^a$
WF100	21.01 $\pm 0.1^b$	23.93 $\pm 0.15^a$	1.34 $\pm 0.07^a$	33.90 $\pm 0.09^a$

Values are expressed as mean \pm SD. Values in the same column with different letters are significantly different ($P < 0.05$).

Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

treatments fostered microbial activity, and as a result, increased the amount of BV and TSS, especially in WF75 and WF100. The control group without wheat flour had the highest transparency, the lowest BV and TSS.

TSS is one of the most crucial physical factors to assess the development of bioflocs (Ebeling et al., 2006; Luo et al., 2020). In our study, BFT treatments showed TSS ranging from ~ 80 to ~ 280 mg/L, corroborating with levels found in other *P. vannamei* studies, e.g. 194–315 mg/L (Hassan et al., 2023), 86–468 mg/L (Irani et al., 2023), and 123–328 mg/L (Panigrahi et al., 2018). Although TSS levels increased significantly in biofloc treatments (with wheat flour addition), they remained within the physiological and physical range for *P. vannamei* (Schweitzer et al., 2024). BV is a practical method for quantifying bioflocs due to its simplicity and less time consuming (Xu et al., 2016; Hassan et al., 2023). Similar to TSS, the addition of wheat flour in water boosted the biofloc formation, enhancing the growth of heterotrophic microorganisms. Same pattern was observed by Vinasyam et al. (2024) using wheat bran.

Regarding other water quality parameters, results observed in all treatments were within acceptable ranges for Pacific white shrimp culture (Samocha and Prangnell, 2019). Shrimp often require DO levels > 5 mg/L, and concentrations below 3.7 mg/L are considered critical for a functioning metabolism (Kumar et al., 2017; Khanjani et al., 2024b). The lowest DO level was observed in WF100 treatment, probably due to the higher activity of microbial communities in this treatment. BFT demands DO for both microbial respiration and metabolic needs of shrimp (De Schryver et al., 2008). Regarding pH, WF75 and WF100 recorded the lowest levels likely due to (i) higher heterotrophic respiration (Xu et al., 2016), and (ii) increased metabolism of nitrifying bacteria (Ebeling et al., 2006; Khanjani et al., 2024b), increasing CO₂ levels in biofloc treatments (Hassan et al., 2022).

Wheat flour significantly reduced TAN levels in the water column. The control group showed higher TAN and NO₂ values, but lower NO₃ values. Carbohydrates added to *P. vannamei* in BFT helped to reduce TAN concentrations (Panigrahi et al., 2018; Chakrapani et al., 2022; Hassan et al., 2023) due to heterotrophic pathway (Emerenciano et al., 2017). Several authors have documented the initial increase in TAN during the first days of the culture and its conversion to nitrite, then to nitrate (Ren et al., 2019; Long et al., 2023). In this process, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are involved (Xu et al., 2021; Khanjani et al., 2024b). In our study, the use of mature biofloc water as a inoculum fostered the nitrifying development, contributing to a better toxic N-compounds control. Moreover, the addition of wheat flour supported the heterotrophic assimilation of TAN, and as a result less loads of nitrite were observed, especially in WF75 and WF100. Wheat flour may also served as a substrate for nitrifiers, resulting in higher nitrate levels with increasing levels of carbohydrate addition in water. According to the results of the present study, adding wheat flour to the water in a biofloc system improves the water quality in *P. vannamei* cultivation tanks.

4.2. Growth and survival performance

Growth performance was higher in biofloc treatments compared to control (clear-water, water exchange) group, with highest values obtained in WF75 and WF100. This trend showcases the ability of *P. vannamei* to consume the microbial particles (Panigrahi et al., 2019), in this case wheat flour-based bioflocs. *P. vannamei* constantly consumes biofloc as an additional food source generated by the microbial community, resulting in positive performance outcomes (Wasielensky et al., 2006; Addo et al., 2023). Biofloc nutrient-rich particles contain essential nutrition such as crude protein, unsaturated fatty acids, vitamins, and minerals, which improved the nutritional status and growth performance of farmed shrimp (Tabarrok et al., 2020), as well as carotenoids, chlorophylls, phytosterols, bromophenols, amino sugars and antibacterial compounds (Ju et al., 2008; Zhao et al., 2016; Dorothy et al., 2021), and combined with the artificial feed form a complex food chain consumed by shrimp (Addo et al., 2023).

In our study, the lowest (1.35, 1.34) and highest (1.77) FCRs were measured in WF50, WF75, and CW groups, respectively. Wheat flour

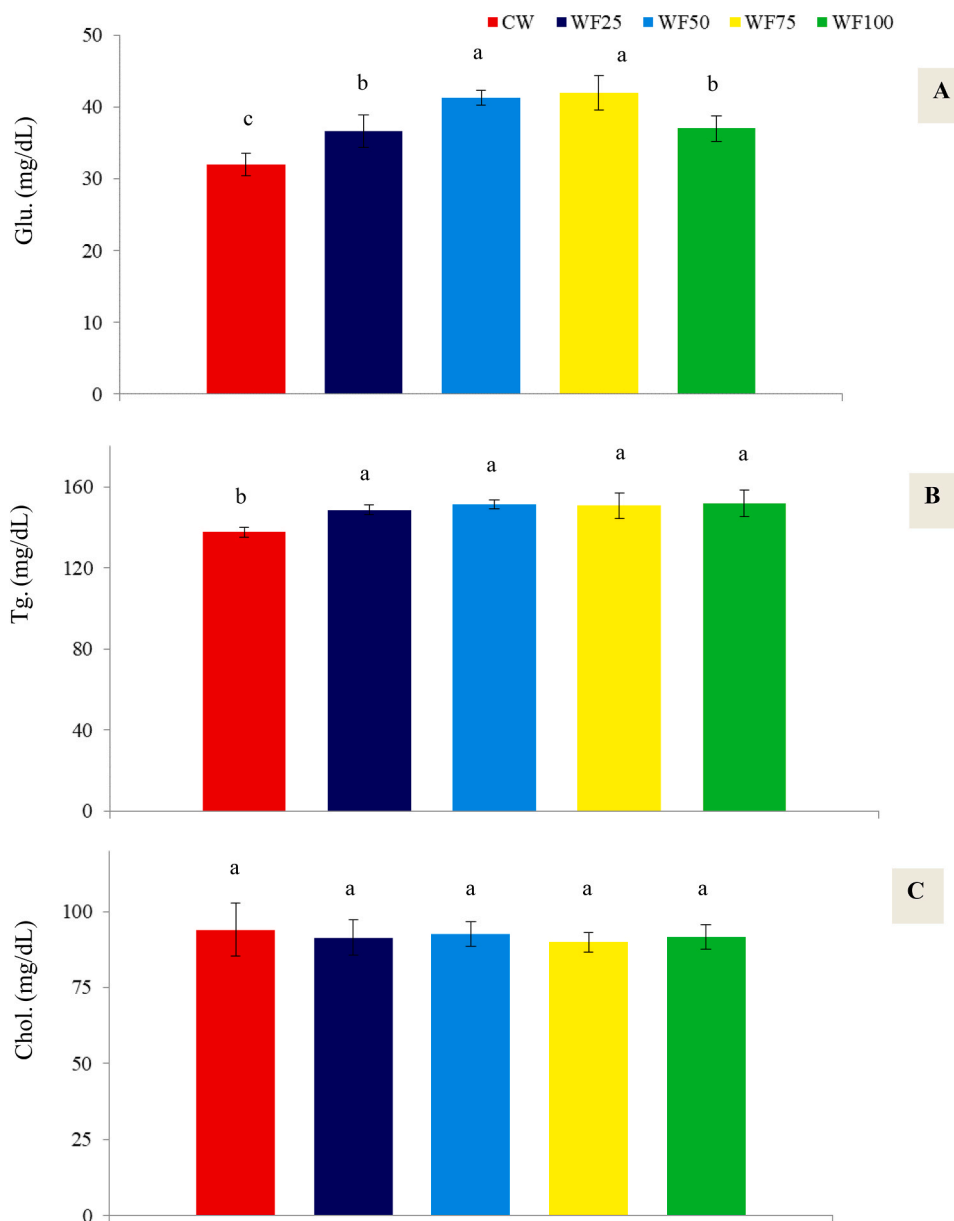


Fig. 3. Hemolymph biochemical parameters of *P. vannamei* including glucose (Glu), triglyceride (Tg), and cholesterol (Chol) under the influence of adding wheat flour (relative to daily feed), at the end of the experiment (49 days). Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

added to the water improved the FCR, corroborated by other BFT studies using diverse carbon sources (Da Silveira et al., 2022; Irani et al., 2023). FCRs for *P. vannamei* were reported as 1.41–1.56 (Ekasari et al., 2014a), 1.03–1.34 (Amjad et al., 2022), and 1.45–1.59 (He et al., 2023), which are consistent with our results.

According to Tabarrok et al. (2020) a reduced FCR was associated with BFT systems' ability to supply essential nutrients, such as crude protein, unsaturated fatty acids, vitamins, and minerals, which improved the nutritional status and growth performance of farmed aquatic animal. Additionally, bioflocs can improve shrimp digestion and absorption of feed by colonizing their guts with extracellular enzymes producing bacteria (Panigrahi et al., 2019).

Shrimp survival in biofloc treatments was higher compared to control group, and WF50 (93.83 %) and WF 75 (93.20 %) as best performing treatments. Some sort of turbidity (more similar to wild conditions compared to clear-water), complementary nutrition and more

suitable water quality (Dorothy et al., 2021) may have contributed to an improved survival rates in BFT treatments. In other studies, addition of carbohydrates also affected the shrimp survival rates (Tinh et al., 2021; Das et al., 2023). *P. vannamei* survival in biofloc systems were reported as 89.0–90.7 (Hussain et al., 2021), 89.7–92.8 (Xu et al., 2022), and 87.92–100 (Irani et al., 2023) which are consistent with the present study. Additionally, bioflocs can also enhance shrimp digestion and assimilation, resulting in improved growth and survival as observed in biofloc groups (Anand et al., 2014), especially WF75 and WF100.

4.3. Proximate composition of shrimp and bioflocs

Wheat flour addition to *P. vannamei* culture water in biofloc treatments impacted body composition in terms of protein, lipid, and ash. Increasing crude protein content in shrimp may be associated with an increase in their utilization and retention of dietary protein (Ghosh,

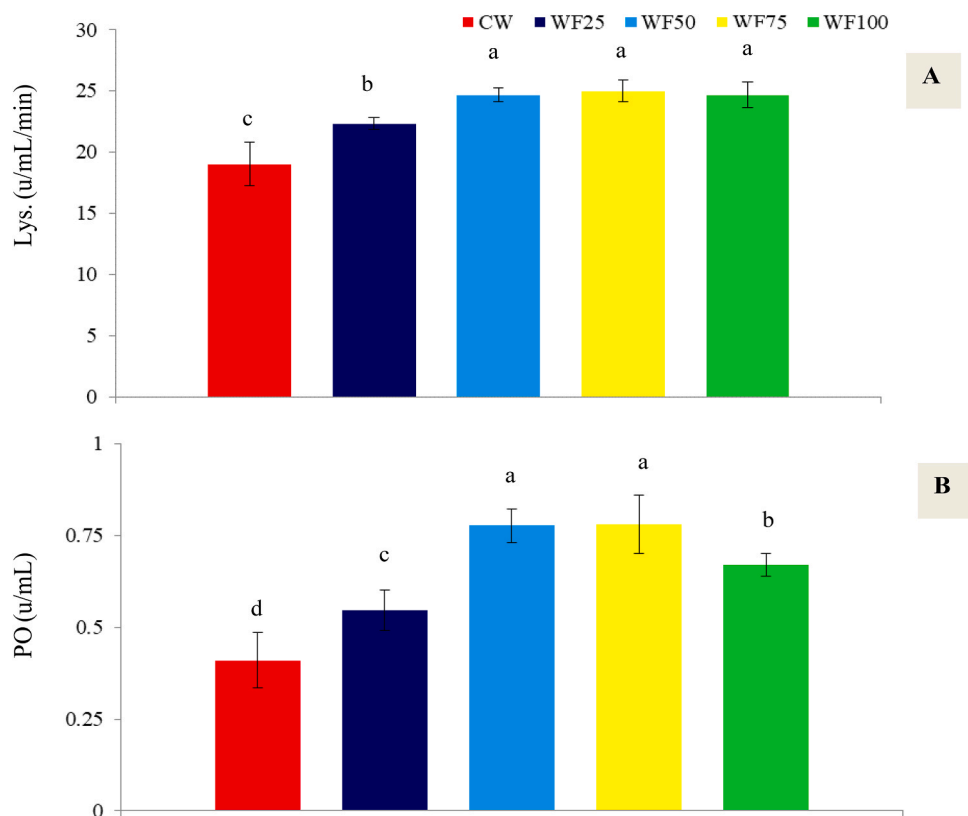


Fig. 4. Immunity parameters in the haemolymph of *P. vannamei* including lysozyme (Lys.) and phenoloxidase (PO) under the influence of adding wheat flour (relative to daily feed), at the end of the experiment (49 days). Experimental groups include the following: CW: without adding wheat flour, WF25: adding wheat flour 25 % of daily feed, WF50: adding wheat flour 50 % of daily feed, WF75: adding wheat flour 75 % of daily feed, WF100: adding wheat flour 100 % of daily feed.

2018). Similarly, greater lipid content and ash was observed as compared to control, probably due to an increased in lipid retention from biofloc-based live food items (Izquierdo et al., 2006; Abbaszadeh et al., 2022); and high availability of minerals (Tacon et al., 2002; Ju et al., 2008), respectively. Vinasiam et al. (2024) reported that *P. vannamei* body protein, lipid, and ash contents were 71.0–74.5 %, 3.8–4.3, and 14.1–17.3 % of DW, respectively. According to Irani et al. (2023), *P. vannamei* bodies contained 17.43–20.79 % protein, 1.09–3.71 lipid, and 1.21–1.53 % ash of wet weight, respectively. Our current results (~75 % protein, ~6–7 % lipids and ~11–12 % ash) are corroborated by other studies (Tinh et al., 2021; Vinasiam et al., 2024), however type of samples (e.g. whole animal or peel off) may impact the results.

Regarding the proximate composition of bioflocs, increasing levels of wheat flour impacted the biofloc chemical characteristics. Farmed shrimp can benefit from the nutritional profile of bioflocs (El-Sayed, 2021), and factors such as environmental conditions, amount and type of carbon sources, bacteria communities, particle size, and stocking density can influence the nutritional composition of bioflocs (Ekasari et al., (2014b); Generally, bioflocs contain 20–45 % protein, 1–8 % lipid, and 15–60 % ash (Khanjani et al., 2023a). For instance, WF25 (23.5 %) had the lowest protein content compared to other biofloc treatments (~23.9 %). Bauer et al. (2012), Lara et al. (2017), and Jimenez-Ordaz et al. (2021) observed biofloc protein content ranging from 23.39 %, 26.39–29.54 %, and 23.81–28.12 % (base of % DW) in *P. vannamei* culture.

In our study, bioflocs presented lipid levels ranging between 1.25 % and 1.34 % dry weight, with no significant differences among treatments. These low levels corroborated with other studies when wheat is used as a carbon source (Rajkumar et al., 2016; Mahanand et al., 2013; Hosain et al., 2021). Other studies reported 0.92 % DW in *P. vannamei* culture (Rajkumar et al., 2016), 1.1 % DW in *Labeo rohita* culture

(Mahanand et al., 2013), and 0.82 % DW in *Macrobrachium rosenbergii* culture (Hosain et al., 2021). In terms of ash, increasing levels of wheat flour lead to a higher levels of ash, with values ranging from 33.43 % to 33.90 %. High ash content in bioflocs are related to high amount of salts from substrates, microbial and suspended particles (Bauer et al., 2012), and abundance of acid-insoluble oxides and mixed silicates (Binalshikh-Abubkr et al., 2021). In *P. vannamei* biofloc-based culture, Rajkumar et al. (2016), Lara et al. (2017), and Khanjani and Sharifinia (2022) reported biofloc ash values of 14.88–25.04 %, 27.86–43.25 %, and 33.96–35.15 %, respectively.

4.4. Haemolymph biochemical and immunity parameters

In *P. vannamei*, bioflocs have been reported to improve health, and serve as immunostimulants (Xu and Pan, 2013; Miao et al., 2017). Glucose changes in shrimp hemolymph can be related to stress and tissue-based glycogenolysis, occurring primarily in the hepatopancreas, muscle, and blood cells. Fluctuations in shrimp glucose are observed due to management and environment conditions (Martinez-Porchas et al., 2020; Guemez-Sorhouet et al., 2019; Kumar et al., 2017).

Compared with the control group (32.0 mg/dL), *P. vannamei* reared in biofloc had a higher plasma glucose level, with values in WF50 (41.33 mg/dL) and WF75 (42.0 mg/dL) treatments, suggesting a better nutritional status. This trend can be explained due to nutritional diversity of biofloc (live food, bacteria, and detritus) with variable carbohydrate levels and impacting plasma glucose levels (Abbaszadeh et al., 2022). Kumar et al. (2017) found that the serum glucose level was higher in the biofloc system than in the control group in *Penaeus monodon* using molasses and rice flour as carbon sources.

Triglycerides is required for membrane maintenance, and triglycerides stored in adipocytes are metabolized and used for energy (Niu et al., 2008). In the biofloc treatments, the hemolymph triglyceride

levels were higher compared to control, probably due to bioflocs ingestion containing several external enzymes, enhancing digestion and increasing plasma levels (Abbaszadeh et al., 2022). In terms of cholesterol, this key component of shrimp cell membranes and subcellular structures is precursor of steroid and molting hormones such as ecdysone (Guo et al., 2024). In shrimp lipids are transported by the hemolymph, and concentration of cholesterol can be an useful indicator of shrimp's lipid metabolism (catabolism and oxidation), with factors such as environment, diet and culture conditions as key modulators (Martinez-Porchas et al., 2020; Hussain et al., 2021). In the present study, hemolymph cholesterol levels were 90–94 mg/dL and no significant difference was found between treatments. Same trend was observed by Abbaszadeh et al. (2022) in *P. vannamei*.

Among invertebrates, lysozyme plays an important role in non-specific immunity against pathogen invasion (Liu et al., 2018). In the present study, lysozyme activity was higher in biofloc treatments (~25 u/mL/min), compared to the control group (~19 u/mL/min), consistent with previous research in *P. vannamei* (Panigrahi et al., 2019) and *Penaeus indicus* (Panigrahi et al., 2020). Regarding phenoloxidase (PO), major enzyme in shrimp immunity, higher activity was observed in biofloc treatments (especially WF50 and WF75) compared to control group. In shrimp cultured with biofloc, Panigrahi et al. (2020) found that consuming microbial floc stimulated shrimp PO activity and improved their innate immunity. It appears that the stimulation effect of the biofloc system is a general characteristic of the system (Panigrahi et al., 2019; 2020), with the degree of stimulation depending on the amount of carbon source (wheat flour) added. A biofloc farming environment contains immune stimulants like peptidoglycans, beta-glucans, and lipopolysaccharides that function as probiotics. Combining poly beta hydroxybutyrate with biofloc produces short-chain fatty acids with probiotic properties, which further boosts the immune response of farmed shrimp (Panigrahi et al., 2020; Khanjani et al., 2023b).

5. Conclusion

In our experimental conditions, the adoption of biofloc technology with increasing levels of wheat flour in water improved the quality of water, growth performance, feed efficiency, body composition, as well as the biochemical and immunity parameters (lysozyme and phenoloxidase) of *P. vannamei* juveniles. Wheat flour added to the amount (50–75 % of daily feed) improved performance. More research (e.g. intestinal microbiome) is encouraged to better understand the role of wheat flour on shrimp performance and health.

Authorship contributions

Conception and design of study: Mohammad Hossein Khanjani, Jamshid Eslami, Drafting the manuscript: Mohammad Hossein Khanjani, Jamshid Eslami, Revising the manuscript after reviewers evaluation: Mohammad Hossein Khanjani, Jamshid Eslami, Maurício Gustavo Coelho Emerenciano.

Funding

This project no 4813–03–06–38929 and with the benefit of the research credits of the Ministry of science, research and technology, Iran, for the academic staff members who have been recruited in the last 5 years (University of Jiroft).

CRedit authorship contribution statement

Mohammad Hossein Khanjani: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization, Formal analysis, Methodology, Project administration. **Jamshid Eslami:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Maurício**

Gustavo Coelho Emerenciano: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Data availability

No data was used for the research described in the article.

References

- Abbaszadeh, A., Keyvanshokoh, S., Yavari, V., Naderi, M., 2019. Proteome modifications of Pacific white shrimp (*Litopenaeus vannamei*) muscle under biofloc system. *Aquac. Nut.* 25 (2), 358–366.
- Abbaszadeh, A., Mozanzadeh, M.T., Qasemi, A., Oujifard, A., Nafsi Bahabadi, M., 2022. Effects of the addition of *Calanopia elliptica*, *Artemia franciscana*, and *Brachionus rotundiformis* in a nursery biofloc system on water quality, growth, gut morphology, health indices, and transcriptional response of immune and antioxidant-related genes in *Penaeus vannamei*. *Aquac. Int.* 30, 653–676.
- Addo, F.G., Zhang, S., Manirakiza, B., Ma, Y., Yuan, S., Alklaf, S.A., Guo, S., Abakari, G., 2023. Brown sugar addition enhanced nutrient removal rates, growth performance, and bacterial community in a rice straw-based biofloc shrimp culture system. *Aquaculture* 567, 739274.
- Alves, G.F.O., Fernandes, A.F.A., Alvarenga, E.R., Turra, E.M., Sousa, A.B., Teixeira, E.A., 2017. Effect of the transfer at different moments of juvenile Nile tilapia (*Oreochromis niloticus*) to the biofloc system in formation. *Aquaculture* 479, 564–570.
- Amjad, K., Dahms, H.U., Ho, C.H., Wu, Y.C., Lin, F.Y., Lai, H.T., 2022. Probiotic additions affect the biofloc nursery culture of white shrimp (*Litopenaeus vannamei*). *Aquaculture* 560, 738475.
- Anand, P.S.S., Kohli, M.P.S., Kumar, S., Sundaray, J.K., Dam Roy, S., Venkateshwarlu, G., Sinha, A., Pailan, G.H., 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture* 418, 108–115.
- AOAC (Association of Official Analytical Chemists), 2012. Official method of analysis, 19th edition. AOAC, Washington, D.C.
- APHA, 2012. Standard Methods for the Examination of Water and Wastewater (22nd ed.). American Public Health Association, Washington, DC, USA.
- Bauer, W., Prentice-Hernandez, C., Borges-Tesser, M., Wasielesky, W., Poersch, L.H.S., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei* carnivorous fish. *Aquaculture* 342–343, 112–116.
- Binalshikh-Abubkr, T., Hanafiah, M.M., Das, S.K., 2021. Proximate chemical composition of dried shrimp and tilapia waste bioflocs produced by two drying methods. *J. Mar. Sci. Eng.* 9, 193.
- Chakrapani, S., Panigrahi, A., Sundaresan, J., Mani, S., Palanichamy, E., Rameshbabu, V. S., Krishna, A., 2022. Utilization of complex carbon sources on biofloc system and its influence on the microbial composition, growth, digestive enzyme activity of pacific white shrimp, *Penaeus vannamei* culture. *TRJFAS18813 Turk. J. Fish. Aquat. Sci.* 22 (4). <https://doi.org/10.4194/TRJFAS18813>.
- Da Silveira, L.G.P., Rosas, V.T., Kruppenauer, D., Poersch, L.H., Wasielesky Jr, W., 2022. Comparison between horizontal and vertical substrate in shrimp superintensive culture in bioflocs system. *Aquac. Eng.* 96, 102218.
- Dai, P., Li, D., Sui, J., Kong, J., Meng, X., Luan, S., 2023. Prediction of meat yield in the Pacific whiteleg shrimp *Penaeus vannamei*. *Aquaculture* 577, 739914.
- Das, R.R., Panigrahi, A., Sarkar, S., Saravanan, A., Biju, I.F., Ambikanandham, K., Jayanthi, M., Kannappan, S., 2023. Growth, survival, and immune potential of post larvae of Indian white shrimp, *Penaeus indicus* (H. Milne Edwards, 1937) in different salinities with biofloc system (BFT) during nursery phase. *Aquac. Int.* 31, 273–293.
- De Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bioflocs technology: the added value for aquaculture. *Aquaculture* 277, 125–137.
- de Souza, D.M., Suita, S.M., Romano, L.A., Wasielesky, W., Ballester, E.L.C., 2012. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. *Aquac. Res.* 1–8. <https://doi.org/10.1111/j.1365-2109.2012.03223.x>.
- Dorothy, M.S., Vungarala, H., Sudhagar, A., Reddy, A.K., Rani Asanaru Majeedkutti, B., 2021. Growth, body composition and antioxidant status of *Litopenaeus vannamei* juveniles reared at different stocking densities in the biofloc system using inland saline groundwater. *Aquac. Res.* 52, 6299–6307.
- Ebeling, J.M., Timmons, M.B., Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture* 257, 346–358.
- Effendy, I., Samy, A.D., Deen, Sambhu, C., 2016. Semi intensive and semi biofloc methods for the culture of Indian white prawn, *Fenneropenaeus indicus* in highdensity polyethylene liner ponds. *Hayati J. Biosci.* 23, 106–110.
- Ekasari, J., Angela, D., Waluyo, S.H., Bachtiar, T., Surawidjaja, E.H., Bossier, P., De Schryver, P., 2014b. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture* 426–427, 105–111.
- Ekasari, J., Azhar, M.H., Enang, H., Sri Nuryati, S., De Schryver, P., Bossier, P., 2014a. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish. Shellfish Immunol.* 41, 332–339.
- Ellis, A.E., 1990. Lysozyme Assays. *Techniques in Fish Immunology* 101–103.

- El-Sayed, A.F.M., 2021. Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. *Rev. Aquac.* 13 (1), 676–705.
- Emerenciano, M.G.C., Khanjani, M.H., Sharifinia, M., Miranda-Baeza, A., 2025. Could biofloc technology (BFT) pave the way toward a more sustainable aquaculture in line with the circular economy? *Aquac. Res. Artic.* ID 1020045. <https://doi.org/10.1155/are/1020045>.
- Emerenciano, M.G.C., Martínez-Córdova, L.R., Martínez-Porchas, M., Miranda-Baeza, A., 2017. In: Tuti, H. (Ed.), *Water quality*. Intech Open, London, pp. 91–109.
- FAO, 2022. *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*. Rome. FAO. <https://doi.org/10.4060/cc0461en>.
- FAO, 2024. *In brief to the state of world fisheries and aquaculture 2024. Blue transformation in action*, Rome. <https://doi.org/10.4060/cd0690en>.
- Ghosh, A.K., 2018. Effect of feeding level on growth, body composition, fatty acid profile, and nutrient accumulation in shrimp (*Litopenaeus vannamei*). *Aquac. Int.* 26 (2), 405–417.
- Guemez-Sorhouet, E., Villarreal, H., Racotta, I.S., Naranjo, J., Mercier, L., 2019. Zootechnical and physiological responses of whiteleg shrimp (*Litopenaeus vannamei*) postlarvae reared in bioflocs and subjected to stress conditions during nursery phase. *Aquac. Res.* 50, 1198–1211.
- Guo, H., Hua, H., Wang, J., Qiang, W., Xiang, X., Liu, W., Jiang, G., 2024. The role of cholesterol during the ovarian maturation and lipid metabolism of female Chinese Mitten Crab (*Eriocheir sinensis*). Article ID 9933600 *Aquac. Nut.* 2024, 16. <https://doi.org/10.1155/2024/9933600>.
- Hassan, S.A.H., Sharawy, Z.Z., El Nahas, A.F., Hemed, S.A., El-Haroun, E., Abbas, E.M., 2022. Carbon sources improve water quality, microbial community, immune-related and antioxidant genes expression, and survival of challenged *Litopenaeus vannamei* Postlarvae in biofloc system. *Aquac. Res.* 53, 5902–5914.
- Hassan, S.A.H., Sharawy, Z.Z., Hemed, S.A., El Nahas, A.F., Abbas, E.M., Khalil, H.S., Verdegem, M., 2023. Sugarcane bagasse improved growth performance, digestive enzyme activity, microbial dynamics, and mRNA transcripts of immune-, growth, and antioxidant-related genes of *Litopenaeus vannamei* in a zero-water exchange system. *Aquac. Rep.* 33, 101788.
- He, X., Abakari, G., Tan, H., Liu, W., Luo, G., 2023. Effects of different probiotics (*Bacillus subtilis*) addition strategies on a culture of *Litopenaeus vannamei* in biofloc technology (BFT) aquaculture system. *Aquaculture* 566, 739216.
- Hosain, Md.E., Amin, S.M.N., Arshad, A., Kamarudin, M.S., Karim, M., 2021. Effects of carbon sources on the culture of Giant river prawn in biofloc system during nursery phase. *Aquac. Rep.* 19, 100607.
- Hussain, A.S., Mohammad, D.A., Sallam, W.S., Shoukry, N.M., Davis, D.A., 2021. Effects of culturing the Pacific white shrimp *Penaeus vannamei* in biofloc vs synbiotic systems on the growth and immune system. *Aquaculture* 542, 736905.
- Irani, M., Islami, H.R., Nafisi Bahabadi, M., Hosseini Shekarabi, S.P., 2023. Production of Pacific white shrimp under different stocking density in a zero-water exchange biofloc system: effects on water quality, zootechnical performance, and body composition. *Aquac. Eng.* 100, 102313.
- Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O., Tacon, A., 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. *Aquac. Nut.* 12, 192–202.
- Jimenez-Ordaz, F.J., Cadena-Roa, M.A., Pacheco-Vega, J.M., Rojas-Contreras, M., Tovar-Ramírez, D., Arce-Amezquita, P.M., 2021. Microalgae and probiotic bacteria as biofloc inducers in a hyper-intensive Pacific white shrimp (*Penaeus vannamei*) culture. *Lat. Am. J. Aquat. Res.* 49 (1), 155–168.
- Jones, C.M., Ng, W.K., King, M., 2010. Alsever's solution: a review of its history, chemistry, and production. *Transfus. Med. Rev.* 24, 259–267.
- Ju, Z.Y., Forster, I.P., Conquest, L., Dominy, W., 2008. Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. *Aquac. Nut.* 14, 533–543.
- Khanjani, M.H., Alizadeh, M., Mohammadi, M., Sarsangi Aliabad, H., 2021. Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: growth performance, carcass analysis, digestive and hepatic enzyme activity. *Iran. J. Fish. Sci.* 20 (2), 490–513.
- Khanjani, M.H., Mohammadi, A., Emerenciano, M.G.C., 2024b. Water quality in biofloc technology (BFT): an applied review for an evolving aquaculture. *Aquac. Int.* 32, 9321–9374.
- Khanjani, M.H., Sharifinia, M., Emerenciano, M.G.C., 2023b. A detailed look at the impacts of biofloc on immunological and hematological parameters and improving resistance to diseases. *Fish. Shellfish Immunol.* 137, 108796.
- Khanjani, M.H., Sharifinia, M., Hajirezaee, S., 2023a. Biofloc: a sustainable alternative for improving the production of farmed cyprinid species. *Aquac. Rep.* 33, 101748.
- Khanjani, M.H., Sharifinia, M., 2022. Biofloc technology with addition molasses as carbon sources applied to *Litopenaeus vannamei* juvenile production under the effects of different C/N ratios. *Aquac. Int.* 30, 383–397.
- Khanjani, M.H., Sharifinia, M., 2024. Feeding Nile tilapia with varying levels of biofloc: effect on growth performance, survival rate, digestive and liver enzyme activities, and mucus immunity. *Aquac. Int.* 32, 8171–8194.
- Khanjani, M.H., Sharifinia, M., Akhavan-Bahabadi, M., Emerenciano, M.G.C., 2024c. Probiotics and phytochemicals as dietary and water supplements in biofloc aquaculture systems (Article ID). *Aquac. Nut.*, 3089887. <https://doi.org/10.1155/anu/3089887>.
- Khanjani, M.H., Torfi Mozanade, M., Sharifinia, M., Emerenciano, M.G.C., 2024a. Broodstock and seed production in biofloc technology (BFT): an updated review focused on fish and penaeid shrimp. *Aquaculture* 579 (30), 740278.
- Kim, Y.S., Kim, S.E., Kim, S.J., Jung, H.K., Park, J., Jeon, Y.J., Kim, D.H., Kang, J.H., Kim, K.H., 2021. Effects of wheat flour and culture period on bacterial community composition in digestive tracts of *Litopenaeus vannamei* and rearing water in biofloc aquaculture system. *Aquaculture* 531, 735908.
- Koyama, M., Nagao, N., Syukri, F., Rahim, A.A., Toda, T., Tran, Q.N.M., Nakasaki, K., 2020. Ammonia recovery and microbial community succession during thermophilic composting of shrimp pond sludge at different sludge properties. *J. Clean. Prod.* 251, 119718.
- Krull, E.S., 2019. Calculation of nitrogen-to-protein conversion factors: a review with a focus on soy protein. *JAOCs* 96 (4), 339–364.
- Kumar, S., Anand, P.S.S., De, D., Deo, A.D., Ghoshal, T.K., Sundaray, J.K., Ponniah, A.G., Jithendran, K.P., Raja, R.A., Biswas, G., Lalitha, N., 2017. Effects of biofloc under different carbon sources and protein levels on water quality, growth performance and immune responses in black tiger shrimp *Penaeus monodon* (Fabricius, 1978). *Aquac. Res.* 48, 1168–1182.
- Lara, G., Krummenauer, D., Abreu, P.C., Poersch, L.H., Wasielesky, W., 2017. The use of different aerators on *Litopenaeus vannamei* biofloc culture system: effects on water quality, shrimp growth, and biofloc composition. *Aquac. Int.* 25 (1), 147–162.
- Liu, G., Ye, Z., Liu, D., Zhu, S., 2018. Inorganic nitrogen control, growth, and immunophysiological response of *Litopenaeus vannamei* (Boone, 1931) in a biofloc system and in clear water with or without commercial probiotic. *Aquac. Int.* 26, 981–999.
- Liu, T., Zhang, G., Feng, Y., Kong, C., Ayisi, C.L., Huang, X., Hua, X., 2019. Dietary soybean antigen impairs growth and health through stress-induced non-specific immune responses in Pacific white shrimp, *Litopenaeus vannamei*. *Fish. Shellfish Immunol.* 84, 124–129.
- Long, L., Liu, H., Lu, S., 2023. Effects of low salinity on growth, digestive enzyme activity, antioxidant and immune status, and the microbial community of *Litopenaeus vannamei* in biofloc technology aquaculture systems. *J. Mar. Sci. Eng.* 11, 2076.
- Luo, G., Xu, J., Meng, H., 2020. Nitrate accumulation in biofloc aquaculture systems. *Aquaculture* 520, 734675.
- Mabrore, R.S., Zidan, A.F.A., Tahoun, A., Mola, H.R.A., Abo- State, H., Suloma, A., 2021. Feeding frequency affect feed utilization of tilapia under biofloc system condition during nursery phase. *Aquac. Rep.* 19, 100625.
- Maciel de Lima, P.C., de Andrade, R.J.V., Elins Moreira da Silva, A., Campos, C.V.F. da S., Oliveira, C.Y.B., Olivera Gálvez, A., Brito, L.O., 2022. Effects of different molasses application rates on planktonic composition in low salinity biofloc culture of Nile tilapia, *Oreochromis niloticus* fingerlings. *Chem. Ecol.* 38 (10), 913–934.
- Mahanand, S.S., Moullick, S., Rao, P.S., 2013. Optimum formulation of feed for rohu, *Labeo rohita* (Hamilton), with biofloc as a component. *Aquac. Int.* 21 (2), 347–360.
- Mansour, A.T., Ashry, O.A., Ashour, M., Alsaqufi, A.S., Ramadan, K.M.A., Sharawy, Z.Z., 2022. The optimization of dietary protein level and carbon sources on biofloc nutritive values, bacterial abundance, and growth performances of whiteleg shrimp (*Litopenaeus vannamei*) juveniles. *Life* 12 (6), 888. <https://doi.org/10.3390/life12060888>.
- Martinez-Porchas, M., Ezquerro-Brauer, M., Mendoza-Cano, F., Higuera, J.E.C., Vargas-Albore, F., Martinez-Cordova, L.R., 2020. Effect of supplementing heterotrophic and photoautotrophic biofloc, on the production response, physiological condition and post-harvest quality of the whiteleg shrimp, *Litopenaeus vannamei*. *Aquac. Rep.* 16, 100257.
- Miao, S., Hu, J., Wan, W., Han, B., Zhou, Y., Xin, Z., Sun, L., 2020. Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. *Aquaculture* 525, 735280.
- Miao, S., Zhu, J., Zhao, C., Sun, L., Zhang, X., Chen, G., 2017. Effects of C/N ratio control combined with probiotics on the immune response, disease resistance, intestinal microbiota and morphology of giant freshwater prawn (*Macrobrachium rosenbergii*). *Aquaculture* 476, 125–133.
- Monroy-Dosta, M.C., Lara-Andrade, R.D., Castro-Mejía, J., Castro-Mejía, G., Coelho-Emerenciano, M.G., 2013. Microbiology community composition and abundance associated to biofloc in tilapia aquaculture. *Rev. Biol. Mar. Oceanogr.* 48 (3), 510–520.
- Nayak, B., Kumar, S., Collins, P.L., Samal, S.K., 2008. Molecular characterization and complete genome sequence of avian paramyxovirus type 4 prototype strain duck/Hong Kong/D3/75. *Virology* 375 (1), 1–12.
- Nguyen, T.T.T., Foyals, M.J., Gupta, S.K., Tay, A., Fotedar, R., Gagnon, M.M., 2024. Effects of carbon source addition in rearing water on sediment characteristics, growth and health of cultured marron (*Cherax cainii*). *Sci. Rep.* 14, 1349.
- Niu, J., Liu, Y.J., Tian, L.X., Mai, K.S., Yang, H.J., Ye, C.X., Zhu, Y., 2008. Effects of dietary phospholipid level in cobia (*Rachycentron canadum*) larvae: growth, survival, plasma lipids and enzymes of lipid metabolism. *Fish. Physiol. Biochem.* 34, 9–17.
- Pamanna, D., Chandrasekhara Rao, A., Reddy, D.R.K., Madhavan, N., Venkatesan, V., Nehru, E., Kumar, P.R., 2017. Growth performance of *Litopenaeus vannamei* in biofloc treatments grown with different carbon sources. *Biochem. Cell. Arch.* 17 (2), 589–593.
- Panigrahi, A., Das, R.R., Sivakumar, M.R., Saravanan, A., Saranya, C., Sudheer, N.S., Kumaraguru Vasagam, K.P., Mahalakshmi, P., Kannappan, S., Gopikrishna, G., 2020. Bio-augmentation of heterotrophic bacteria in biofloc system improves growth, survival, and immunity of indian white shrimp *Penaeus indicus*. *Fish. Shellfish Immunol.* 98, 477–487.
- Panigrahi, A., Saranya, C., Sundaram, M., Kannan, V., Das, R.R., Kumar, R.S., Rajesh, P., Otta, S.K., 2018P. Carbon: Nitrogen (C:N) ratio level variation influences microbial diversity of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. *Fish. Shellfish Immunol.* 81, 329–337.
- Panigrahi, A., Sundaram, M., Chakrapani, S., Rajasekar, S., Syama Dayal, J., Chavali, G., 2019. Effect of carbon and nitrogen ratio (C: N) manipulation on the production performance and immunity of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) in a biofloc-based rearing system. *Aquac. Res.* 50, 29–41.
- Papadopoulos, D.K., Alvanou, M.V., Lattos, A., Ouroulis, K., Giantsis, I.A., 2024. Tropical shrimp biofloc aquaculture within greenhouses in the mediterranean: preconditions, perspectives, and a prototype description. *Fishes* 9, 208.

- Putra, I., Efendi, I., Lukisowati, I., Tang, U.M., Rusliadi, R., Manik, R.A., 2020. Effects of various carbon doses on tilapia (*Oreochromis sp*) culture with biofloc technology. IOP Conf. Ser. Earth Environ. Sci. 430, 012028.
- Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V., Purushothaman, C.S., 2016. Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). Aquac. Res. 47 (11), 3432–3444.
- Raza, B., Zheng, Z., Yang, W., 2024. A review on biofloc system technology, history, types, and future economical perceptions in aquaculture. Animals 14, 1489.
- Ren, W., Li, L., Dong, S., Tian, X., Xue, Y., 2019. Effects of C/N ratio and light on ammonia nitrogen uptake in *Litopenaeus vannamei* culture tanks. Aquaculture 498, 123–131.
- Rind, K.H., Habib, S.S., Ujan, J.A., Fazio, F., Naz, S., Batool, A.I., Ullah, M., Attaullah, S., Khayyam, K., Khan, K., 2023. The effects of different carbon sources on water quality, growth performance, hematology, immune, and antioxidant status in cultured Nile tilapia with biofloc technology. Fishes 8, 512.
- Samocha, T.M., Prangnell, D.I., 2019. Chapter 7 - Water Quality Management. In: Samocha, T.M. (Ed.), Sustainable Biofloc Systems for Marine Shrimp. Academic Press, pp. 133–151.
- Schweitzer, R., Baccarat, R.F.C., Gaona, C.A.P., Wasielesky, W., Arantes, R., 2024. Concentration of suspended solids in superintensive culture of the Pacific white shrimp *Litopenaeus vannamei* with biofloc technology (BFT): a review. Rev. Aquac. 16 (2), 785–795.
- Söderhäll, K., Hall, L., 1984. Lipopolysaccharide-induced activation of prophenoloxidase activating system in crayfish haemocyte lysate. BBA-Bioenergetics 797, 99–104.
- Sun, Y., Zhang, J., Dong, D., Li, M., Yang, X., Song, X., Li, X., 2024. Effects of carbon source addition strategies on water quality, growth performance, and microbial community in shrimp BFT aquaculture systems. Aquaculture 578, 740027.
- Tabarrok, M., Seyfabadi, J., Salehi Jouzani, G., Younesi, H., 2020. Comparison between recirculating aquaculture and biofloc systems for rearing juvenile common carp (*Cyprinus carpio*): Growth performance, haemato-immunological indices, water quality and microbial communities. Aquac. Res. 51, 4881–4892.
- Tacon, A.G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., Decamp, O.E., 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquac. Nut. 8, 121–137.
- Tinh, T.H., Momoh, T.A., Kokou, F., Hai, T.N., Schrama, J.W., Verreth, J.A.T., Verdegem, M.C.J., 2021. Effects of carbohydrate addition methods on Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 543, 736890.
- Vinasiam, A., Ekasari, J., Schrama, J.W., Verdegem, M.C.J., Kokou, F., 2024. Wheat bran addition methods in Pacific white shrimp (*Litopenaeus vannamei*) biofloc systems. Aquac. Eng. 106, 102437.
- Wasielesky, W., Atwood, H., Stokes, A., Browdy, C.L., 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258, 396–403.
- Wei, Y., Liao, S.A., Wang, A., 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture 465, 88–93.
- Xu, Z., Guan, W., Xie, D., Lu, W., Ren, X., Yuan, J., Mao, L., 2019. Evaluation of immunological response in shrimp *Penaeus vannamei* submitted to low temperature and air exposure. Dev. Comp. Immunol. 100, 103413.
- Xu, W.J., Morris, T.C., Samocha, T.M., 2016. Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. Aquaculture 453, 169–175.
- Xu, W.J., Pan, L.Q., 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture 412–413, 117–124.
- Xu, W., Wen, G., Su, H., Xu, Y., Hu, X., Cao, Y., 2022. Effect of input C/N ratio on bacterial community of water biofloc and shrimp gut in a commercial zero-exchange system with intensive production of *Penaeus vannamei*. Microorganisms 10, 1060. <https://doi.org/10.3390/microorganisms10051060>.
- Xu, W., Xu, Y., Su, H., Hu, X., Xu, Y., Li, Z., Wen, G., Cao, Y., 2021. Production performance, inorganic nitrogen control and bacterial community characteristics in a controlled biofloc-based system for indoor and outdoor super-intensive culture of *Litopenaeus vannamei*. Aquaculture 531, 735749.
- Zhao, D., Pan, L., Huang, F., Wang, C., Xu, W., 2016. Effects of different carbon sources on bioactive compound production of biofloc, immune response, antioxidant level, and growth performance of *Litopenaeus vannamei* in zero-water exchange culture tanks. J. World Aquac. Soc. 47 (4), 566–576.