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Influence of Different Stocking Density and C/N ratio on Growth Performance, Feed Utilization and Digestive Enzyme Activities of the Pacific White Shrimp (*Litopenaeus vannamei*) in a Biofloc System

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ABSTRACT

The purpose of the following study was to use the "biofloc system" in different stocking densities and C/N ratios, analyzing the zootechnical performance, the nutritional composition of the formed biofocs, digestive enzymes and the economic evaluation of the white shrimp for 56 days. For this study, 36 tanks were used. Stocking densities were 10, 20, and 30 shrimp/100L (average initial weight, 0.6± 0.23g). Molasses was added as a carbon source to culture tanks to achieve C/N ratios of 10, 15, or 20 for each stocking density group, along with the daily amount of commercial feed. Following 56 days of culture, the maximum growth rate of shrimp was produced in the tanks with the C/N ratio (15:1) at all the stocking densities ($P < 0.05$). On the contrary, both the C/N ratio and stocking densities increased, while the feed conversion ratio (FCR) decreased ($P < 0.05$). The BFT system showed little effect on the shrimp's body. However, the increasing C/N ratio (20:1) showed a significant change in the crude lipid in the shrimp's body. Molasses addition led to a considerable enhancement in the formation of the produced biofloc. Overall, all the protease, lipase and amylase activities were influenced by the microorganisms applied in the BFT system. However, the C/N ratio (15:1) recorded the best digestive enzymes activities in the hepatopancreas of cultured shrimp. The study's findings show that adjusting the C/N ratio (15:1) with low or medium stocking density can have a considerable impact on shrimp performance, feed utilization, and digestive enzymes. Using BFT system as an *in-situ* food source produces benefits that are similar to those of conventional systems, without the associated economic costs.

INTRODUCTION

Shrimp farming, one of the main aquaculture industries, has a number of obstacles, such as needing to use less antibiotics indiscriminately, regulate feed excess, and increase storage densities. Higher expenses, declining water quality, and the increased danger of disease outbreaks are the results of these problems (**Qiu *et al.*, 2017)**. The Pacific whiteleg shrimp, or *Litopenaeus vannamei,* is the most important farmed crustacean species globally **(FAO, 2022)**. As a result of severe infectious diseases, the shrimp aquaculture has incurred major economic consequences. Notably, both of bacterial and viral diseases (e.g. WSSV) greatly impeded the sustainable aquaculture development **(Tassanakajon *et al.*, 2018)**. It is crucial to create green and organic technology in order to fulfill environmental concerns and achieve sustainable manufacturing **(Panigrahi *et al.*, 2018)**. In terms of these technological advances, biofloc technology (BFT) has shown to be a promising means of securing the sustainability of shrimp aquaculture **(Sharawy *et al.*, 2022)**.

Biofloc technology (BFT) has great promise for reinforcing the water quality in ponds, by getting rid of dangerous nitrogenous substances and adding an *in-situ* microbial protein supply; a natural food source to promote growth development and decrease diseases **(Khanjani *et al.*, 2020)**. Furthermore, culture water from biofloc systems has a probiotic impact and also is rich with numerous polysaccharides, carotenoids, chlorophylls, and vitamins (A, D, E, and K) that might enhance the shrimp’s health raised in it **(Emerenciano *et al.*, 2013, 2017)**. According to reports, the innate immune system of crustaceans is activated by lipopolysaccharides (LPS), peptidoglycans, and β-1,3-glucans found in the cell walls of bacteria and fungi **(Kumar *et al.*, 2019)**. Numerous studies have demonstrated that the biofloc based culture system, a diverse collection of microorganisms that enhances hemocyte phagocytic activity, prophenoloxidase activity, and superoxide dismutase activity, strengthens the shrimp's immunity system **(Esparza-Leal *et al.*, 2020)**. Crucially, a number of studies found that the use of BFT might enhance the growth performance of cultivated shrimp in various stocking densities, mainly because it would encourage the growth of bioflocs **(Tinh *et al.*, 2023)**. In addition to meeting part of the nutritional needs of the shrimp, it was believed that the biofloc might also positively affect the activity of the digestive enzymes in the shrimp and manufacture certain microbial extracellular enzymes, which might be beneficial in the digestion and utilization of feed **(Mansour *et al.*, 2022a, b)**.

The carbon to nitrogen ratio (C/N) is essential in a water-based environment as it helps convert harmful biological nutrients molecules into beneficial bacterial cells (single-cell protein), which can directly serve as food for the organisms being cultivated **(Avnimelech, 1999)**. Moreover, the production of the protein-rich microbial cells requires nitrogen. When the organic substrates are digested and the C/N ratio is high, inorganic nitrogen becomes trapped within the bacterial cell. Given that bacterial cells have a C/N ratio of 5:1 and that bacteria have a 40–60% efficiency of conversion, a C/N ratio of 10 or higher in the feed is necessary for the growth of heterotrophic microorganisms **(Panigrahi *et al.*, 2019)**. The bio-volume of total heterotrophic bacteria (THB), phytoplankton, rotifers, and crustaceans in the water column was considerably enhanced by raising the C/N ratio from 10 to 20 **(Asaduzzaman *et al.*, 2010)**.

Heterotrophic bacteria (THB) utilized the additional carbon sources to increase their yield. **Hargreaves (2013)** observed that during the culture period, the primary cause of the rise in THB count in the water column was the increased feed and carbohydrate utilization due to the animals' growing biomass. Furthermore, several studies have indicated that adjusting the C/N ratio in diets can enhance the biofloc community, without compromising its nutritional quality **(Tinh *et al.,* 2023)**. Although BFT system have developed and have effectively been used in intensive culture methods for various shrimp species, it is most notably applied to the white-leg shrimp (*L. vannamei*) (**Tinh *et al.,* 2023; Gustilatov *et al.,* 2024)**. Nevertheless, additional research into the nutritional composition of biofloc is needed for the continued advancement of BFT, as there is limited understanding of how to adjust it to maximize the nutritional benefits for the farmed shrimp. Aligned with this approach, this research sought to investigate the optimum level of various stocking densities and C/N ratios of shrimp (*L. vannamei*) using a BFT system". The investigation involved the analyses of the zootechnical performance, the nutritional composition of the formed biofocs, digestive enzymes and the economic evaluation.

MATERIALS AND METHODS

1. Shrimp and experimental design

This study was performed at the Suez Canal Aquaculture Company- Qantara Sharq - Ismailia - Egypt for 56 days. Postlarvae (*Litopenaeus vannamei*) were bought from a Diba-Damietta-Egypt triangle commercial shrimp hatchery. The shrimp specimens were acclimated in an indoor fiberglass tank (6m^2, 5 tons) for 15 days before the feeding started at temperature of 25-28°C, pH 7.8-8, and salinity of 28-30ppt. Moreover, the samples were fed four times daily (9, 11, 13, and 15hr) using a commercial food (38% CP).

The experiment was conducted in indoor fiberglass tanks (100 L capacity). The tanks were filled with seawater previously filtered using a sand filter to remove undesired elements and floating particles. The seawater was then diluted with tap water to achieve a salinity of 30 ppt. A total of 720 healthy shrimp (initial body weight: 0.6 ± 0.23 g) were selected and placed in 36 indoor, round fiberglass tanks (100 L each, in triplicates), where they were fed a basal diet.

The experimental design included three stocking densities (D1 = 10 shrimp/100 L, D2 = 20 shrimp/100 L, and D3 = 30 shrimp/100 L) and three C/N ratios (C/N 10:1 [CN10], C/N 15:1 [CN15], and C/N 20:1 [CN20]), both with and without a Biofloc Technology (BFT) system. The twelve treatments were designated as follows: CD1, D1 + CN10, D1 + CN15, D1 + CN20, CD2, D2 + CN10, D2 + CN15, D2 + CN20, CD3, D3 + CN10, D3 + CN15, and D3 + CN20.

Each tank was equipped with four air stone hose diffusers connected to a five-horsepower blower, providing continuous aeration throughout the experiment. A 12-hour light/12-hour dark photoperiod cycle was maintained in all tanks. Approximately 40-55% of the water in each control tank without the BFT system was exchanged every 3 days to remove excess feed and feces. In contrast, the water in the BFT tanks was maintained at zero water exchange for 56 days, with dechlorinated freshwater added only to compensate for evaporation losses. To replicate the natural habitat and minimize shrimp stress, each tank was covered with black plastic sheets to limit light infiltration.

2. Biofloc set-up

Biofoc inoculum was prepared by mixing old shrimp tank and molasses as a carbon source to inculcate the BFT treatments. Until the biofloc volume achieved 20ml L^{-1} in Imhoff cone, each of BFT tank was inoculated at a rate of 5L. According to the estimation provided by **De Schryver *et al.* (2008)**, molasses (50% carbon) was applied once daily to the BFT tanks in order to keep the intended C/N ratio throughout the experiment.

3. Diet preparation

Table (1) shows the chemical composition of basal diet and molasses. Shrimp were fed three times daily at 9, 11, 13, and 15hr at 10% of initial weight with experimental diets (gradually modified to 3% at the end of experiment). The daily feeding ration for each treatment was determined and adjusted by estimating the average biomass sampled every two weeks. A pre-measured carbon source was thoroughly mixed with a water sample from the tank in a glass beaker, and then evenly distributed across the tank surfaces at 14:00 hours.

Table 1. Biochemical composition of the experimental feed and carbon source

Parameter	Commercial diet	Molasses
Moisture	**8.6**	**22.67**
Crude protein (CP)	**38.4**	**6.30**
Crude lipid (CL)	**9.5**	**4.01**
Ash	**10.7**	**16.20**
Fiber	**11.8**	**3.75**
Nitrogen-free extract (NFE)	**29.6**	**69.74**

4. Biochemical composition of biofloc and shrimp

Following by the standard methods **AOAC (1995)**, the bio-chemical composition of the biofloc and shrimp's body was determined. In simple terms, a consistent weight was established at 105°C using oven drying to determine the moisture content. Crude protein and crude lipid were estimated using Kjeldahl system and Soxtec system, respectively. Ash content was determined by incinerating the material in a muffle furnace for six hours at 600°C. Additionally, crude fiber was determined using Fibertec by sequential digestion with H_2SO_4 and NaOH.

5. Digestive enzyme activity in the digestive tract

At the end of the experiment, five shrimps were randomly sampled from each replicate for the measurement of the activity of digestive enzyme in the hepatopancreas of shrimp. The shrimp organs were separated and homogenized in clean distilled water before being weighed and homogenized separately with cooled buffer phosphate (pH 7, 0.65%, 1:10 w/v). The supernatant was used for enzyme tests after being centrifuged (3000g for 1 minute at 4°C). Samples were immediately transferred into sterile containers and refrigerated at -80°C until use. Protease activity was determined by the casein digestion method of **Drapeau (1976)**. Additionally, lipase activity was determined based on the study of **Cherry and Crandall (1932)**, and amylase activity was measured by the 3, 5-using dinitrosalicylic acid (DNS) method **(Rick & Stegbauer, 1974)**.

6. Growth rates of the shrimp

The performance indicators assessed for *L. vannamei* include final body weight (FBW), weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR, $\%.day^{-1}$), feed efficiency (FE), protein efficiency ratio (PER), and survival rate (S%). The growth rate was calculated using the following formulas of **Tekinay and Davis (2001)**.

- $\textbf{Weight gain (WG)} = \text{FBW (g)} - \text{IBW (g)}.$
- $\textbf{Specific growth rate (SGR,\%/day)} = \frac{(\text{Log FBW} - \text{logIBW})}{\text{Rearing period in days}} \times 100$
- $\textbf{Feed conversion ratio (FCR)} = \frac{\text{Diet consumed (g)}}{\text{Weigt gain (g)}}$
- $\textbf{Protein efficiency ratio (PER)} = \frac{\text{WG (g)}}{\text{Protein fed (g)}}$
- $\textbf{Feed efficiency (FE)} = \frac{\text{WG (g)}}{\text{Diet consumed (g)}}$
- $\textbf{Survival (S\%)} = \frac{\text{Final shrimp no.}}{\text{Initial shrimp no.}} \times 100$

7. Economic evaluation

Feed and shrimp costs were factored into the economic evaluation calculation. Based on the local market prices, the cost of diets was computed in L.E. by **Eid *et al.* (2017)** using the following equations:

1. **Cost /kg diet (LE)** = Cost per Kg diet L.E.
2. **Consumed feed to produce 1kg shrimp (kg) =**
   Feed intake per shrimp per period / Final weight per shrimp kg/kg.
3. **Feed cost per kg fresh shrimp (LE)** = St. 1 × St. 2.
4. **Relative % of feed cost / kg shrimp** $= \frac{\text{Respective figure for St.3}}{\text{Higest figure in this step}}$.
5. **Feed cost /1Kg gain (LE)** = Feed intake per Kg Gain × St. 1.
6. **Relative % of feed cost of Kg gain** $= \frac{\text{Respective figures for st.5}}{\text{Higest figure in this step}}$

8. Data analysis

Shrimp performance, biofloc composition, digestive enzyme activity, and economic value were analyzed using a two-way analysis of variance, followed by Duncan's Multiple Range Test to identify differences between treatments. All significance tests were conducted at a $P<0.05$ level. The analyses were performed using the IBM SPSS 19.0 software, and the results are presented as Means±SD.

RESULTS

1. Growth rates of the shrimp

A 56-day feeding trial using the BFT system was conducted to evaluate the growth indicators of *L. vannamei*. According to the two-way ANOVA, shrimp growth rates and survival were significantly impacted by stocking densities and the C/N ratio. The BFT treatments showed significant differences ($P< 0.05$) in final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), food conversion ratio (FCR), and survival rate (S%), as presented in Table (2).

FBW, WG, SGR, FE, and PER were significantly higher ($P< 0.05$) in shrimp reared at low and medium stocking densities with a C/N ratio of 15 (CN15) compared to the control treatments. However, shrimp growth rate significantly declined ($P< 0.05$) when reared at high stocking density with a C/N ratio of 20 (CN20) compared to other treatments. Conversely, the food conversion ratio (FCR) was significantly higher ($P< 0.05$) in the control group than in the BFT treatments, with the lowest FCR recorded in shrimp reared at low and medium stocking densities with CN15.

Additionally, different stocking densities and C/N ratios affected growth parameters differently. The survival rate (S%) was significantly improved in the BFT treatments compared to the control ($P < 0.05$), ranging from 70 to 90%. Increasing the C/N ratio significantly ($P < 0.05$) improved shrimp survival.

2. Whole-body proximate analysis

The whole-body composition of shrimp is shown in Table (3). According to the two-way ANOVA, stocking density and C/N ratio had no noteworthy impact on the shrimp's protein content ($P > 0.05$), which ranged from 55.40 to 62.72%. Significant differences ($P < 0.05$) were observed in moisture, crude lipid, and ash content between shrimp fed with BFT and those in the control. In contrast, rising the C/N ratio dramatically decreased ($P < 0.05$) the crude lipid content in shrimp. The highest lipid content was recorded in the lowest CN10 with low and high stocking density, and ranged between 20 to 23.44%. While, the ash content ranged from 17.76 to 24.6%.

Table 2. Growth indicators of shrimp *L. vannamei* raised in a BFT system at different stocking density and C/N ratios for 56 days

Treatment	Parameters							
	IBW (g)	FBW (g)	WG (g)	SGR (%/day)	FE (g)	PER (g)	FCR (g)	S%
CD_1	$0.60\pm.23$	$4.19\pm.05^{g}$	$3.59\pm.05^{g}$	$3.47\pm.006^{h}$	$0.48\pm.006^{cde}$	$18.42\pm.006^{f}$	$2.09\pm.006^{b}$	70 ± 1.15^{d}
D_1+CN10	$0.60\pm.23$	$5.17\pm.05^{e}$	$4.57\pm.04^{e}$	$3.85\pm.006^{c}$	$0.67\pm.006^{b}$	$25.46\pm.001^{d}$	$1.49\pm.006^{f}$	80 ± 1.15^{b}
D_1+CN15	$0.60\pm.23$	$6.11\pm.06^{a}$	$5.51\pm.05^{a}$	$4.14\pm.006^{a}$	$0.98\pm.006^{a}$	$37.24\pm.001^{a}$	$1.02\pm.006^{j}$	85 ± 1.15^{a}
D_1+CN20	$0.60\pm.23$	$3.17\pm.05^{h}$	$2.57\pm.04^{h}$	$2.97\pm.006^{i}$	$0.35\pm.006^{ef}$	$13.3\pm.006^{g}$	$1.59\pm.011^{e}$	80 ± 1.15^{b}
CD_2	$0.60\pm.23$	$4.87\pm.06^{de}$	$4.27\pm.05^{de}$	$3.74\pm.006^{e}$	$0.56\pm.006^{cd}$	$21.35\pm.006^{e}$	$1.78\pm.006^{c}$	70 ± 1.15^{d}
D_2+CN10	$0.60\pm.23$	$4.82\pm.05^{c}$	$4.22\pm.04^{c}$	$3.72\pm.006^{e}$	$0.78\pm.006^{ab}$	$29.0\pm.006^{b}$	$1.2\pm.006^{i}$	75 ± 1.15^{c}
D_2+CN15	$0.60\pm.23$	$5.94\pm.03^{b}$	$5.33\pm.02^{b}$	$4.09\pm.006^{b}$	$0.98\pm.006^{a}$	$37.24\pm.006^{a}$	$1.01\pm.006^{j}$	90 ± 1.15^{a}
D_2+CN20	$0.60\pm.23$	$4.14\pm.03^{g}$	$3.54\pm.02^{g}$	$3.45\pm.006^{g}$	$0.67\pm.22^{b}$	$25.46\pm.006^{d}$	$1.25\pm.006^{h}$	80 ± 1.15^{c}
CD_3	$0.60\pm.23$	$3.06\pm.04^{i}$	$2.46\pm.03^{i}$	$2.91\pm.006^{j}$	$0.24\pm.006^{f}$	$9.28\pm.006^{h}$	$4.09\pm.011^{a}$	70 ± 1.15^{d}
D_3+CN10	$0.60\pm.23$	$4.29\pm.06^{f}$	$3.69\pm.05^{f}$	$3.51\pm.006^{f}$	$0.55\pm.006^{cd}$	$21.01\pm.006^{e}$	$1.66\pm.006^{d}$	75 ± 1.15^{c}
D_3+CN15	$0.60\pm.23$	$4.95\pm.03^{d}$	$4.35\pm.02^{d}$	$3.77\pm.006^{d}$	$0.76\pm.006^{b}$	$28.88\pm.001^{c}$	$1.31\pm.006^{g}$	80 ± 1.15^{b}
D_3+CN20	$0.60\pm.23$	$4.13\pm.03^{g}$	$3.53\pm.02^{g}$	$3.44\pm.006^{g}$	$0.78\pm.006^{ab}$	$29.64\pm.001^{b}$	$1.2\pm.006^{h}$	80 ± 1.15^{b}

Values for each treatment are presented as Means±SD from 3 replicate tanks.

3. Nutritional values of biofloc

The nutritional values of different bioflocs are shown in Table (4). According to the two-way ANOVA, the C/N ratio had an enormous impact on the nutritional content of the biofloc, with the CP content showing an increase at a CN15. The CP content varied from 39 to 45.98%. Additionally, CL and NFE contents also considerably increased ($P< 0.05$) at the higher CN20. However, there was significant enhancment ($P<0.05$) in the ash content with the CN10. The CL content varied from 3.6 to 4.4%, and ash content varied from 21.17 to 23.95%, while NFE content varied from 29.17 to 34.25%. The CN15 treatments showed no noteworthy difference in CL content compared to the CN10 treatments. The results indicate that, raising the CN15 considerably increases the protein and lipid content in BFT, while reducing the nitrogen-free extract and ash content. Furthermore, no significant interaction effect ($P> 0.05$) was detected among the C/N ratios and stocking densities on the composition of biofloc.

Table 3. Whole-body proximate analyses (%) of shrimp *L. vannamei* reared in a BFT system at different stocking densities and C/N ratios for 56 days

Treatment	**Parameters**			
	DM %	**Crude protein %**	**Crude lipid %**	**Ash%**
CD$_1$	**28.53±.005^{b}**	**57.31±.05d**	**20.14±.005^{c}**	**22.55±.005^{c}**
D$_1$+CN10	**30.51±.007^{a}**	**58.31±.05c**	**23.13±.005^{a}**	**18.56±.005^{fg}**
D$_1$+CN15	**21.82±.005^{g}**	**59.30±.05b**	**21.19±.005^{bc}**	**19.51±.005de**
D$_1$+CN20	**26.54±.005^{d}**	**59.00±.57b**	**20.16±.005^{c}**	**17.76±.005^{h}**
CD$_2$	**21.82±.005^{g}**	**55.40±.01e**	**20.00±.57^{c}**	**24.60±.05^{a}**
D$_2$+CN10	**27.84±.005^{c}**	**60.74±.05b**	**20.16±.005^{c}**	**18.10±.05^{gh}**
D$_2$+CN15	**23.38±.005^{e}**	**59.33±.05b**	**20.09±.005^{c}**	**19.00±.5^{ef}**
D$_2$+CN20	**27.84±.005^{c}**	**60.74±.02b**	**20.13±.005^{c}**	**19.13±.005de**
CD$_3$	**26.5±.005^{d}**	**55.30±.05e**	**20.15±.005^{c}**	**23.93±.005^{b}**
D$_3$+CN10	**26.54±.005^{d}**	**59.00±.57b**	**23.44±.005^{a}**	**17.76±.005^{h}**
D$_3$+CN15	**23.18±.005^{e}**	**62.72±.01^{a}**	**20.10±.05^{c}**	**19.60±.05^{d}**
D$_3$+CN20	**23.38±.005^{e}**	**62.74±.07^{a}**	**20.10±.05^{c}**	**19.16±.005de**

Values for each treatment are presented as Means±SD from 3 replicate tanks.

Table 4. Nutritional values of biofloc (%) produced in shrimp *L. vannamei* reared at different stocking densities and C/N ratios after 56-day trial

Treatment	Parameters				
	DM	Crude protein (CP)	Crude lipid (CL)	Ash	NFE
CD$_1$	**	**	**	**	**
D$_1$+CN10	9.56 ± .28^{bc}	44.88 ± .005^{bc}	4.2 ± .05^{bc}	21.17 ± .005^{d}	29.75 ± .005^{f}
D$_1$+CN15	8.35 ± .02^{d}	45.98 ± .005^{a}	3.8 ± .05^{e}	21.19 ± .005^{d}	29.3 ± .17^{f}
D$_1$+CN20	10.2 ± .11^{ab}	39.00 ± .057^{f}	4.4 ± .05^{a}	23.14 ± .005^{b}	33.46 ± .005^{b}
CD$_2$	**	**	**	**	**
D$_2$+CN10	9.5 ± .05^{bc}	40.63 ± .017^{b}	3.98 ± .005^{e}	23.90 ± .05^{a}	31.49 ± .005^{d}
D$_2$+CN15	10.95 ± .01^{a}	45.16 ± .005^{b}	4.1 ± .05^{cd}	22.19 ± .005^{c}	29.85 ± .005^{f}
D$_2$+CN20	10.0 ± .57^{abc}	39.50 ± .05^{ef}	4.1 ± .05^{cd}	22.15 ± .005^{c}	34.25 ± .005^{a}
CD$_3$	**	**	**	**	**
D$_3$+CN10	9.0 ± .57^{cd}	39.75 ± .02^{e}	4.0 ± .05^{d}	23.95 ± .01^{a}	32.3 ± .17^{c}
D$_3$+CN15	6.63 ± .28^{ae}	44.38 ± .005^{c}	3.6 ± .05^{f}	21.18 ± .005^{d}	30.34 ± .005^{e}
D$_3$+CN20	10.5 ± .28^{ab}	39.25 ± .005^{e}	4.3 ± .05^{ab}	22.30 ± .17^{c}	34.15 ± .005^{a}

Values for each treatment are presented as Means±SD from 3 replicate tanks.

4. Digestive enzyme activity in the digestive tract

Protease, lipase, and amylase activities are presented in Table (5). According to a two-way ANOVA, stocking densities and C/N ratios showed considerable improvement ($P< 0.05$) on digestive enzyme activities. The findings show that digestive enzyme activities increased with higher C/N ratios. The BFT treatments considerably improved the hepatopancreas's digestive enzyme activity ($P< 0.05$) when compared to the control. Protease activity didn’t differ considerably amongst the BFT treatments. Nevertheless, lipase and amylase activities recorded the highest value in CN15 with low and medium stocking density than the other treatment. Moreover, the various stocking densities, C/N ratio, and digestive enzymes showed a positive correlation ($P< 0.05$).

Table 5. Digestive enzyme activity in the hepatopancreas of *L. vannamei* reared at different stocking densities and C/N ratios for 56 days

Treatment	**Parameters**		
	Protease	**Lipase**	**Amylase**
CD_1	**13.89 ± .58^{b}**	**0.7 ± .58^{d}**	**4.00 ± .58^{e}**
D_1+CN10	**38.3 ± .58^{a}**	**1.7 ± .58^{a}**	**12.03 ± .58^{d}**
D_1+CN15	**38.5 ± .58^{a}**	**1.8 ± .58^{a}**	**20.2 ± .58^{a}**
D_1+CN20	**36.75 ± .58^{a}**	**0.98 ± .5^{c}**	**15.23 ± .58^{bc}**
CD_2	**13.50 ± .58^{b}**	**0.8 ± .58^{cd}**	**3.94 ± .58^{e}**
D_2+CN10	**37.3 ± .58^{a}**	**1.3 ± .17^{b}**	**12.35 ± .58^{d}**
D_2+CN15	**38.93 ± .58^{a}**	**1.83 ± .17^{a}**	**21.18 ± .58^{a}**
D_2+CN20	**36.90 ± .58^{a}**	**0.8 ± .58^{cd}**	**15.86 ± .58^{bc}**
CD_3	**12.75 ± .58^{b}**	**0.8 ± .58^{cd}**	**4.76 ± .58^{e}**
D_3+CN10	**37.05 ± .58^{a}**	**0.78 ± .006^{cd}**	**14.42 ± .58^{c}**
D_3+CN15	**37.00 ± .58^{a}**	**0.85 ± .11^{cd}**	**16.50 ± .58^{b}**
D_3+CN20	**36.85 ± .97^{a}**	**1.0 ± .07^{c}**	**15.23 ± 1.83^{bc}**

Values for each treatment are presented as Means±SD from 3 replicate tanks.

4. Economic evaluation

Table (6) presents the results of the economic evaluation of the diets. The cost analysis was based on the prices of food components sourced from the local market in 2023. Despite adjustments to stocking density and C/N ratios, the economic value of the diet per kilogram remained variable. The economic analysis indicated that the control treatments with high stocking density had the highest feed cost per kilogram of fresh shrimp gain, with a higher relative percentage of feed cost per kilogram of shrimp compared to the BFT treatments. However, the best economic evaluation was in BFT treatments high at density with CN20, followed by low and medium density with CN15.

Table 6. The economic evaluation of *L. vannamei* reared at different stocking densities and C/N ratios for 56 days

Treatment	Parameter						
	Cost/1Kg/diet(LE/kg)	(Fi)(g/g)	Feed cost per kg (LE/kg)	Relative % of feed cost/ kg Shrimp)	FCR (g/g)	Feed cost /1Kg fresh Shrimp (LE)	Relative % of feed cost/ kg shrimp
CD$_1$	**45**	**7.50 ±.05^{b}**	**0.35± .03^{b}**	**13.83±.34^{b}**	**2.09±0.006^{b}**	**0.73±.53^{b}**	**39.7±.5^{b}**
D$_1$+CN10	**45**	**6.81±.0^{d}**	**0.31±.02^{d}**	**12.25±.42^{d}**	**1.49±0.006^{f}**	**0.46±.5^{f}**	**25±.5^{e}**
D$_1$+CN15	**45**	**5.62 ±.05^{f}**	**0.25± .01^{d}**	**100±.32^{a}**	**1.02±0.006^{j}**	**2.58±.54^{h}**	**14.13±.5^{h}**
D$_1$+CN20	**45**	**7.27 ±.05^{c}**	**0.33± .05^{c}**	**13.04±.54^{e}**	**1.59±0.011^{e}**	**0.52±.53^{e}**	**14.13±.5^{h}**
CD$_2$	**45**	**7.60±.05^{b}**	**0.34± .02^{b}**	**13.44±.5^{d}**	**1.78±0.006^{c}**	**0.61±.57^{d}**	**32.61±.5^{c}**
D$_2$+CN10	**45**	**5.39±.05^{g}**	**0.24± .03^{d}**	**9.49±.43^{f}**	**1.2 ±0.006^{i}**	**0.29±.56^{g}**	**15.8±.5^{g}**
D$_2$+CN15	**45**	**5.43 ±.05^{g}**	**0.24± .04^{d}**	**9.49±.42^{f}**	**1.01±.006^{j}**	**0.24±.55^{h}**	**13.04±.5^{i}**
D$_2$+CN20	**45**	**5.32 ±.05^{g}**	**0.24± .04^{d}**	**9.49±.5^{e}**	**1.25±0.006^{h}**	**0.3±.54^{g}**	**16.30±.5^{g}**
CD$_3$	**45**	**10.0±.05^{a}**	**0.45± .03^{a}**	**17.79±.55^{a}**	**4.09±0.011^{a}**	**1.84 ±.55^{a}**	**100±.5^{a}**
D$_3$+CN10	**45**	**6.13±.05^{e}**	**0.28± .03^{d}**	**11.07±.56^{e}**	**1.66±0.006^{d}**	**0.46±.65^{e}**	**25±.5^{e}**
D$_3$+CN15	**45**	**5.70±.05^{f}**	**0.26± .03^{c}**	**10.28± .53^{c}**	**1.31±0.006^{g}**	**0.34±.5^{c}**	**18.5±.5^{f}**
D$_3$+CN20	**45**	**4.51±.05^{h}**	**0.20± .03^{e}**	**7.91±.34^{f}**	**1.2±0.006^{h}**	**0.24±.5^{g}**	**13.4 ±.5^{i}**

Values for each treatment are presented as means ± SD from 3 replicate tanks.

DISCUSSION

Shrimp exhibited better performance at a C/N ratio of 15 with low stocking density. A negative correlation exists between shrimp developing and staying healthy, and stocking density has been observed in both conventional systems and unconventional systems (e.g. recirculating and BFT systems) **(Tinh *et al.*, 2023)**. Declines in growth rate and survival of cultivated animals kept at high stocking densities may be linked to higher rivalry for food and space, as well as lower water quality **(Esparza-Leal *et al.*, 2020; Tinh *et al.*, 2023)**. According to previous research, cultivated shrimp have decreased the immunological indicators and activity of digestive enzyme at high stocking densities **(Said *et al.*, 2022; Sharawy *et al.*, 2022)**; this might help clarify our findings.

In this study, the aim of adding molasses to all BFT treatments was to raise the C/N ratio (10, 15, and 20) and encourage the growth of healthy microbe populations and heterotrophic bacteria that arise in the tanks. The biofloc, which the shrimp can continually ingest as a natural food supply, is often formed by these dense active bacteria and suspended organic particles **(Kent *et al.*, 2011)**. Additionally, it strengthens the immunological parameters, microbial dynamics, and aquatic environment of the farmed shrimp and fish **(Shourbela *et al.*, 2021; Solanki *et al.*, 2023)**. Our findings confirmed that in the biofloc-based shrimp culture system, varying stocking densities and C/N ratios considerably promote growth. However, the S% was greatly higher in BFT treatments (75–90%) than the control (70%). Moreover, the CN15 with all stocking densities had the highest growth compared to the control. Similar findings about the effectiveness of BFT system on survival and growth in farmed shrimp were depicted in previous studies **(Xu *et al.*, 2016; Panigrahi *et al.*, 2018)**. **Panigrahi *et al.* (2018)** suggested that the development and sustainability of shrimp may be considerably enhanced by microalgae in the BFT system.

Our findings show that, all of the BFT treatments had considerably ($P< 0.05$) improved growth, SGR, FE, FCR, and PER than the control, and the performance was better in the CN15 treatments. While, the FCR values were considerably enhanced in the BFT treatments (1.01–1.66) than the control (1.78–4.09). The FCR was lower in low, medium and high stocking density with CN15. According to a similar model, **Wasielesky *et al.* (2013)** reported that the BFT system notably declined the FCR values (ranging from 0.95 to 1.61). This reason may be due to the selection of the optimal C/N ratio optimization for the healthy development of shrimp in BFT systems. According to this study, a CN15 can exist in a BFT without having an adverse effect on water quality or shrimp growth.

In our results, we observed that the raising CN20 and stocking density notably declined the performance of shrimp. This finding is consistent with that of **Tinh *et al.* (2023)**, who observed a considerable decrease in shrimp growth with various storage and rising C/N ratio. Shrimp farming systems are complex, and the interactions between various factors within these systems have attracted considerable attention from researchers. However, **Moss and Moss (2004)** observed substantial impacts on shrimp development from both the cultivation conditions (i.e. the addition of substrates) and stocking densities. Meanwhile, **Guemez-Sorhouet *et al.* (2019)** showed the effects of culture system interactions and various storage on shrimp growth. In clear-water systems, where NO_2 and NO_3 levels were higher than in other systems, shrimp's development was not inversely linked with various storage as it was in biofloc and biofloc with substrate systems **(Guemez-Sorhouet *et al.*, 2019)**. This may be because biofloc is recognized for its ability to enhance shrimp feed utilization through the addition of vital amino acids, vitamins, lipids, and minerals. Additionally, it increases the activity of digestive enzymes, which leads to better nutritional digestion inside the shrimp's digestive system **(Gustilatov *et al.*, 2024)**.

Whole-body proximate composition was found to be little affected by BFT treatments. Despite the fact that the increasing C/N ratio (20:1) was shown to significantly affect crude lipid content. Similarly, bioflocs had no discernible impact on the CP content of the shrimp's body, but there were slight variations in the CL and ash contents **(Xu & Pan 2012; Yun *et al.*, 2015; Mansour *et al.*, 2022a)**. The CL content of the shrimp's body was much lower in CN20, as opposed to the results in the control. This finding could suggest that, while feeding shrimp around their maintenance level, lipids were used more favorably as an energy source. Nonetheless, when compared to the control, the whole body shrimp's crude ash content in BFT was significantly higher. Given the high ash content observed in this study, the elevated whole body ash content of the shrimp in BFT can be attributed to the consistent availability of mineral-rich elements provided by the bioflocs **(Yun *et al.*, 2015; Mansour *et al.*, 2022a)**.

The nutritional values of microbial flocs vary depending on the carbon source, proximal feed composition, environmental conditions, culture time, and other factors **(Mansour *et al.*, 2022a; Solanki *et al.*, 2023**). In our investigation, biofloc samples collected from various BFT treatments exhibited nutrient contents ranging from 39 to 45.98% CP, 3.6 to 4.4% CL, and 21.17 to 23.95% ash. The amount of carbon supply has an impact on the nutrient contents of microbial floc, as seen by the considerable differences in nutritional components of bioflocs across the various BFT treatments in this study. The CP and ash content of the CN15 treatments were comparable to those of **Xu and Pan (2014)**, the most effective nutritional values for the bioflocs collected in CN15 were discovered when sucrose was used as a carbon source with different dietary protein levels and C/N ratios for the cultivation of *L. vannamei*. However, **Tinh *et al.* (2021)** indicated that biofloc CP content ranged from 39 to 43%, based on the type of carbon source used. In this study, the various storage and C/N ratio had the greatest impact on the biofloc nutritional contents **(Tinh *et al.*, 2023)**.

Studies have shown that increased bioflocs have positive benefits on shrimp nutrition **(Xu *et al.*, 2012)**. It's conceivable to propose that the nutritional components and extracellular enzymes of bioflocs are associated with their nutritive contribution **(Xu & Pan, 2012)**. In addition, **Ju *et al.* (2008a, b)** revealed that the amounts of crude protein, crude lipid, and ash in the bioflocs recovered from white shrimp systems varied according to the dominant microorganisms (e.g. bacteria, diatoms, or chlorophytes) in the microbial community. While, **Xu and Pan (2014)** and **Wang *et al.* (2016)** postulated that the microorganisms that comprise the biofloc likely have an impact on its nutritional value. This indicates that the microorganisms of the bioflocs depends on the protein levels and C/N ratio, and this may have caused the variations in the nutritional contents of biofloc. Additionally, **Mansour *et al.* (2022a, b)** showed that the BFT system influenced the activities of proteases and amylases. These bioflocs enzymes may aid in the digestion and absorption of feed by the shrimp by mouldering the proteins, carbohydrates, and other essential components into smaller units.

During the shrimp production process, appropriate stocking density has been consistently linked to excellent health, and healthy animals were repeatedly seen to eat substantially more and have a more active appetite than unhealthy ones **(Wang *et al.*, 2016)**. There has to be an essential link between the digestive enzymes and growth performance. Bioflocs can serve as an extra food source to enhance the growth rates of farmed shrimp. They can not only augment microbial nutrition but also aid in the digestion and use of the feed **(Xu & Pan, 2012)**. The microorganisms that make extracellular products to augment endogenous digestive enzymes in the intestinal tract of aquatic animals for the purpose of breaking down proteins and carbs lend assistance to this. This may help with feed digestion, absorption, and utilization **(Zafar *et al.*, 2022)**. The greater total digestive enzyme activity was seen in the BFT treatments in comparison to the control. However, the best activities of protease, lipase and amylase were recorded in shrimp reared in CN15 at low and medium stocking density. This also holds true for increased nutritional intake and improved activity of the digestive enzymes; shrimp development is enhanced and the feed conversion ratio (FCR) is decreased in the BFT treatments **(Mansour *et al.*, 2022b; Gustilatov *et al.*, 2024)**.

In general, it is possible that the elevated the activities of digestive enzyme in the shrimp's digestive tissues during the BFT treatments improved the feed's digestion and absorption, which in turn may have improved the shrimps' growth and feed utilization. As the stocking density grew in our investigation, the digestive enzyme activity all decreased, which is consistent with the white shrimp's poor development performance at the high stocking density. These results are consistent with those of **Liu *et al.* (2017)**, who discovered that in spite of the probiotic impact of the biofloc, digestive enzyme activity were decreased in the high stocking density group.

Shrimp farming holds the highest economic value globally **(FAO, 2022)**. This study aimed to determine the optimal stocking density and C/N ratios for shrimp raised in a biofloc system to achieve maximum economic benefit. Additionally, all growth indicators showed notable improvement in BFT treatments with low and medium density at CN15. However, the best economic evaluation was at high density with CN20, this is due the shrimp feed on the biofloc more than relying on commercial diet. **Burford *et al.* (2003)** revealed that more than 29% of the diets that *L.vannamei* ate on a daily basis may have been biofloc. The only stocking density that significantly impacted this study, regardless of feeding rate, may be linked to the optimal floc particle aggregation observed in the high-density groups. Similar findings were reported by **Zaki *et al.* (2020)**, who noted that at high densities, the surface area available for bacterial growth increases, leading to a greater production of biofloc due to the larger surface area and particle size of the floc.

CONCLUSION

Finally, we demonstrate in our work that shrimp's growth and activities of digestive enzymes and biofloc proximate composition can be influenced similarly by the C/N ratio and various storage. On the other hand, the best parameters were in low and medium stocking density with C/N ratio (15:1).

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