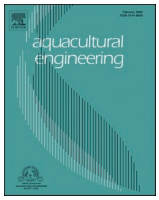
Aquacultural Engineering 106 (2024) 102437

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aque



Wheat bran addition methods in Pacific white shrimp (*Litopenaeus vannamei*) biofloc systems

Apriana Vinasyiam [a,b], Julie Ekasari [b], Johan W. Schrama [a], Marc C.J. Verdegem [a,*], Fotini Kokou [a]

[a] Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen University and Research, the Netherlands
[b] Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Indonesia

ARTICLE INFO

Keywords:
Carbon supplementation
Pacific white shrimp
Digestibility
Growth performance
Microbial activity

ABSTRACT

Incorporating non-starch polysaccharides (NSP) as a slowly degradable carbohydrate source in pelleted feed can simplify biofloc system management, compared to external carbon addition. NSP-containing ingredients like wheat bran synchronizes carbon input with the system organic matter dynamics compared to simple sugars. This study evaluated the effect of two methods of addition of an NSP-rich carbon source, wheat bran, to a Pacific white shrimp (*Litopenaeus vannamei*) biofloc system; supplementation via feed (wheat bran diet; WBdiet) and direct addition to the water (CONdiet+WB). The amount and composition of the total nutrient input to each mesocosm of the treatments was the same. A digestibility and a growth experiment were carried out separately for 35 and 42 days, respectively. The nutrient digestibility was overall lower in shrimp fed the WBdiet compared to the CONdiet, but without an effect on shrimp final weight, production, and survival. When comparing the methods of addition, neither of the approaches altered the shrimp nutritional quality. As expected, a difference in faecal C:N ratio was observed; 21 vs. 17 for WBdiet and CONdiet, respectively. The difference in faeces composition did not affect biofloc quantity, quality, and chlorophyll-a concentrations, and resulted in comparable water quality and microbial activity. Our study concluded that both NSP-addition methods, either via feed or via the water, were equally effective. When compared to the traditional addition of carbohydrate to the water, supplementing NSP-containing carbon sources via the feed can be a more efficient approach in terms of labour and feeding management practices.

1. Introduction

Zero water exchange biofloc technology (BFT) systems have been employed the past 35 years to maintain water quality, improve nutrient use efficiency and promote shrimp growth (Avnimelech et al., 2015; Panigrahi et al., 2019; Santhana Kumar et al., 2018; Tinh, Momoh, et al., 2021). This cultivation method relies mainly on heterotrophic bacteria to immobilize toxic inorganic nitrogen species into bacterial biomass, using organic carbon as energy source. These microorganisms produce extracellular polysaccharides (EPS), aggregating different microbial groups (bacteria, archaea, algae, protists, zooplankton, fungi) and particles into biofloc (More, Yadav, Yan, Tyagi, and Surampalli, 2014; Wilén, Onuki, Hermansson, Lumley, and Mino, 2008). The carbon to nitrogen (C:N) ratio of a 38 – 42 % protein shrimp diet is smaller than 10, while an optimal growth of heterotrophic bacteria requires a C:N ratio of 10–20 (Avnimelech et al., 2015). Therefore, an external carbon source is usually added to the water in the biofloc system to increase the C:N ratio of the feed to avoid carbon deficiency.

Under conventional biofloc management, extra carbohydrates such as molasses or starch are added directly into the water to stimulate biofloc formation and maintain water quality (Khanjani, Alizadeh, Mohammadi, and Sarsangi Aliabad, 2021; Miao, Sun, Bu, Zhu, and Chen, 2017; Panigrahi et al., 2019; Xu, Morris, and Samocha, 2018). Administrating these carbohydrates, however, requires extra labour and skills from the farmer to provide the right amount of carbon to maintain the water quality in line with the feed input. A more simple method can be to feed one single pellet that combines both the diet and the carbon source (Tinh, Momoh, et al., 2021). If an easily digestible carbon source is incorporated in the pelleted feed, most will be digested by the culture species. When using a source like wheat bran that contains a high

* Correspondence to: Zodiac building, De Elst 1, Wageningen 6708 WD, the Netherlands.
E-mail addresses: apriana@apps.ipb.ac.id (A. Vinasyiam), j_ekasari@apps.ipb.ac.id (J. Ekasari), johan.schrama@wur.nl (J.W. Schrama), marc.verdegem@wur.nl (M.C.J. Verdegem), fotini.kokou@wur.nl (F. Kokou).

https://doi.org/10.1016/j.aquaeng.2024.102437
Received 24 February 2024; Received in revised form 18 June 2024; Accepted 20 June 2024
Available online 21 June 2024

fraction of indigestible non-starch polysaccharides (NSP), a major fraction of the NSP will be excreted with the faeces and become available to heterotrophic bacteria in the water column, stimulating biofloc development (Braga, Magalhães, Hanson, Morris, and Samocha, 2016; López-Elías et al., 2015). Moreover, administrating easily digestible carbohydrate to the water will rapidly induce excessive biofloc production (El-Husseiny, Goda, Mabroke, and Soaudy, 2018; Serra, Gaona, Furtado, Poersch, and Wasielesky, 2015). By providing carbohydrates rich in NSP through the pelleted feed, the carbon input may become better synchronized with the total organic matter flow through the biofloc system, as it requires more time for decomposition into simple sugars (Avnimelech et al., 2015; Ekasari, Hanif Azhar, et al., 2014; Serra et al., 2015). Therefore, comparing the input of NSP-rich carbohydrates via the feed or via the water is worth investigating to optimize BFT management practices.

In this experiment, addition of wheat bran to a Pacific white shrimp biofloc rearing system, either via the feed or via the water column, was compared. First, a digestibility trial was conducted to assess the feed digestibility for the shrimp and the composition of the faeces. Subsequently, in a growth experiment, shrimp performance (growth, production, survival, body composition) and biofloc system performance (biofloc quantity and quality, water quality, carbon C, nitrogen N and phosphorus P mass balances) of the two wheat bran administration methods (treatments) were compared. The nutrient input per mesocosm was the same in both treatments.

2. Material and methods

2.1. Experimental feed and general design

The experiment involved two treatments differing in the supply of wheat bran (WB) to the ponds. In the first treatment, all wheat bran was included in the diet and thus all WB was assumed to pass through the shrimp ('WBdiet' treatment). In the second treatment, 30 % of the WB was taken out of 'WBdiet', which resulted in the CON diet. This 30 % WB was added directly to the water of the biofloc system. Therefore, the two experimental diets had an identical composition except for the inclusion level of wheat bran (WB) (Table 1). The CONdiet was aimed to be representative for a commercial shrimp diet regarding ingredients used and nutrient content. Yttrium oxide (Y_2O_3) was used as a marker for nutrient digestibility. The diet formulation and nutritional compositions of both diets are shown in Table 1. Both diets were produced by steam pelleting at a die of 2 mm. This was done by Research Diet Service (Wijk bij Duurstede, The Netherlands). The diets were stored at 4 ^{0}C before and during the experiment.

Two experiments were conducted at the animal research facility Carus of Wageningen UR, including a digestibility study and a shrimp and biofloc system performance assessment. The digestibility study compared the apparent digestibility coefficient and faeces composition when feeding equal rations of the WBdiet and CONdiet to Pacific white shrimp (*Litopenaeus vannamei*). In the shrimp and biofloc system performance experiment the treatments were WBdiet and CONdiet+WB, as explained above.

2.2. Digestibility experiment

Fourteen aquaria, part of one recirculating aquaculture system (RAS) containing a sedimentation unit for particle removal, a trickling filter, a degassing unit, a sump, and a tube with UV light for water sterilization. Each aquarium was equipped with an aeration stone and an aquarium pump to create continuous water movement. The inflow of water was sourced from the sump. Thirty shrimp (6.0±2.0 g/ind) were stocked per aquarium and reared for five weeks. Shrimp were fed overnight for 8–10 hours using a mechanical belt feeder. The amount of feed fed daily was calculated using the Eq. 1* and 2* (Tinh, Momoh, et al., 2021). Faeces was collected in the morning, five days a week. There was no water exchange during faeces collection. Prior to faeces collection at 07.00 in the morning, shrimp were fed the assigned diet at 1–2 g/aquarium. One hour later, any carapaces, faeces, and debris were siphoned out. Shrimp were given an additional hour to excrete new faeces. Afterwards, faeces were siphoned from the aquarium bottom, and were collected in 500-ml glass bottles placed on ice. After rinsing with demineralized water to remove salt, faeces from the same diet were pooled into an aluminium container and stored at −20 °C. To remove water, faeces were dried at 70°C for 72 hours, then kept in an open container at room temperature for another six hours, and later used as a faeces sample for the proximate analysis.

Table 1
Diet formulation and analysed nutrient composition.

		CONdiet	Wheat bran	WBdiet
Ingredients				
Fishmeal	%	17	-	12
Soyabean meal	%	10	-	7
Wheat	%	17	-	12
Wheat flower	%	25	-	17
Wheat bran	%	14	100	40
Wheat gluten	%	8	-	6
Soya lecithin	%	3	-	2
Salmon oil	%	0.8	-	0.6
Monocalcium phosphate	%	2	-	1
Limestone ($CaCO_3$)	%	0.7	-	0.5
Cholesterol	%	0.4	-	0.3
Premix	%	1	-	1
L-Lysine HCl	%	0.2	-	0.1
DL-Methionine	%	0.2	-	0.8
L-Threonine	%	0.2	-	0.1
Yttrium oxide	%	0.03	-	0.02
Nutritional composition				
Dry matter	g/kg	927	891	919
Ash	g/kg dm	71	54	68
Crude protein	g/kg dm	348	194	303
Fat	g/kg dm	65	52	61
Carbohydrate*	g/kg dm	515	700	568
Energy	kJ/g dm	19.8	19.5	19.5
Phosphorus	g/kg dm	13.0	13.3	13.4
Calcium	g/kg dm	13.4	1.1	10.4
Magnesium	g/kg dm	2.8	4.7	3.4
Yttrium	g/kg dm	0.17	0.00	0.2
C:N ratio		8.2	14.5	9.4

CN ratio = carbon to nitrogen ratio. *Calculated by subtracting ash, crude protein, and fat from dry matter.

$$Feeding\ rate(\%Individual\ Body\ Weight(IBW)) = 0.0861 * \left(IBW(gwet\ weight)^{-0.428}\right) * 100 \quad (1)$$

$$FCR = 0.001 * IBW(gwet\ weight)^2 + 0.0681 * IBW(gwet\ weight) + 0.5386 \quad (2)$$

2.3. Growth experiment

2.3.1. Biofloc inoculant production

Three weeks prior to the start of the experiment, post-larvae (PL) shrimp from CreveTec, Belgium, were stocked in three 1.000-L tanks, containing 750 L each, at a density of approximately 500 shrimp per tank. Shrimp were fed a commercial shrimp starter diet (CP 41 %) twice daily to satiation. Corn starch was added to the water following feeding to raise the C:N ratio (g/g) of the total nutrient input to 20:1 to promote biofloc formation. The rearing tanks had a 12 h dark/light cycle using artificial lighting (LEP, Gavita Pro 270e; HI, Gavita Hortistar 600 SE EU). Continuous aeration was provided from one aeration stone hanging in the centre of the tank and from a circular aeration pipe close to the bottom, maintaining the dissolved oxygen level above 6 mg/L. Temperature was maintained at 24±1 °C using an aquarium heater Schego® (600 W) placed in each tank, while salinity was kept at 23±2 ppt during

the inoculant production period.

2.3.2. Mesocosm set-up and water quality monitoring

Six identical mesocosm tanks were used (3 per treatment). At the start of the experiment, shrimp from the biofloc inoculant production tanks were harvested for the growth experiment. Meanwhile, the pre-maturated biofloc water was pooled in one large tank with continuous aeration and water circulation to ensure homogenous mixing, as well as to keep the biofloc in suspension. The biofloc water was evenly divided among six clean 1.000-L mesocosm tanks, resulting in a volume of ca. 350 L per tank. Subsequently, 400 L brackish water was added to each tank, bringing the final water volume to 750 L per tank. Shrimp with a body weight of 0.27±0.05 g was stocked at a density of 150 individuals per tank. During the experiment, the water was continuously aerated and exposed to a 12 h dark/light cycle.

Water temperature, salinity, pH, and oxygen were monitored daily at 08:00 am using electronic probes. The dissolved oxygen (DO) concentration was maintained above 6 mg/L, salinity at 25±1 ppt and the pH above 7.5. At the start of the experiment, the temperature was increased approximately 1 °C daily, from 24±1 °C to 27±1 °C. During the experiment, 12 L of fresh water was added weekly to each tank to compensate evaporation loss. The concentrations of dissolved ammonia, nitrite and nitrate were checked daily using Merck MQuant® kits.

2.3.3. Feeding management

Shrimp were fed each day for 8–10 hours using mechanical belt feeders. The amount the CONdiet fed daily was calculated using formula 1* (Tinh, Momoh, et al., 2021). The daily shrimp weight gain was calculated by dividing the feed input by the FCR using Eq. 2* (Tinh, Momoh, et al., 2021). The daily feeding rate of the CONdiet declined from 15.1 % BW on day 1 (D1) to 3.9 % BW on D42. In CONdiet+WB treatment tanks, wheat bran was added to the water from a mechanical belt feeder approximately one hour after feeding. Each day, the mass of WBdiet fed was equal to the mass of CONdiet+WB fed.

2.3.4. Sample collection during grow-out experiment

Shrimp was collected for proximate composition analyses at the start (D1) and end of the growth experiment (D42). Two hundred shrimp were collected as initial sample, while on D42 all the shrimp in each tank were harvested, counted, and weighed. The samples were subsequently freeze-dried 72 hours and reweighed. Throughout the experiment, 20 g portions from the WBdiet, the CONdiet, and the wheat bran were collected each week. Each collected portion was then placed in a designated container assigned to its respective feed type. These containers were consistently maintained at a temperature of 4 °C. At the end of the experiment, the proximate composition of both the diets and wheat bran was determined.

Biofloc was sampled on D1, D21 and D42. Before sampling, 1.5 µm pore size fiber glass filters were dried at 70 °C for minimum 3 hours. Dried filters were stored in a desiccator for 1 hour and then weighed to determine the empty filter weight. To collect biofloc sample, 10 L of biofloc water was siphoned to a plastic bucket from the centre of each biofloc tank. Collected biofloc water was mixed thoroughly using an electric disperser IKA Ultra-Turrax® at 300 rpm for 30 seconds and distributed into four 1.5-L plastic jars of which two were stored for back-up. The other jars were immediately processed. While processing, the water in each jar was continuously mixed at 250 rpm using a magnetic stirrer. Batches of 100 ml biofloc water were filtered through 1.5 µm pore size fiber glass filters using a vacuum pump to collect biofloc. Subsequently, 100 ml demineralized water was passed twice through the fibre glass filters to remove salt and to collect biofloc particles stuck on the inner wall of the filtration chamber. After filtration, each filter with biofloc was folded and stored in a clean plastic tube at −20 °C until further analysis.

Prior to proximate analysis, biofloc-filled filters with biofloc were dried at 70 °C for minimum 72 hours, transferred to a desiccator for 1 hour and weighed. The biofloc mass was obtained by deducting the weight of the full and empty filter. Unfiltered biofloc samples were used to measure chlorophyll-a and microbial activity. Fifty ml of biofloc water from the center of each tank was collected by siphoning and stored in a sterile plastic tube. For chlorophyll-a measurement, samples were stored at −20 °C until analysis. Samples to measure biofloc microbial activity were analyzed immediately after collection.

The filtrate water from the biofloc filtration was used as water sample. Fifty ml of filtrate water of each tank was acidified to a pH of 2–3 by adding 2 N HCl. Subsequently, 10 ml of acidified water was filtered using a 0.45 µm syringe filter and stored in a clean plastic tube at room temperature. Samples were analysed within 24 hours after collection.

2.4. Sample analysis

Proximate analysis was conducted on shrimp, faeces, diet and biofloc samples to determine their nutrient composition, including dry matter (DM), ash, minerals, crude protein, fat, energy, carbon, and nitrogen content. To measure the DM content, each sample was oven-dried at 103°C for at least 4 hours until a constant weight (ISO-6496, 1999). After DM determination, the samples were incinerated at 550°C for minimum 4 hours until constant weight (ISO-5984, 2002). The ash samples were analysed for phosphorous (P), calcium (Ca), magnesium (Mg), copper (Cu) and yttrium (Y) using plasma-mass spectrophotometry (ICP OES), following the NEN-15510 (2017) procedure. Crude protein analysis was determined using the Kjeldahl method according to (ISO-5983, 2005), while the energy content was determined by bomb calorimetry through direct combustion (IKA® werke C7000; IKA Analysentechnik, Weitershem, Germany) (ISO-9831, 1998). The fat analysis was performed according to the Soxhlet method (ISO-6492, 1999). The carbon and nitrogen content were determined using a DUMAS analyzer (Leco CN 628, Leco Instrumente GmbH., Germany) (Tinh, Momoh, et al., 2021).

The total suspended solids (TSS) represents the mass of biofloc particles (DM) per liter of rearing water, while volatile suspended solids (VSS) specifically refers to the organic portion (ash-free DM) of the VSS (APHA, 1995). Chlorophyll-a was determined following standard methods for examination of water and wastewater (APHA, 1995). The microbial activity was determined by performing H_2O_2 degradation assays according to Pedersen, Rojas-Tirado, Arvin, and Pedersen (2019). Dissolved nutrients were analysed using a segmented analyzer (SAN ++, Skalar Analytical B.V.) measuring concentrations of total carbon (TC), total inorganic carbon (TIC), total organic carbon (TOC), total nitrogen (TN), total inorganic nitrogen (TIN), total organic nitrogen (TON), total ammonia nitrogen (TAN), nitrate- nitrite nitrogen (NOx-N) and orthophosphate (PO_4-P).

2.5. Data calculation and analysis

Table 2 presents the formulas used to calculate the apparent digestibility coefficient (ADC), shrimp growth and feed utilization parameters, and nutrient balances. Statistical analysis was performed with IBM SPSS Statistics 26 software (IBM Corporation, NY, USA). The data homogeneity was assessed using Levene's test, while normality distribution was assessed using Shapiro-Wilk's test. Statistical analysis comparing faeces compositions, ADC, shrimp growth, and body composition between treatments was conducted using one-way ANOVA. Meanwhile, biofloc parameters and water quality were analysed using repeated measure ANOVA with treatment as main factor and sampling day as repeated factor. A significance level (α-value) of 0.05 was employed to determine significant differences. Post-hoc analysis was performed using Tuckey HSD. The correlation between microbial activity and VSS as well as between microbial activity and chlorophyll-a concentration was determined.

Table 2
Formulae for data calculation.

Parameter	Formula
ADC (%)	$1-\{(nut.conc_{faeces\ (g/kg)} / nut.conc_{diet\ (g/kg)}) \times (Y.conc_{diet\ (g/kg)} / Y.conc_{faeces\ (g/kg)})\} \times 100$
SGR (% BW/day)	$(\ln W_f - \ln W_i) / t * 100$
Production $_{kg/m^3}$	$(BM_{f\ (g)} - BM_{i\ (g)}) /$ (water volume $_{m^3}$ x 1000)
Survival (%)	Final number of shrimps / initial number of shrimps * 100
FCR	total diet input $_{(g)}$ / $(BM_{f\ (g)} - BM_{i\ (g)})$
FCR_{system}	(total diet input $_{(g)}$ + total wheat bran input $_{(g)}$) / $(BM_{f\ (g)} - BM_{i\ (g)})$
PER	$(BM_{f\ (g)} - BM_{i\ (g)})$ / dietary protein input $_{(g)}$ x 1000
PER_{system}	$(BM_{f\ (g)} - BM_{i\ (g)})$ / total protein input (diet + wheat bran) $_{(g)}$
PUE (%)	(retained protein $_{(g)}$ / dietary protein input $_{(g)}$) × 100
PUE_{system} (%)	(retained protein $_{(g)}$ / total protein input (diet + wheat bran) $_{(g)}$) × 100
EUE (%)	(retained energy $_{(kJ)}$ / dietary energy input $_{(kJ)}$) × 100
EUE_{system} (%)	(retained energy $_{(kJ)}$ / total energy input (diet + wheat bran) $_{(kJ)}$) × 100
Total nutrient input $_{(g)}$	$nut_{diet\ (g)} + (nut_{shrimp\_i\ (g)} + nut_{biofloc\_i\ (g)} + nut_{water\_i\ (g)})$
Nutrient loss $_{(g)}$	total nutrient input $_{(g)} - (nut_{shrimp\_f\ (g)} + nut_{biofloc\_f\ (g)} + nut_{water\_f\ (g)})$

ADC = apparent digestibility coefficient, GR = growth rate, SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio, PUE = protein utilization efficiency, EUE = energy utilization efficiency, $Nut.conc_{faeces}$ and $Nut.conc_{diet}$ represent the nutrient concentrations in faeces and diet, $Y.conc_{diet\ (g/kg)}$ and $Y.conc_{faeces\ (g/kg)}$ indicate the yttrium concentration in diet and faeces, W_i = initial body weight, W_f = final body weight, BM = biomass, BM_i = initial BM, BM_f = final BM, t = number of days, total diet input and total wheat bran input refer to total amount of diet and wheat bran given during experiment, water volume = total volume of water in the tank, $nut_{diet\ (g)}$ is total nutrient in the diet, $nut_{shrimp\_i\ (g)}$, $nut_{biofloc\_i\ (g)}$, and $nut_{water\_i\ (g)}$ are the initial nutrient present in the shrimp, biofloc, and water, respectively, $nut_{shrimp\_f\ (g)}$, $nut_{biofloc\_f\ (g)}$, and $nut_{water\_f\ (g)}$ are the final amount of nutrient present in shrimp, biofloc, and water, respectively.

3. Results

3.1. Shrimp performance and digestibility

Diet treatment effects on shrimp faecal composition and ADC are presented in Table 3 and Table 4, while in Table 5 and Table 6 the effects on shrimp growth performance and body composition are shown. The shrimp faecal composition was affected by treatment ($P<0.05$). Shrimp consuming the WBdiet had 14 % less protein and 5 % more carbohydrate in the faeces than shrimp eating the CONdiet. This resulted in a faecal C:N ratio of 21 and 17 in shrimp consuming the WBdiet and CONdiet, respectively (Table 3). Consumption of the WBdiet reduced the ADC of crude protein by 4 %, fat by 10 % and carbohydrate by 14 % (Table 4). No differences in shrimp individual growth and total production parameters were observed between dietary treatments ($P<0.05$, Table 5), except for FCR and EUE: 1.24 vs. 0.89 and 21 vs. 27 for the WBdiet and CONdiet+WB treatments, respectively ($P>0.05$). To be noted, FCR and EUE only considered the amounts of WBdiet and CONdiet fed, not the amount of WB fed separately. When summing the amounts of CONdiet and WB fed into CONdiet+WB treatment tanks, the resulting FCR_{system} and EUE_{system} were similar between treatments ($P>0.05$). The PER showed a trend ($P=0.051$) for being higher in CONdiet+WB fed treatment tanks, while PER_{system} was similar between the dietary treatments ($P<0.05$). On average, survival was 87 %, with a higher variability observed in WBdiet fed tanks (standard deviation - sd of 10) than in CONdiet+WB fed tanks (sd of 3). In both treatments, shrimp grew on average from 0.27 g to 5.5 g within 42 days, realizing an SGR of 7.15 % body weight day-1 and a total harvested shrimp biomass of 710 g ($P>0.05$, Table 5). No differences were observed in body composition between the dietary treatments, except for magnesium content, which was 6 % smaller in shrimp fed the WBdiet, compared to shrimp fed the CONdiet with separate WB addition to the water ($P<0.05$, Table 6).

3.2. Biofloc

Biofloc composition and microbial activity are summarized in Table 7. No differences were observed between the WB addition treatments in biofloc quantity (e.g., TSS and VSS) and quality, nor in biofloc chlorophyl-a content and microbial activity ($P<0.05$). In addition, there was no treatment x time interaction effect observed for all biofloc parameters. However, the biofloc composition changed during the experiment ($P<0.05$) for all parameters, except for the calcium content ($P>0.05$). Overall, all parameter values on the nutritional content of biofloc showed an increasing trend. TSS, VSS, chlorophyll-a content and biofloc microbial activity increased faster between D1 and D21 than between D21 and D42 (Table 7). Correlation analysis showed that microbial activity was positively associated with the VSS concentration (R^2= 0.82–0.84) and with total chlorophyll-a (R^2= 0.72–0.74) (Fig. 1).

3.3. Water quality

The effect of WB addition method on water quality is summarized in Table 8. No differences were observed between WB addition treatments in dissolved nutrient concentrations ($P>0.05$), but the water quality changed over time for all parameters ($P<0.05$). The NH4-N concentration was low overall and still decreased over time, meanwhile other dissolved nitrogen substances including NOx-N (which mostly consisted of NO3-N), TIN and TON increased ($P<0.05$). The dissolved carbon substances (TIC, TOC, and TC) showed a declining trend during the culture period ($P<0.05$). The PO4-P concentration doubled during the

Table 3
Effect of the wheat bran addition method on the proximate composition of faeces of Pacific white shrimp within 42-days culture period.

Parameter		CONdiet			WBdiet			P-value
		mean		sd	mean		sd	
Crude protein	g/kg afdm	185	±	**2a**	159	±	**1b**	**0.000**
Fat	g/kg afdm	84	±	**1a**	75	±	**1b**	**0.000**
Energy	kJ/g afdm	23	±	0.2	23	±	0.1	0.329
Carbohydrate*	g/kg afdm	731	±	**3b**	766	±	**1a**	**0.000**
Phosphorus	g/kg dm	29	±	**0.2a**	25	±	**0.5b**	**0.000**
Calcium	g/kg dm	53	±	**0.5a**	36	±	**0.7b**	**0.000**
Magnesium	g/kg dm	9	±	**0.1b**	10	±	**0.1a**	**0.003**
Yttrium	g/kg dm	1	±	**0.01a**	0.3	±	**0.01b**	**0.000**
Carbon (C)	g/kg afdm	540	±	7	551	±	9	0.399
Nitrogen (N)	g/kg afdm	31	±	**0.5a**	26	±	**0.5b**	**0.000**
C:N ratio	(g/g)	17	±	**0.5b**	21	±	**0.7a**	**0.002**

Values are the mean ± standard deviation (sd) of each diet (CONdiet = control diet and WBdiet = wheat bran diet). The data are obtained from the digestibility experiment. DM = dry matter, C:N ratio = carbon to nitrogen ratio, P-value = probability value. Per row, different letters in bold indicate significant difference ($P<0.05$). *Calculated by subtracting ash, crude protein, and fat from ash dry matter.

Table 4
Effect of the dietary wheat bran addition on the apparent digestibility coefficient (ADC) of nutrient in Pacific white shrimp within 42-days culture period.

Parameter		CONdiet			WBdiet			P-value
		mean		sd	mean		sd	
Crude protein	%	84	±	**1a**	81	±	**2b**	**0.001**
Fat	%	62	±	**1a**	56	±	**1b**	**0.005**
Energy	%	66	±	**1a**	58	±	**1b**	**0.000**
Carbohydrate	%	50	±	**2a**	43	±	**3b**	**0.001**
Phosphorus	%	5	±	6.2	8	±	4	0.188
Carbon (C)	%	65	±	0.3**a**	57	±	1.8**b**	**0.024**
Nitrogen (N)	%	83	±	0.2	81	±	0.8	0.056

Values are the mean and the standard deviation (sd) of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet), P-value = probability value. Different letters in bold show significant difference (P<0.05).

Table 5
Effect of the wheat bran addition method on the growth performance of Pacific white shrimp reared in a biofloc system within 42-days culture period.

Parameter		CONdiet+WB			WBdiet			P-value
		mean		std	mean		std	
Initial weight	g/ind	0.27			0.27			
Final weight	g/ind	5.6	±	0.3	5.4	±	0.4	0.901
Final biomass	g	719	±	55	701	±	57	0.722
Specific growth rate (SGR)	%/day	7.2	±	0.1	7.1	±	0.2	0.886
Production	kg/m^3	0.90	±	0.07	0.88	±	0.08	0.725
Survival	%	88	±	3	87	±	10	0.867
Feed conversion ratio (FCR)		0.89	±	0.07**b**	1.24	±	0.11**a**	**0.009**
FCR_{system}		1.21	±	0.10	1.24	±	0.11	0.723
Protein efficiency ratio (PER)		3.5	±	0.3	2.9	±	0.3	0.051
PER_{system}		2.9	±	0.2	2.9	±	0.3	0.894
Protein utilization ratio (PUE)	%	58	±	5	49	±	4	0.066
PUE_{system}	%	49	±	4	49	±	4	0.919
Energy utilization ratio (EUE)	%	27	±	**2a**	21	±	**2b**	**0.016**
EUE_{system}	%	20	±	2	21	±	2	0.782

Values are the mean and the standard deviation (sd) of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet), P-value = probability value. Different letters in bold show significant difference (P<0.05).

Table 6
Effect of the wheat bran addition method on the body composition of the Pacific white shrimp reared in a biofloc system within 42-days culture period.

Parameter		D1			D42					P-value
					CONdiet+WB			WBdiet		
		mean		sd	mean		sd	mean	sd	
Dry matter (dm)	g/kg	226	±	0.02	223	±	4.99	227	± 1.43	0.245
Ash	g/kg dm	173	±	1	148	±	4	141	± 4	0.088
Crude protein	g/kg dm	710	±	2	745	±	20	744	± 1	0.903
Fat	g/kg dm	38	±	1	39	±	3	43	± 5	0.285
Energy	kJ/g dm	18.4	±	0.1	19.9	±	0.1	20.3	± 0.3	0.066
Carbohydrate*	g/kg dm	79	±	1	67	±	19	72	± 2	0.309
Phosphorus	g/kg dm	14.0	±	0.1	12.2	±	0.1	11.9	± 0.3	0.101
Calcium	g/kg dm	42	±	0.4	36	±	2.5	33	± 0.5	0.071
Magnesium	g/kg dm	3.2	±	0.05	3.3	±	0.02**a**	3.1	± 0.10**b**	**0.021**
Carbon (C)	g/kg dm	427	±	2	454	±	4	457	± 2	0.252
Nitrogen (N)	g/kg dm	121	±	1.2	121	±	0.8	120	± 0.4	0.102
C:N ratio (mass)		3.5	±	0.01	3.7	±	0.05	3.8	± 0.00	0.104

Values are the mean and the standard deviation (sd) of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet). C:N ratio = carbon to nitrogen ratio, P-value = probability value. different letters in bold show significant difference (P<0.05). *Calculated by subtracting ash, crude protein, and fat from dry matter.

experiment, increasing faster towards the end of the experiment (P<0.05, Table 8).

3.4. Nutrient balance

The nutrient mass balances, expressed as a percentage to the total nutrient input (feed + stocking nutrient) comprising carbon, nitrogen and phosphorous content of the biofloc on each sampling day (D1, D21 and D42), are shown in Table 9. At the end of culture period (D42) the majority of carbon, and nitrogen present was in the biofloc, followed by the shrimp and water in both treatments. Meanwhile, phosphorous distribution was highest in the biofloc and the lowest in the water. The distribution share in the biofloc were comparable between the 2 treatments, with the exception for carbon which showed a lower percentage in the WBdiet group. Overall, shrimp accumulated between 16 % and 21 %, 29–3449 % and 12–16 % of the carbon, nitrogen and phosphorous fed during the 42-day culture period. The distribution share of nutrient retained in shrimp was not significantly different between the 2 groups. On average 39 %, −4 % and 3 % of carbon, nitrogen and phosphorous, respectively, was unaccounted in the WBdiet treatment at D42.

Table 7
Effect of the wheat bran addition method on biofloc quantity, quality, and chlorophyll-a concentration within 42-days culture period.

		WB addition method (Treatment)		SEM treatment	Time			SEM Time	P-value		
Parameter	Unit	CON+WB	WBdiet		D1	D21	D42		Treatment	Time	Treatment*time
Crude protein	g/kg afdm	697	703	20	532**b**	814**a**	753**a**	27	0.873	**0.000**	0.607
Energy	g/kg afdm	28.3	27	1.3	15.1**b**	36.0**a**	31.5**a**	2.5	0.484	**0.001**	0.550
Phosphorus	g/kg dm	13.7	13.6	0.5	14.4**a**	11.4**b**	15.2**a**	0.6	0.822	**0.003**	0.135
Calcium	g/kg dm	50	51	2.4	50	49	52	2.4	0.957	0.700	0.381
Magnesium	g/kg dm	11.8	12.4	0.5	**4.7b**	17.2**a**	14.4**a**	0.9	0.520	**0.000**	0.567
Carbon (C)	g/kg afdm	553	541	10	349**a**	641**b**	650**b**	18	0.448	**0.000**	0.193
Nitrogen (N)	g/kg afdm	93	92	2.1	62**b**	108**a**	107**a**	3.5	0.691	**0.000**	0.276
C:N ratio (mass)		5.9	5.9	0.05	5.6**b**	5.9**a**	6.1**a**	0.06	0.503	**0.002**	0.912
TSS	mg/L	585	597	15	340**c**	651**b**	781**a**	17	0.611	**0.000**	0.676
VSS	mg/L	356	373	13	269**c**	377**b**	448**a**	3.1	0.398	**0.000**	0.532
Chl-a water	mg/m^3	183	193	13	104**c**	206**b**	254**a**	8	0.594	**0.000**	0.943
Chl-a particulate	mg/m^3	196	205	7	110**c**	226**b**	265**a**	8	0.450	**0.000**	0.290
Chl-a total	mg/m^3	378	398	18	214**c**	432**b**	519**a**	13	0.487	**0.000**	0.628
Microbial activity		0.931	1.009	0.036	0.780**c**	0.993**b**	1.138**a**	0.032	0.202	**0.002**	0.685

Values are the mean of three sampling times of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet) and the mean of two treatments of each sampling times (D1 = day-1, D21 = day-21, and D42 = day-42). Dm = dry matter, WB = wheat bran, TSS = total suspended solid, VSS = volatile suspended solid, Chl-a = chlorophyll-a, SE = standard error, -value = probability value. For each factor (diet or time), different letters in bold show significant difference (P<0.05). (there is no unit of ma because.)

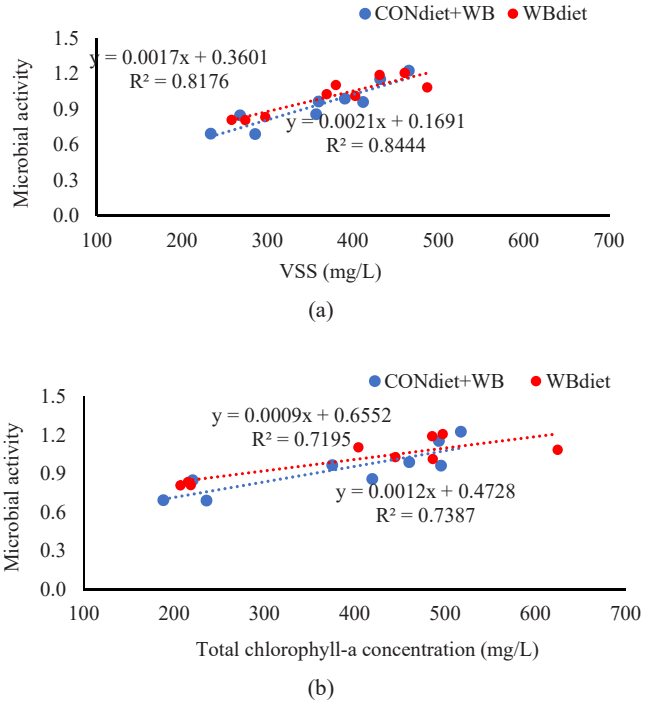


Fig. 1. Linear regression between (a) microbial activity and volatile suspended solid (VSS); (b) microbial activity and total chlorophyll-a concentration. Values are the mean of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet) in all sampling times (D1 = day-1, D21 = day-21, and D42 = day-42). R^2 = coefficient of determination.

4. Discussion

4.1. Nutrient digestibility and shrimp performance

The inclusion of wheat bran increased the NSP level in the diet which reduced the apparent digestibility. According to (Sinha, Kumar, Makkar, De Boeck, and Becker, 2011), dietary NSP increases the digesta viscosity and the digesta passage velocity, and reduces the mixing of digestive enzymes with feed in the fish gut. Yang et al. (2009) reported that 30 % incorporation of various plant-based ingredients, such as soyabean meal, peanut meal and wheat gluten meal reduced the nutrient ADCs in Pacific white shrimp. However, the ADC values reported in their study were higher than in our study because we included more NSP in the diet.

In our study, 14 % wheat bran was also included in the CONdiet, to reduce the difference in digestibility with the WBdiet, which partially explains the small differences in protein and carbohydrate apparent digestibility between diets (4–15 %; Table 4). The average carbohydrate ADC for Pacific white shrimp in this study (43–50 %) was lower than reported for Nile tilapia (65–72 %), averaged from studies using diverse feed formulations (Maas, Verdegem, Wiegertjes, and Schrama, 2020). Related to the longer gut, the gut passage time is much longer in Nile tilapia than in Pacific white shrimp and consequently there is a longer exposure time for fermentation of carbohydrates in Nile tilapia, which might explain the difference in ADC between both species (Beseres, Lawrence, and Feller, 2006; Kabir, Verdegem, Verreth, Phillips, and Schrama, 2020). Therefore, Nile tilapia might cope better with NSP than Pacific white shrimp. In the present study, NSP digestibility was not analyzed. More research is needed on NSP digestibility in shrimp, to be able to finetune the formulation of NSP-rich shrimp diets.

Assuming 100 % diet intake, the protein intake of shrimp fed the WBdiet and the CONdiet was estimated. Shrimp fed the WBdiet via water consumed 18 % more protein (Table S1) with a 4 % lower apparent digestibility (Table 4), resulting in a 13 % higher digestible protein intake compared to shrimp in the CONdiet+WB treatment (Table S2). However, the shrimp production and shrimp protein content were similar (P>0.05), most likely because shrimp in CONdiet+WB treatment tanks consumed more biofloc. Krummenauer et al. (2020), using stable isotopes, estimated that biofloc contributed 35–86 % to the N-retention by Pacific white shrimp in biofloc systems. That biofloc contributes to shrimp production is also suggested comparing biofloc and non-biofloc studies. In biofloc studies the reported SGR fall in the range of 2.1–10.1 % body weight gain/day, FCR in the range of 0.9–2.6, and PER in the range of 2.2–3.8 g body weight gain/g protein intake (Braga et al., 2016; Panigrahi et al., 2018, 2020; Rajkumar et al., 2016; Tinh, Hai, Verreth, and Verdegem, 2021; Tinh, Momoh, et al., 2021). In contrast, in non-biofloc studies on average the performance is less, with reported ranges for SGR of 1.6–4.1 % body weight gain/day, for FCR of 1.5–2.8 and for PER of 1.3–1.8 g body weight gain/g protein intake (Mansour et al., 2022; Panigrahi et al., 2018, 2020; Rajkumar et al., 2016; Ruvalcaba-Márquez et al., 2021). One limitation of this study is the absence of an economic analysis of both treatments, to provide a more comprehensive view from a business perspective.

Tinh, Momoh, et al. (2021) did a similar study, comparing inclusion in the pelleted feed versus separate addition of corn starch. In contrast to our results, including the carbohydrate in the pelleted diet was less effective than separate addition. Corn starch is highly digestible, and

Table 8
Effect of the wheat bran addition method on the water quality within 42-days culture period.

Parameter	Unit	WB addition (treatment)		SEM treatment	Time			SEM time	P-value		
		CON+WB	WBdiet		D1	D21	D42		Treatment	Time	Treatment. x time
NH_4-N	(mg/L)	0.026	0.023	0.004	0.039**a**	0.019**b**	0.018**b**	0.002	0.633	**0.000**	0.514
NO_x-N	(mg/L)	19.3	20.2	0.73	18.2**b**	18.5**b**	22.6**a**	0.58	0.467	**0.001**	0.755
TIN	(mg/L)	19.4	20.2	0.73	18.2**b**	18.5**b**	22.6**a**	0.58	0.467	**0.001**	0.755
TON	(mg/L)	1.71	1.68	0.06	1.26**b**	1.55**b**	2.29**a**	0.11	0.746	**0.000**	0.910
TN	(mg/L)	21.1	21.9	0.69	19.5**b**	20.0**b**	25.0**a**	0.64	0.459	**0.001**	0.785
TIC	(mg/L)	12.7	12.5	0.46	13.8**b**	15.5**a**	8.5**c**	0.28	0.856	**0.000**	0.459
TOC	(mg/L)	15.7	15.9	0.21	14.0**c**	15.5**a**	17.9**b**	0.37	0.574	**0.000**	0.958
TC	(mg/L)	28.4	28.4	0.39	27.9**b**	30.9**a**	26.4**b**	0.51	0.925	**0.001**	0.874
PO_4-P	(mg/L)	3.36	3.27	0.09	2.1**c**	2.7**b**	5.2**a**	0.12	0.501	**0.000**	0.602

Values are the mean of three sampling times of each treatment (CONdiet+WB = control diet + direct addition of wheat bran and WBdiet = wheat bran diet) and the mean of two treatments of each sampling times (D1 = day-1, D21 = day-21, and D42 = day-42). WB = wheat bran, NO_x-N = total of NO_2-N and NO_3-N, TON = total organic nitrogen; TIN = total inorganic nitrogen; TN = total nitrogen; TIC = total inorganic carbon; TOC = total organic carbon; TC = total carbon, P-value = probability value. For each factor (diet or time), different letters in bold show significant difference ($P<0.05$).

when included in the pelleted feed, the shrimp digest it, resulting in faeces with a low C:N ratio providing less energy for the microbiota in the biofloc system. When applying the corn starch via the water, the microbiota in the biofloc tank benefit, and contribute more to water quality maintenance, and shrimp and biofloc production. By incorporating an NSP-rich carbohydrate in the pelleted feed, however, a large fraction of the dietary carbohydrate may be transferred to the biofloc tank through the faeces, benefiting biofloc performance and contribution to shrimp growth. As a result, there was no difference in shrimp and system performance when administrating the NSP-rich carbohydrate through either the pelleted feed (WBdiet treatment) or by introducing it into the water in the biofloc tank (CONdiet+WB treatment) (Table 5 and Table 9).

4.2. Biofloc quantity, quality, and activity

A high fibre content in the diet increases the TSS concentration in the rearing system (Braga et al., 2016; López-Elías et al., 2015). In this study, the way wheat bran was introduced in the rearing system did not affect the quantity (e.g., TSS and VSS) of biofloc present ($P>0.05$; Table 7). Tinh, Momoh, et al. (2021) also found similar TSS and VSS concentrations between dietary treatments with four times higher stocking density than in our experiment using corn starch instead of wheat bran as carbon source. In this study, shrimp coped well with the biofloc concentration in the biofloc rearing system, as seen from a high survival (above 85 %; Table 5) for the type of culture system used (Hamidoghli et al., 2018). If the culture period would have been longer causing the TSS concentration to raise above 800 mg/L then water exchange or partial biofloc harvesting might become necessary (Gaona, de Almeida, Viau, Poersch, and Wasielesky, 2017; Ray, Drury, and Cecil, 2017; Ray, Lewis, Browdy, and Leffler, 2010; Schveitzer et al., 2013).

The proximate content of the biofloc, including ash, crude protein, energy and minerals in this study (Table 7) was similar between wheat bran addition method and comparable with reported contents in literature (Ekasari, Angela, et al., 2014; Santhana Kumar et al., 2018; Tacon et al., 2002). The nutritive value of biofloc makes it a good supplemental natural food (Kuhn, Lawrence, Crockett, and Taylor, 2016; Wang et al., 2015), although it should be checked for deficiencies. For instance, in a study by Ju et al. (2008) biofloc was deficient in arginine and lysine. In our study, the concentration ranges of Cu (0.09–2.1 g/kg DM), Mg (4.1–12.3 g/kg DM), and P (12.3–31.5 g/kg DM) aligned closely with the range documented by Kuhn, Boardman, Lawrence, Marsh, and Flick Jr (2009) and Rajkumar et al. (2016), except for the Ca content, which was twofold higher in our study. This discrepancy could potentially be attributed to difference in salt removal during sample washing.

Wheat bran addition methods did not affect the algal growth (seen from the chlorophyl-a concentration) and the microbial activity in the system. Our study measured microbial activity using H_2O_2 degradation analysis, principally measuring microbial enzymatic activity (peroxidases and catalases) (Iwase et al., 2013; Mishra and Imlay, 2012; Rojas-Tirado, Pedersen, Vadstein, and Pedersen, 2018). Dead microbiota are not contributing, as suggested by Arvin and Pedersen (2015) who measured no H_2O_2 degradation after autoclaving water samples showing previously microbial activity. We observed significant correlations between microbial activity and TSS, VSS and chlorophyl-a concentration (Fig. 1). Pedersen et al. (2019) reported a significant correlation between microbial activity and biological oxygen demand (BOD) and Rojas-Tirado et al. (2018) between microbial activity and feed load. In our study, the concentrations of both VSS and chlorophyl-a were positively correlated with bacterial activity. The latter is confusing, as algae under stress produce extracellular reactive oxygen species (ROS) and hydrogen peroxide (Diaz, Plummer, Tomas, and Alves-de-Souza, 2018), which might reduce the net H_2O_2 degradation recorded with the H_2O_2 test. A possible explanation is that epiphytic bacteria on algae contribute to the antioxidative defences of the algae, which is beneficial to the growth of both algae and bacteria (Hünken, Harder, and Kirst, 2008). More work is needed to fine-tune the hydrogen peroxide test for use in mixotrophic biofloc systems, with algae and heterotrophic and nitrification bacteria present.

If measurements of microbial activity would the further standardized and results better interpreted, then it is a simple, cost-effective, rapid, and relatively accurate approach for assessing the microbial activity in water samples, including biofloc samples. Microbial activity measurements are less time consuming than TSS, VSS and BOD5 analyses, and thus this will allow to obtain insight in biofloc development and activity faster than presently possible.

The biofloc ash content increased over time while the protein level decreased ($P<0.05$; Table 7). This trend was consistent with previous studies (Ju et al., 2008). Post- feeding waste, shrimp and plankton exoskeletons, and minerals (e.g. acid soluble oxides and mixed silicates) mainly contribute to ash accumulation (Tacon et al., 2002). The chlorophyl-a concentration, an indicator of algal presence, doubled during the first half of the culture period, and continued to increase until the end of the experiment, although more slowly. A similar pattern was observed in the biofloc for the amount Mg, an important component of chlorophyll-a, although the increase stabilized during the second half of the culture period (Marchand, Heydarizadeh, Schoefs, and Spetea, 2018; Salman et al., 2023). In spite of observed increase in chlorophyl-a, the authors observed a change in colour from green to brownish, during the culture period, concurring with observations of (Ju et al., 2008), who reported a negative correlation between the brownness of biofloc water and the alga: bacteria ratio.

Besides the algae, both autotrophic and heterotrophic bacteria contributed to water quality management in the biofloc rearing system, as seen from the low TON and the increasing accumulation of NOx-N throughout the culture period, as suggested by (Correia et al., 2014).

Table 9
Effect of the wheat bran addition method on the distribution of nitrogen, carbon and phosphorous biofloc a system at D21 and D42.

	Unit	Carbon							Nitrogen							Phosphorous						
		CONdiet+WB			WBdiet			P-value	CONdiet+WB			WBdiet			P-value	CONdiet+WB			WBdiet			P-value
		mean		sd	mean		sd		mean		sd	mean		sd		mean		sd	mean		sd	
Input																						
Stocking nutrient (shrimp+biofloc+water)	g DM	78	±	12	86	±	7		27		2	29		0.8		5.0		0.5	5.4		0.5	
Total feed D1 to D21	g DM	113			113				12			12				3.2			3.3			
Total feed D1 to D42	g DM	347			348				38			37				9.8			10.1			
D21																						
Shrimp	%	24	±	3	19	±	1	0.070	30	±	3	27	±	1	0.165	16	±	2	14	±	1	0.088
Biofloc	%	85	±	2**a**	66	±	2**b**	**0.001**	67	±	2**a**	58	±	2**b**	**0.005**	71	±	2**a**	68	±	2**b**	**0.050**
Water	%	14	±	1**a**	11	±	0**b**	**0.007**	41	±	3	37	±	3	0.187	29	±	1**a**	23	±	0.3**b**	**0.001**
Unaccounted	%	-22	±	5**b**	3	±	4**a**	**0.003**	-38	±	3**b**	-22	±	0.2**a**	**0.000**	-16	±	2**b**	-4	±	2**a**	**0.004**
D42																						
Shrimp	%	21	±	3	16	±	1	0.044	34	±	4	29	±	2	0.146	16	±	2	12	±	1	0.039
Biofloc	%	52	±	5**a**	40	±	4**b**	**0.026**	50	±	5	45	±	4	0.295	77	±	18	54	±	2	0.092
Water	%	6	±	0**a**	4	±	0**b**	**0.002**	31	±	2	29	±	3	0.367	32	±	2**a**	25	±	3**b**	**0.022**
Unaccounted	%	21	±	3**b**	39	±	3**a**	**0.003**	-15	±	2**b**	-4	±	5**a**	**0.023**	-26	±	18**b**	9	±	1**a**	**0.029**

Here too, more research is needed on how to keep a mature biofloc system maintaining the water quality, finding a balance between formation and degradation of biofloc, and keeping nutrients in the system. In this study, working with mature biofloc, the nitrogen and phosphorous supplied stayed in the system and carbon loss was low (Tinh, Hai, et al., 2021; Tinh, Momoh, et al., 2021). The challenge is to keep the nutrients accumulated in biofloc and water biological available and to steer them though the microbial food web towards shrimp production.

5. Conclusion

When feeding a biofloc system with the same nutrient inputs, it does not matter if carbohydrate to raise the C:N ratio of nutrient inputs is provided via the feed or via the water. Shrimp production and biofloc system performance were not affected by the way the carbohydrate was administrated. Simplifying biofloc system management for farmers to administrating daily one input, in this case the WBdiet, reduces on-farm labour and room for human error, while ensuring homogenous carbon and energy delivery to the microbiota in the biofloc rearing tank through shrimp faeces.

CRediT authorship contribution statement

apriana vinasyiam: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julie Ekasari:** Writing – review & editing, Supervision. **Fotini Kokou:** Writing – review & editing, Supervision. **Johan Schrama:** Writing – review & editing, Methodology, Conceptualization. **Marc Verdegem:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vinasyiam reports financial support was provided by LPDP Indonesian Endowment Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

The authors are thankful to the Lembaga Penyalur Dana Pendidikan (LPDP, Indonesian Endownment Fund for Education Agency) for the financial support for this PhD project (ID number 201703223010569). Appreciations are also given to Bruno Costa and Jonas Fuchs who contributed in the experiment and sample analysis. Also, sincere gratitude are given to our colleagues at Aquaculture and Fisheries Group and Carus Animal Research Facilities, Wageningen University and Research for their help during the experiment and the writing process of this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaeng.2024.102437.

References

APHA, 1995. Standard Methods for the Examination of Water and Wastewater: 19th Edition. American Public Health Administration Inc, New York.

Arvin, E., Pedersen, L.F., 2015. Hydrogen peroxide decomposition kinetics in aquaculture water. Aquac. Eng. *64*, 1–7. https://doi.org/10.1016/j.aquaeng.2014.12.004.

Avnimelech, Y., De Schryver, P., Emmereciano, M., Kuhn, D., Ray, A.J., Taw, N., 2015. In: Tomasso, J. (Ed.), Biofloc Technology, a practical guide book. Third Edition. The World Aquaculture Society. Baton Rouge, Louisiana, United States.

Beseres, J.J., Lawrence, A.L., Feller, R.J., 2006. Practical equivalence of laboratory and field measurements of gut passage time in two penaeid shrimp species. Mar. Ecol. Prog. Ser. *309*, 221–231. https://doi.org/10.3354/meps309221.

Braga, A., Magalhães, V., Hanson, T., Morris, T.C., Samocha, T.M., 2016. The effects of feeding commercial feed formulated for semi-intensive systems on Litopenaeus vannamei production and its profitability in a hyper-intensive biofloc-dominated system. Aquac. Rep. *3*, 172–177. https://doi.org/10.1016/j.aqrep.2016.03.002.

Correia, E.S., Wilkenfeld, J.S., Morris, T.C., Wei, L., Prangnell, D.I., Samocha, T.M., 2014. Intensive nursery production of the Pacific white shrimp Litopenaeus vannamei using two commercial feeds with high and low protein content in a biofloc-dominated system. Aquac. Eng. *59*, 48–54. https://doi.org/10.1016/j.aquaeng.2014.02.002.

Diaz, J.M., Plummer, S., Tomas, C., Alves-de-Souza, C., 2018. Production of extracellular superoxide and hydrogen peroxide by five marine species of harmful bloom-forming algae. J. Plankton Res. *40* (6), 667–677. https://doi.org/10.1093/plankt/fby043.

Ekasari, J., Angela, D., Waluyo, S.H., Bachtiar, T., Surawidjaja, E.H., Bossier, P., De Schryver, P., 2014. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture *426-427*, 105–111. https://doi.org/10.1016/j.aquaculture.2014.01.023.

Ekasari, J., Hanif Azhar, M., Surawidjaja, E.H., Nuryati, S., De Schryver, P., Bossier, P., 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish. Shellfish Immunol. *41* (2), 332–339. https://doi.org/10.1016/j.fsi.2014.09.004.

El-Husseiny, O.M., Goda, A.M.A.E., Mabroke, R.S., Soaudy, M., 2018. Complexity of carbon sources and the impact on biofloc integrity and quality in tilapia (Oreochromis niloticus) tanks. AACL Bioflux *11* (3), 846–855.

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W., 2017. Effect of different total suspended solids levels on a Litopenaeus vannamei (Boone, 1931) BFT culture system during biofloc formation. Aquac. Res. *48* (3), 1070–1079. https://doi.org/10.1111/are.12949.

Hamidoghli, A., Yun, H., Shahkar, E., Won, S., Hong, J., Bai, S.C., 2018. Optimum dietary protein-to-energy ratio for juvenile whiteleg shrimp, Litopenaeus vannamei, reared in a biofloc system. Aquac. Res. *49* (5), 1875–1886. https://doi.org/10.1111/are.13643.

Hünken, M., Harder, J., Kirst, G.O., 2008. Epiphytic bacteria on the Antarctic ice diatom Amphiprora kufferathii Manguin cleave hydrogen peroxide produced during algal photosynthesis. Plant Biol. *10* (4), 519–526. https://doi.org/10.1111/j.1438-8677.2008.00040.x.

Iwase, T., Tajima, A., Sugimoto, S., Okuda, K.I., Hironaka, I., Kamata, Y., Mizunoe, Y., 2013. A simple assay for measuring catalase activity: A visual approach. Sci. Rep. *3* https://doi.org/10.1038/srep03081.

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., Kuo, W.C., & Horgen, F.D. (2008). Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. 118-133. doi:10.1111/j.1365-2109.2007.01856.x.

Kabir, K.A., Verdegem, M.C.J., Verreth, J.A.J., Phillips, M.J., Schrama, J.W., 2020. Dietary non-starch polysaccharides influenced natural food web and fish production in semi-intensive pond culture of Nile tilapia. Aquaculture *528*. https://doi.org/10.1016/j.aquaculture.2020.735506.

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. https://doi.org/10.22092/ijfs.2021.123873.

Krummenauer, D., Abreu, P.C., Poersch, L., Reis, P.A.C.P., Suita, S.M., dos Reis, W.G., Wasielesky Jr., W., 2020. The relationship between shrimp (Litopenaeus vannamei) size and biofloc consumption determined by the stable isotope technique. Aquaculture *529*. https://doi.org/10.1016/j.aquaculture.2020.735635.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick Jr, G.J., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture *296* (1-2), 51–57.

Kuhn, D.D., Lawrence, A.L., Crockett, J., Taylor, D., 2016. Evaluation of bioflocs derived from confectionary food effluent water as a replacement feed ingredient for fishmeal or soy meal for shrimp. Aquaculture *454*, 66–71. https://doi.org/10.1016/j.aquaculture.2015.12.009.

López-Elías, J.A., Moreno-Arias, A., Miranda-Baeza, A., Martínez-Córdova, L.R., Rivas-Vega, M.E., Márquez-Ríos, E., 2015. Proximate composition of bioflocs in culture systems containing hybrid red tilapia fed diets with varying levels of vegetable meal inclusion. North Am. J. Aquac. *77* (1), 102–109. https://doi.org/10.1080/15222055.2014.963767.

Maas, R.M., Verdegem, M.C.J., Wiegertjes, G.F., Schrama, J.W., 2020. Carbohydrate utilisation by tilapia: a meta-analytical approach. Rev. Aquac. https://doi.org/10.1111/raq.12413.

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 (Chic., Ill.) *12* (6). https://doi.org/10.3390/life12060888.

Marchand, J., Heydarizadeh, P., Schoefs, B., Spetea, C., 2018. Ion and metabolite transport in the chloroplast of algae: lessons from land plants. Cell. Mol. Life Sci. *75* (12), 2153–2176. https://doi.org/10.1007/s00018-018-2793-0.

Miao, S., Sun, L., Bu, H., Zhu, J., Chen, G., 2017. Effect of molasses addition at C:N ratio of 20:1 on the water quality and growth performance of giant freshwater prawn (Macrobrachium rosenbergii). Aquac. Int. *25* (4), 1409–1425. https://doi.org/10.1007/s10499-017-0124-3.

Mishra, S., Imlay, J., 2012. Why do bacteria use so many enzymes to scavenge hydrogen peroxide? Arch. Biochem. Biophys. *525* (2), 145–160. https://doi.org/10.1016/j.abb.2012.04.014.

More, T.T., Yadav, J.S.S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2014. Extracellular polymeric substances of bacteria and their potential environmental applications. J. Environ. Manag. *144*, 1–25. https://doi.org/10.1016/j.jenvman.2014.05.010.

Panigrahi, A., Saranya, C., Sundaram, M., Vinoth Kannan, S.R., Das, R.R., Satish Kumar, R., Otta, S.K., 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. Shellfish Immunol. *81*, 329–337. https://doi.org/10.1016/j.fsi.2018.07.035.

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* (1), 29–41. https://doi.org/10.1111/are.13857.

Panigrahi, A., Sivakumar, M.R., Sundaram, M., Saravanan, A., Das, R.R., Katneni, V.K., Gopikrishna, G., 2020. Comparative study on phenoloxidase activity of biofloc-reared pacific white shrimp Penaeus vannamei and Indian white shrimp Penaeus indicus on graded protein diet. Aquaculture *518*. https://doi.org/10.1016/j.aquaculture.2019.734654.

Pedersen, L.F., Rojas-Tirado, P., Arvin, E., Pedersen, P.B., 2019. Assessment of microbial activity in water based on hydrogen peroxide decomposition rates. Aquac. Eng. *85*, 9–14. https://doi.org/10.1016/j.aquaeng.2019.01.001.

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. https://doi.org/10.1111/are.12792.

Ray, A.J., Lewis, B.L., Browdy, C.L., Leffler, J.W., 2010. Suspended solids removal to improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture *299* (1-4), 89–98. https://doi.org/10.1016/j.aquaculture.2009.11.021.

Ray, A.J., Drury, T.H., Cecil, A., 2017. Comparing clear-water RAS and biofloc systems: shrimp (Litopenaeus vannamei) production, water quality, and biofloc nutritional contributions estimated using stable isotopes. Aquac. Eng. *77*, 9–14. https://doi.org/10.1016/j.aquaeng.2017.02.002.

Rojas-Tirado, P., Pedersen, P.B., Vadstein, O., Pedersen, L.F., 2018. Changes in microbial water quality in RAS following altered feed loading. Aquac. Eng. *81*, 80–88. https://doi.org/10.1016/j.aquaeng.2018.03.002.

Ruvalcaba-Márquez, J.C., Álvarez-Ruíz, P., Zenteno-Savín, T., Martínez-Antonio, E., Goytortúa-Bores, E., Casillas-Hernández, R., Magallón-Barajas, F.J., 2021. Performance, immune response, and oxidative stress parameters of Litopenaeus vannamei fed diets containing varying carbohydrate/protein, lipid/protein, and energy/protein ratios. Aquac. Rep. *21* https://doi.org/10.1016/j.aqrep.2021.100771.

Salman, J.M., Grmasha, R.A., Stenger-Kovács, C., Lengyel, E., Al-sareji, O.J., Al-Cheban, A.M.A.A., Meiczinger, M., 2023. Influence of magnesium concentrations on the biomass and biochemical variations in the freshwater algae, Chlorella vulgaris. Heliyon *9* (1). https://doi.org/10.1016/j.heliyon.2023.e13072.

Santhana Kumar, V., Pandey, P.K., Anand, T., Bhuvaneswari, G.R., Dhinakaran, A., Kumar, S., 2018. Biofloc improves water, effluent quality and growth parameters of Penaeus vannamei in an intensive culture system. J. Environ. Manag. *215*, 206–215. https://doi.org/10.1016/j.jenvman.2018.03.015.

Schveitzer, R., Arantes, R., Costódio, P.F.S., do Espírito Santo, C.M., Arana, L.V., Seiffert, W.Q., Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. Aquac. Eng. *56*, 59–70. https://doi.org/10.1016/j.aquaeng.2013.04.006.

Serra, F.P., Gaona, C.A.P., Furtado, P.S., Poersch, L.H., Wasielesky, W., 2015. Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of Litopenaeus vannamei. Aquac. Int. *23* (6), 1325–1339. https://doi.org/10.1007/s10499-015-9887-6.

Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition – a review. Food Chem. *127* (4), 1409–1426. https://doi.org/10.1016/j.foodchem.2011.02.042.

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. Nutr. *8* (2), 121–137. https://doi.org/10.1046/j.1365-2095.2002.00199.x.

Tinh, T.H., Hai, T.N., Verreth, J.A.J., Verdegem, M.C.J., 2021. Effects of carbohydrate addition frequencies on biofloc culture of Pacific white shrimp (Litopenaeus vannamei). Aquaculture *534*. https://doi.org/10.1016/j.aquaculture.2020.736271.

Tinh, T.H., Momoh, T.A., Kokou, F., Hai, T.N., Schrama, J.W., Verreth, J.A.J., Verdegem, M.C.J., 2021. Effects of carbohydrate addition methods on Pacific white shrimp (Litopenaeus vannamei). Aquaculture *543*, 736890. https://doi.org/10.1016/j.aquaculture.2021.736890.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Zheng, Z., 2015. Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed

on the growth performance of crucian carp, Carassius auratus. Aquaculture *443*, 98–104. https://doi.org/10.1016/j.aquaculture.2015.03.015.

Wilén, B.M., Onuki, M., Hermansson, M., Lumley, D., Mino, T., 2008. Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability. Water Res. *42* (8-9), 2300–2308. https://doi.org/10.1016/j.watres.2007.12.013.

Xu, W.J., Morris, T.C., Samocha, T.M., 2018. Effects of two commercial feeds for semi-intensive and hyper-intensive culture and four C/N ratios on water quality and performance of Litopenaeus vannamei juveniles at high density in biofloc-based, zero-exchange outdoor tanks. Aquaculture *490*, 194–202. https://doi.org/10.1016/j.aquaculture.2018.02.028.

Yang, Q., Zhou, X., Zhou, Q., Tan, B., Chi, S., Dong, X., 2009. Apparent digestibility of selected feed ingredients for white shrimp Litopenaeus vannamei, Boone. Aquac. Res. *41* (1), 78–86. https://doi.org/10.1111/j.1365-2109.2009.02307.x.