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Hatchery performance of Pacific white shrimp, *Penaeus vannamei* in Biofloc technology by using different carbon sources

Kola Suneetha^{1*}, P. Padmavathi¹ and Darwin Chatla¹

Abstract

The present study assessed the hatchery performance of *Penaeus vannamei* between the mysis 1 and postlarva 10 stages, in a zero-exchange biofloc system under three treatments with different carbon sources, fructose, lactose, and dextrose, in a 15:1 fixed C:N ratio with a stocking density of 100 L⁻¹ along with control treatment. The study used a stocking density of 100 L⁻¹. Water quality and survival performance were compared among treatments. The results revealed that adequate water quality parameters were more appropriate for production in the BFT treatment than in the control, and analysis of variance revealed that there were significant differences between treatment groups for NO₂⁻N, NO₃⁻N, and alkalinity ($P < 0.05$). Survival was significantly greater in the BFT treatment group than in the control group. Dextrose exhibited the highest survival rate for PL₁ at 93%, followed by fructose at 88.67% and lactose at 86.33%, while the control group had the lowest survival rate at 79.33% ($P > 0.05$). For PL₅ and PL₁₀, the survival rates were 90.67%, 85.67%, 78.33%, and 66.67% ($P < 0.05$) for dextrose, fructose, lactose, and the control, respectively. The study concluded that dextrose is the most effective carbon source for maintaining the hatchery system.

Keywords Biofloc, Carbon, Water quality, Survival

Introduction

Fisheries and aquaculture are efficient protein production sectors that offer ample opportunities to alleviate poverty, hunger, and malnutrition [1]. Aquaculture has experienced phenomenal growth, with a global production of 178 million tonnes, and in India, the quantity increased from 0.79 MT in 1987 to 16.25 MT in 2022–23 [2]. Globally, India occupies the third position after China and Indonesia, with a share of 7.70% of the world's aquaculture production [1], and has achieved an impressive

double-digit average annual growth rate of more than 10%, surpassing that of any other food production sector in the country over the past decade. It contributes approximately 1.24% to the country's gross value added (GVA) and more than 7.28% to the agricultural GVA (Indian Economic Survey, 2021–22). This sector has also witnessed significant export growth, with a total production of 12.22 MT valued at USD 1.42 billion in 2023 [3].

The vast resources in terms of water bodies and species of fish and shellfish in different agroecological regions of the country provide a wide array of culture systems and practices [4]. However, despite these resources, the rapid growth of the aquaculture industry is hampered because of several limitations, including the poor quality of seeds during the initial stages, raising concerns about the survival and growth of culturing species, which leads to limited productivity [5].

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In the production line, the larval and postlarval stages are crucial components of the hatchery system for producing high-quality seeds. Generally, conventional shrimp hatchery systems are knotted with large amounts of water exchange to maintain adequate water quality parameters [6]. Minimizing water volume utilization in shrimp aquaculture systems may substantially reduce the expenses associated with water (i.e., pumping, collection, filtration, disinfection) and environmental impacts and enhance biosecurity for hatcheries [7]. Hence, the challenge in shrimp hatcheries is to determine a favorable water quality parameter with minimal/zero water exchange [8]. Furthermore, high stocking densities demand a greater food supply for reared species during the initial stages, raising concerns about survival and growth, which leads to limited productivity [9]. Hence, these concerns in the shrimp, larval, and postlarval stages need to be addressed in terms of technological advancements [10].

Biofloc technology (BFT) is an emerging technique that has gained momentum in recent years, with encouraging performance in aqua farming. Microbial manipulation allows cultured shrimp to grow more successfully [4, 11–16]. Microbial communities consist of microorganisms such as phytoplankton, bacteria, and organic matter, which serve as an extra food source for cultivable species. The fundamental premise of this technique is to recycle nutrients and nitrogenous wastes by manipulating the carbon:nitrogen (C:N) ratio in water to enhance the growth of heterotrophic bacteria [17]. These dense and active bacteria tend to produce biofloc, which shrimp can continually consume as a naturally occurring food supply. [18, 19].

Maintaining a balanced C:N ratio is essential for efficient waste bioconversion and a healthy BFT environment [20]. The selection of an appropriate carbon source can significantly impact floc formation, nutrient assimilation, and overall health and performance of the aquaculture species [21]. Carbon sources can be categorized as simple (easily degradable) or complex (slowly degradable). Simple sources like molasses, sugars, and starches are rapidly broken down by bacteria, while complex sources like brans (rice, wheat) and cellulose decompose at a slower rate. The choice of carbon source depends on various factors including cost, digestibility, and local availability. Commonly used options include molasses, glycerol, tapioca, and various brans [22]. Research is ongoing to identify new and sustainable sources to optimize BFT systems [23].

BFT aid in improving water quality under minimal/zero water exchange systems to maximize biosecurity [4]. In recent years, this technology has been adopted successfully in various aquatic species at different stages

of production, including the broodstock [24–27], hatchery [28, 29], nursery [30–34], and grow-out phases [35–40]. However, there is limited information on the effectiveness of BFTs in the larval and postlarval stages of *P. vannamei*. Therefore, this study aimed to investigate the efficacy of BFTs in these stages by utilizing three different carbon sources.

Materials and methods

Experimental design

The present research was conducted with facilities acquired from the BKMN shrimp hatchery, which is situated in Undavalli (16°50'64" N and 80°57'13" E), Guntur district of Andhra Pradesh, India. The experimental setup included four treatments, consisting of three BFT treatments and a control treatment. The trials were conducted in eight 1000 L capacity HDPE circular tanks with a working volume of 800 L each. All the tanks were in the same capacity, and each group was randomly assigned to replicate. Prior to usage, the tanks were meticulously cleaned and treated with bleaching powder at 5 ppm. Subsequently, they were allowed to undergo dechlorination for 3 days. An aeration setup was installed at the bottom side of all the tanks to maintain proper dissolved oxygen (DO) levels in the water for shrimp and to sustain the suspension of solids produced during cultivation.

The SPF *Penaeus vannamei* shrimp nauplii were procured from the same hatchery after ensuring their disease-free status through specific PCR hatchery tests [41, 42]. The present experiment employed treated water with a salinity of 32 ppt. The larvae were reared at a carbon ratio of 15:1 [43] for three treatments using different carbon sources: BFT-I (fructose – $C_6H_{12}O_6$), BFT-II (lactose – $C_{12}H_{22}O_{11}$), and BFT-III (dextrose – $C_6H_{12}O_6$) and one control (without any addition of carbon source). Each experimental unit was stocked with 80,000 larvae in the mysis-1 stage (M_1), resulting in a stocking density of 100 larvae L^{-1} . The experiment continued for 13 days until the larvae reached postlarval stage 10 (PL₁₀). Throughout the experiment, water in the biofloc experimental units was not exchanged.

Preparation of biofloc before stocking

For floc preparation, the treatment was started as per the protocol established by Avnimelech [43]. In brief, the process began on the first day with adding 1.5 g of ammonium chloride to introduce nitrogen into the system. Subsequently, carbon sources were added on the 3rd and 5th days at a rate of 5.62 g, followed by a doubling of the carbon sources to 11.25 g on the 7th day. The change in the color of the water from clear and transparent to light brown indicated the formation of flocs, which was attributed to the addition of carbon and nitrogen sources

from the external environment. On day 9, nauplii of *P. vannamei* (Mysis 1 stage) were introduced into all the prepared tanks at a density of 100 nos L⁻¹.

Feed management

The larval and postlarval shrimp were fed INVE commercial microencapsulated diets (minimum protein content of 52%, minimum lipid content of 14.5%, maximum fiber content of 3%, and maximum moisture content of 10%) following the manufacturer's recommendations for each larval stage [28, 29]. The feeding schedule involved six daily feedings at specific times (06:00, 08:00, 10:00, 12:00, 14:00, 16:00, and 18:00), and the amount of feed, artemia, and carbon–nitrogen ratio were adjusted for each larval stage (Table 1). The feed quantity was calculated based on floc volume, while the feeding times remained the same throughout the study [43].

Assessment of water quality parameters

The water quality of the experimental systems was checked daily. Water parameters such as temperature (mercury thermometer), pH (laboratory model Elico pH meter), salinity (hand refractometer), total ammonia nitrogen (TAN) using phenol hypochlorite method, dissolved oxygen (DO), ammonia (NH₃-N), nitrite (NO₂-N), nitrate (NO₃-N), and total alkalinity were analyzed following the American Public Health Association guidelines [44].

Estimation of biofloc volume

Biofloc volume was quantified by employing an Imhoff cone daily to understand the dynamics of biofloc generation and to adopt control measures in the case of excess biofloc generation, if any as described by Avnimelech and Kochva [45]. These cones have marked graduations on their outer surface, which can be used to measure the volume of solids that settle from one liter of water in the rearing tank.

Estimation of survival performance

Survival performance (%) was assessed at various larval and postlarval stages and was calculated as follows [46]:

$$SR (\%) = N_t/N_o \times 100$$

where SR = survival (%), N_t = the number of shrimps that survived until the end of the experiment, and N_o = the number of shrimps that were available at the beginning of the experiment.

Statistical analysis

All the values are presented as the mean ± standard deviation (SD) of three replicate analyses. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) was carried out by IBM SPSS software (version 29) at the 0.05 level (*P* < 0.05) of significance.

Table 1 Feeding regimes for *P. vannamei* between the M₁ and PL₁₀ phases

Treatment	Daily Input	Mysis			Post larvae									
		M ₁	M ₂	M ₃	PL ₁	PL ₂	PL ₃	PL ₄	PL ₅	PL ₆	PL ₇	PL ₈	PL ₉	PL ₁₀
BFT-I	Diet (g tank ⁻¹)	0.8	0.8	0.8	0.8	0.96	1.12	1.28	1.44	1.6	1.2	1.6	2	2.2
	Artemia (g tank ⁻¹)	0.696	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046
	N (g tank ⁻¹)	0.04	0.04	0.04	0.04	0.05	0.067	0.075	0.086	0.097	0.072	0.097	0.12	0.14
	Fructose (g tank ⁻¹)	0.6	0.6	0.6	0.6	0.75	1.005	1.125	1.29	1.455	1.08	1.455	1.8	2.1
	C: N	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01
BFT-II	Diet (g tank ⁻¹)	0.8	0.8	0.8	0.8	0.96	1.12	1.28	1.44	1.6	1.2	1.6	2	2.2
	Artemia (g tank ⁻¹)	0.696	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046
	N (g tank ⁻¹)	0.04	0.04	0.04	0.04	0.05	0.067	0.075	0.086	0.097	0.072	0.097	0.12	0.14
	Lactose (g tank ⁻¹)	0.6	0.6	0.6	0.6	0.75	1.005	1.125	1.29	1.455	1.08	1.455	1.8	2.1
	C: N	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01
BFT-III	Diet (g tank ⁻¹)	0.8	0.8	0.8	0.8	0.96	1.12	1.28	1.44	1.6	1.2	1.6	2	2.2
	Artemia (g tank ⁻¹)	0.696	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046
	N (g tank ⁻¹)	0.04	0.04	0.04	0.04	0.05	0.067	0.075	0.086	0.097	0.072	0.097	0.12	0.14
	Dextrose (g tank ⁻¹)	0.6	0.6	0.6	0.6	0.75	1.005	1.125	1.29	1.455	1.08	1.455	1.8	2.1
	C: N	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01	15:01
Control	Diet (g tank ⁻¹)	0.8	0.8	0.8	0.8	0.96	1.12	1.28	1.44	1.6	1.2	1.6	2	2.2
	Artemia (g tank ⁻¹)	0.696	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046	1.046

C Carbon, N Nitrogen

Results and discussion

Water quality parameters

Table 2 summarizes the water quality parameters observed in the study. The water quality parameters in the BFT treatments were similar, and analysis of variance revealed that there were significant differences between treatment groups for $\text{NO}_2^- \text{N}$, $\text{NO}_3^- \text{N}$, and alkalinity ($P < 0.05$). The DO levels of lactose and dextrose treatments showed no significant differences except for the fructose which had significantly lower DO values than that of lactose and dextrose ($P < 0.05$). All the observed parameters remained similar to those found in conventional hatchery systems with high levels of water exchange. The BFT hatchery systems showed similar results with the addition of carbon sources to *P. vannamei*, as reported by de Lorenzo et al. [28].

In shrimp hatchery systems, it is crucial to manage water quality parameters carefully to ensure the optimal survival and viability of the shrimp. Any variations in these parameters beyond a certain range can have a severe impact on production and result in significant economic losses [47]. Temperature is one of the most important factors influencing physiological responses in organisms, such as respiration, metabolism, growth, and reproduction [48]. Cultured shrimp grow best at temperatures ranging from 24 to 32 °C [49]. During the study, the average temperature ranged from 27 °C to 29 °C, which was the optimal temperature for all the treatments. Well-maintained aeration for a sufficient supply of DO is necessary for shrimp and for the formation of biofloc [50]. The lethal DO concentration for *P. vannamei* has been reported to be 1.0 ppm [51]. The DO levels ranged from 5.30 to 6.24 mg L⁻¹ in all the tanks. The lower pH values were possibly a result of high respiration rates by a large number of microorganisms, which may have

increased carbon dioxide concentrations. The permissible pH limit for *P. vannamei* is 7.5 to 8.5 [52]. The pH was significantly lower in the BFT, ranging from 7.54 to 7.83, than in the control (8.05). Similarly, the TAN levels were significantly lower (0.69 to 0.78 mg L⁻¹) than those in the control (1.07 mg L⁻¹). These lower levels may be caused by the inclusion of carbon sources. However, the mean values of pH and TAN in the BFT treatments remained at optimum levels throughout the experiment [46].

Ammonia ($\text{NH}_3^- \text{N}$) and nitrite ($\text{NO}_2^- \text{N}$) are highly toxic to cultured shrimp. High nitrite concentrations have been shown to significantly impact the circulatory and immune systems of aquatic organisms [53]. The concentration of $\text{NH}_3^- \text{N}$ ranged from 0.12 to 0.36 mg L⁻¹. The $\text{NH}_3^- \text{N}$ was significantly lower in the BFT (0.12 mg L⁻¹) than in the control (0.36 mg L⁻¹). The levels of $\text{NO}_2^- \text{N}$ ranged from 0.06 to 1.53 mg L⁻¹. The lowest concentrations were observed in the BFT (0.06 mg L⁻¹), while the highest were found in the control treatment (1.53 mg L⁻¹), which is unfavorable for optimal culture conditions [54]. For a successful *P. vannamei* culture, the optimal $\text{NO}_2^- \text{N}$ concentration is < 1.0 mg L⁻¹ [55]. Nitrate ($\text{NO}_3^- \text{N}$) is an inorganic nitrogen compound formed at the end of the nitrification process. The concentration of nitrate is usually greater than that of ammonia and nitrite [56]. High levels of nitrate have been shown to affect the osmoregulation and oxygen transport of cultured aquatic species [57]. In the BFT treatments, the observed nitrate values ranged from 1.94 to 2.05 mg L⁻¹, lower than those in the control groups, which had a value of 2.77 mg L⁻¹. These results for nitrate are similar to those reported by Furtado et al. [58]. The relatively stable concentrations of $\text{NH}_3^- \text{N}$, $\text{NO}_2^- \text{N}$, and $\text{NO}_3^- \text{N}$ in the BFT treatments may be attributed to effective nitrification processes. Alkalinity, which is the buffering capacity of water, can

Table 2 Water quality parameters of *P. vannamei* between the M₁ and PL₁₀ phases

Parameter	Biofloc treatments			Control
	Fructose	Lactose	Dextrose	
Temperature (°C)	28.34 ± 0.46	29.16 ± 0.33	28.80 ± 0.33	29.63 ± 0.13
DO (mg L ⁻¹)	5.81 ± 0.09 ^{ab}	5.96 ± 0.09 ^c	6.24 ± 0.32 ^c	5.30 ± 0.10 ^a
Salinity (g L ⁻¹)	32.05 ± 0.03	32.27 ± 0.18	32.11 ± 0.06	32.29 ± 0.10
pH	7.54 ± 0.20	7.83 ± 0.14	7.70 ± 0.15	8.05 ± 0.29
TAN (mg L ⁻¹)	0.78 ± 0.04	0.69 ± 0.02	0.72 ± 0.02	1.07 ± 0.34
$\text{NH}_3^- \text{N}$ (mg L ⁻¹)	0.18 ± 0.13	0.12 ± 0.02	0.23 ± 0.17	0.36 ± 0.03
$\text{NO}_2^- \text{N}$ (mg L ⁻¹)	0.13 ± 0.06 ^a	0.16 ± 0.10 ^a	0.06 ± 0.04 ^b	1.53 ± 0.17 ^a
$\text{NO}_3^- \text{N}$ (mg L ⁻¹)	1.94 ± 0.11 ^b	2.05 ± 0.04 ^b	2.00 ± 0.07 ^b	2.77 ± 0.14 ^a
Alkalinity (mg L ⁻¹)	127.92 ± 1.00 ^b	128.17 ± 0.27 ^b	127.08 ± 0.58 ^b	91.38 ± 0.57 ^a

All the values are the means ± SD (standard deviation) of three replicate analyses

Data with different superscript letters in the same row are significantly different ($P < 0.05$)

DO Dissolved oxygen, TAN Total ammonia nitrogen, $\text{NH}_3^- \text{N}$ Ammonia, $\text{NO}_2^- \text{N}$ Nitrite, $\text{NO}_3^- \text{N}$ Nitrate

significantly impact primary productivity [59]. In the present investigation, the alkalinity of the BFT ranged from 127.08–128.17 mg L⁻¹, which was significantly greater than that of the control (91.38 mg L⁻¹).

Biofloc volume

The volume of biofloc increased gradually in all treatments over time (Fig. 1); however, the greatest floc volume was observed for dextrose (1.62 ml L⁻¹), followed by fructose (1.12 ml L⁻¹) and lactose (0.84 ml L⁻¹). Similar levels of floc volume were reported by Panigrahi et al. [46]. However, the lowest floc volume could be because lactose, being a disaccharide, is harder to break down than monosaccharides such as dextrose and fructose, resulting in less floc. Compared to fructose, dextrose is derived from simple starch, which makes it easier to break down and thus forms flocs more easily.

Survival performance

The percentages of the survival rates for different treatments are presented in Table 3. The highest survival rate for PL₁ was observed for dextrose (93%), followed by fructose (88.67%) and lactose (86.33%), while the lowest survival rate was in the control group (79.33%) ($P > 0.05$). For PL₅ and PL₁₀, the trends were similar; the survival rates were 90.67%, 85.67%, 78.33%, and 66.67% for the dextrose, fructose, lactose, and control treatments, respectively. One-way ANOVA showed that there were no significant differences ($P > 0.05$) in the mean percentages of PL₅ and PL₁₀ among the different treatments. However, when comparing PL₅ fructose and dextrose treatments, there were no significant differences except for lactose, which had significantly lower survival values.

In biofloc technology, maintaining a suitable carbon–nitrogen ratio is crucial. The choice of carbohydrate

Table 3 Survival performance of *P. vannamei* between the M₁ and PL₁₀ stages

Larval stage	Treatments (%)			
	Fructose	Lactose	Dextrose	Control
M ₁ Stocked	100 nos L ⁻¹	100 nos L ⁻¹	100 nos L ⁻¹	100 nos L ⁻¹
PL ₁	88.67 ± 2.33	86.33 ± 5.81	93.00 ± 3.61	79.33 ± 2.33
PL ₅	85.67 ± 2.96 ^b	78.33 ± 4.33 ^{ab}	90.67 ± 4.37 ^b	66.67 ± 4.41 ^a
PL ₁₀	80.33 ± 4.70 ^{bc}	71.33 ± 2.40 ^b	86.33 ± 3.84 ^c	53.71 ± 3.39 ^a

All the values are the means ± SD (standard deviation) of three replicate analyses. Data with different superscript letters in the same row are significantly different ($P < 0.05$)

M Mysis, PL Post Larva

source is one of the main factors since different carbon sources have different effects on cultured species [33, 60, 61]. In this study, M₁ to PL₁₀ were reared under three treatments with different carbon sources—fructose, lactose, and dextrose—at a ratio of 15:1, and the control treatment without the addition of a carbohydrate source. The average survival rate was significantly greater in the BFT treatment group (71% to 86%) than in the control group (53%). Similar studies have shown that the survival of shrimp in BFTs ranges from 80 to 100% of that of control shrimp [40, 62, 63]. The current results showed higher survival levels than those of de Lorenzo et al. [28, 29] under the carbon source dextrose, which may be due to the lower stocking density of larvae adapted to the present study. The overall survival in all biofloc-treated groups surpassed the rate appropriate for the species (70%, [64]) and that appropriate for the experimental hatcheries [28, 29, 65, 66]. Based on these results, among all the carbon sources used, fertilization with dextrose can be efficiently maintained in the hatchery system.

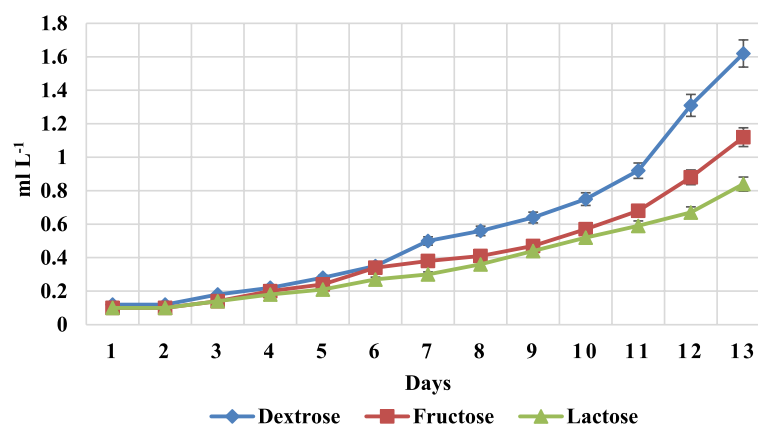


Fig. 1 Floc volume in biofloc treatments

Conclusion

BFT systems have been driven toward increased sustainability in shrimp aquaculture. The types of carbon sources and addition strategies are critical considerations in BFT systems. The current study contributes to a better understanding of the effects of different carbon sources on the *P. vannamei* hatchery system. Based on the present findings, it can be concluded that using dextrose, fructose, and lactose as carbon sources at a ratio of 15:1 without water exchange resulted in adequate water quality. Additionally, *P. vannamei* showed a greater survival rate during the M₁ and PL₁₀ hatchery phases when dextrose was used than during the other treatments.

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Authors' contributions

S.K.; Conceptualization, methodology, investigation, formal analysis, writing - original draft. P.P.; supervision, writing - review & editing. D. C.; Data - curation & preparation. S.K., P.P., and D.C. have read and agreed to the published version of the manuscript.

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Availability of data and materials

This article contains all of the data that were analyzed for this study and can be obtained from the corresponding author if needed.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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