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Effect of Carbon Source Addition Strategies on Water Quality, Growth Performance and Histology in *Penaeus vannamei* and GIF Tilapia (*Oreochromis niloticus*) in Polyculture Model in Lined Pond - BFT Aquaculture Systems

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MJ conducted the trial and analysis, analyzed the data and drafted the manuscript. PC, BA, KR and CA presented the concept and corrected the manuscript. BA designed the study. AU conceptualized the design. PR carried out the statistical analysis.

Key words
Biofloc, GIF tilapia, *Penaeus vannamei*, Polyculture, Lined pond

ABSTRACT

Profitable and environmental friendly culture depends on the innovative and sustainable technology method of production. The experiment was conducted to evaluate the efficiency of biofloc on the water quality, growth performance and histology of *Penaeus vannamei* and Genetically Improved Farmed Tilapia (*Oreochromis niloticus)* in a polyculture model in lined pond. *P. vannamei* (1.06±0.08 g) and GIF tilapia (0.42±0.01 g) were distributed to the 12 m x 10 m x 1.5m lined pond. The *P. vannamei* and GIF tilapia were fed with commercial diet of 36% (sinking) and 24% (floating) crude protein. The growth performance and histology were assessed and the culture trial was conducted for 90 days. The biofloc cultured lined pond yielded the best result compared to the clear water lined pond culture in terms of growth performance and histology. The study found significantly higher weight gain (22.28±0.94 g and 16.28±0.35 g) and survival (63.31±0.47% and 85.90±1.44%) were recorded in *P. vannamei* and GIF tilapia in lined pond based biofloc culture system. No abnormalities were observed in the gut of GIF tilapia, and gut and hepatopancreas of *P. vannamei*, but deformities like congestion of tips of few primary lamellae of gill were noticed in GIF tilapia reared in lined pond based biofloc culture system. One way ANOVA of the data collected clearly affirmed that significant difference ($p<0.05$) was observed among the biofloc culture and clear water culture system.

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INTRODUCTION

Nowadays aquaculture industry is growing rapidly and cutting edge technologies are being practiced to improve quantity and quality production. Generally, aquaculture species retains at least 20–30% of feed nutrients (Avnimelech and Ritvo, 2003); dense aquaculture results in rapid accumulation of organic and inorganic compounds, which disturbs the environment by the discharge of waste water due to water exchange. To overcome the environmental damage and increase sustainable aquaculture production (Avnimelech, 2009), one of the promising technologies that developed was an ecofriendly culture technology known as biofloc technology (BFT) (Martinez-Cordova *et al*., 2017).

BFT is a protein rich live food formed by aggregates of algae, protozoa, bacteria and particulate organic matter and held together in a loose matrix of mucus secreted by bacteria and bound by filamentous microorganisms. Biofloc has two major advantages viz., treating wastes from feeding and providing nutrients from floc consumption. However, certain drawbacks can encounter in the biofloc technology such as high concentration of solids generated, excessive accumulation of suspended solids in the water and the solids removed are an effluent rich in nitrogen and phosphorous compounds.

To overcome the above-mentioned obstacle, the utilization of polyculture model could result in a better development in a BFT system. Polyculture is an aquaculture model, where simultaneous cultivation of different trophic levels in the same environment system, resulting in the conversion of culture residues in to food, for the other species (Chopin *et al*., 2001). The use of polyculture model could contribute to increased productivity and would allow for maximum utilization of nutrients present in BFT system, based on different trophic level species. The interaction between farmed aquatic organisms in polyculture depends mainly on the stocking density and biological characteristics of the species.

Shrimp is the main exporting species in the world, since it is widely cultured in tropical and sub-tropical regions. Some characteristics that made it widely known based on expanding production chain due to its adaptability, rapid growth, disease resistance (Hossain and Islam, 2006) and adaptability to polyculture with fish (Haque *et al*., 2018). Moreover, shrimp with fish culture improves the production efficiency, greater profits (De-Shang and Shuang-Lin, 2000) and improved ecological balance of pond (Uddin *et al*., 2006) and less environmental impact (Santos and Valenti, 2002). In polyculture model, synergistic interactions should be improvements in feed availability and environmental conditions (Milstein, 1992) and antagonistic interactions are competition for food, space, oxygen and other resources.

Above mentioned fundamentals can be matched by the *Penaeus vannamei* and GIF tilapia because of different spatial distribution and feeding habits. *P. vannamei* are benthic in production ponds, omnivorous, eating detritus, waste and feces (D'Abramo and New, 2010). Whereas, GIF tilapia are pelagic, filtering phytoplankton, omnivorous and eating periphyton (Tadesse, 1999). However, the growth and production performance of shrimp and GIF tilapia in polyculture based BFT system in lined pond have been poorly documented to date (Reinoso *et al*., 2019). Therefore, the documentation of this hybrid technique using lined pond based biofloc technology in polyculture model of *P. vannamei* and GIF tilapia is highly essential for further adoption.

MATERIALS AND METHODS

Experimental design and experimental units

The experiment was conducted at Tamil Nadu Dr. J. Jayalalithaa Fisheries University; Pulicat Research Farm Facility (PRFF), Pazhaverkadu, Chennai, Tamil Nadu, India for a period of 90 days during September to December, 2023. It was conducted in four uniform HDPE (High Density Polyethylene Ponds) lined ponds with an area of 0.12 ha (12 X 10 X 1.5 m), by following completely randomized design (CRD) in duplicate, with and without addition of carbon source for development of biofloc and considered as biofloc treatment and clear water treatment, respectively. The biofloc was developed and maintained as per Avnimelech (1999) with minor modifications in two lined ponds. One horse power aerator was placed at the center of the pond in order to maintain a circulation of water.

The shrimp species used in the experiment was white leg shrimp (*P. vannamei)* with an average initial weight of 1.06±0.08 g and stocking density of 60 shrimp/m^3. The shrimp were fed with commercial sinking pellet feed of 36% crude protein level and fed with only 50% of feeding rate compared to actual feeding rate (Table I) in both treatments. GIF tilapia used as a fish species, had an average initial weight of 0.42±0.01 g and was maintained in hapa at the shrimp lined ponds at a density of 5 fish/m^3. GIF tilapia was fed with commercial floating pellet feed of 24% crude protein level. Both the species were fed four times a day (06.00; 10.00; 14.00 and 18.00 Hrs.). Weights of 10% of total number of animals were measured individually at fortnight interval with a view to estimate the animal biomass and to adjust the feeding rate accordingly.

Table I. Feeding rate for *Penaeus vannamei* stocked in a biofloc based lined pond.

Days of culture	Weight of *P. vannamei*	Feeding rate	
		Lin, 1991	Followed
0-15	1.06 g	10%	5%
15-30	3.50 g	6.5%	3.25%
30-45	8.09 g	5%	2.5%
45-60	11.80 g	4.2%	2.1%
60-75	14.80 g	4.0%	2.0%
75-90	17.70 g	3.5%	1.75%

Water quality monitoring

During the experimental period, water temperature (° C), dissolved oxygen, pH and salinity were measured daily. Nitrogen compounds such as ammonia, nitrite and nitrate were analyzed weekly with the double beam UV visible spectrophotometer KLUV-2100 model. Total hardness, calcium hardness, magnesium hardness and alkalinity were determined as per the method described in APHA (2005).

Growth performance analysis of shrimp and GIF tilapia

Fortnightly, growth performance of fish and shrimp was recorded by measuring the 10% of total number of animals, randomly with minimal stress, and the individual final body weight was recorded. The collected animal weight were used to calculate weight gain (g), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), specific growth rate (SGR-%/day), average daily gain (ADG-g/animal/day) and survival rate (%) by adopting standard formulae (Dong *et al*., 2018; Tan *et al*., 2018).

Histology

Ten animals in similar size from biofloc and clear water treatment were euthanized for histology. The hepatopancreas, gut of shrimp and gill, gut of GIF tilapia were collected and immediately fixed in 10% neutralized buffered formalin (NBF), dehydrated in graded ethanol levels, embedded in paraffin wax and blocked at 58°C and sectioned using Rotary microtome (Leica RM2255, India) (Bell and Lightner, 1988). The sections of 6μm were stained with hematoxylin and eosin (H and E) with Microm HMS7. Finally, sections were evaluated under light microscope at 100X (Olympus CX21, India) and photographed for further examination (Krogdahl *et al*., 2003; Wang *et al*., 2017).

Statistical analysis

The results of water quality and growth were analyzed in SPSS Version 24 software using student's t-test. Significance level for the test was set as p>0.05. Histology analyses were analyzed descriptively.

RESULTS AND DISCUSSION

Water quality parameters

Water quality parameters were recorded during the experimental trial are given in Table II. pH, temperature, salinity, alkalinity, total hardness, calcium hardness, magnesium hardness, nitrite and dissolved oxygen did not shown any significant difference between the treatment groups. Mean ammonia and nitrate ranged from 0.33±0.02 to 0.91±0.02 mg/L and 1.21±0.07 to 1.88±0.13 mg/L with the lowest value observed in biofloc based lined pond system.

In case of ammonia and nitrate, significant difference was observed in between the treatments. The decrease level of ammonia and nitrate in biofloc treatment lined pond is due to the occurrence of nitrification process by chemoautotrophic bacteria and the removal of ammonia by heterotrophic bacteria, present in the biofloc system (Ebeling *et al*., 2006). As the addition of carbon source in the biofloc lined pond, having the ability to consume ammonia and nitrate for the growth and multiplication of bacterial population. Polyculture of shrimp and fish in biofloc at lined pond has been known for minimizing the environmental impact of effluents, particularly related to nitrogenous wastes (Martinez-Porchas *et al*., 2010).

Table II. Mean water quality parameters recorded in lined pond with biofloc and clear water in polyculture model.

Parameters	Biofloc	Clear water	p value
pH	8.57±0.09	8.53±0.09	0.017
Temperature (°C)	27.21±0.44	27.28±0.42	0.465
Salinity (ppt)	5.64±0.14	5.40±0.06	0.158
Alkalinity (mg/L)	210.92±1.54	217.00±2.56	0.072
Total hardness (mg/L)	2984.20±17.59	2974.00±27.32	0.793
Calcium hardness (mg/L)	143.75±5.80	135.33±2.57	0.162
Magnesium hardness (mg/L)	902.08±26.50	937.50±5.59	0.226
Ammonia (mg/L)	0.33±0.02^b	0.91±0.02^a	0.000
Nitrite (mg/L)	0.21±0.10	0.40±0.01	0.078
Nitrate (mg/L)	1.21±0.07^b	1.88±0.13^a	0.000
Dissolved oxygen (mg/L)	6.04±0.22	5.78±0.41	0.611

Values were expressed as mean ± standard error of mean (SEM). Values in the same row with different superscripts are significantly different at p< 0.05.

Table III. Growth performance of *P. vannamei* and GIF tilapia reared using lined pond maintained with biofloc and clear water in polyculture model.

	Penaeus vannamei			GIF tilapia		
	Biofloc	Clear water	p-value	Biofloc	Clear water	p-value
Initial weight (g)	1.06±0.08	1.06±0.08	-	0.42±0.01	0.42±0.01	-
Final weight (g)	23.34±0.90[a]	15.68±0.66[b]	0.000	16.70±0.35[a]	9.39±0.50[b]	0.000
Average body weight gain (g)	22.28±0.94[a]	14.62±0.71[b]	0.000	16.28±0.35[a]	8.97±0.50[b]	0.000
FCR	0.84±0.03[b]	1.29±0.06[a]	0.000	1.04±0.02[b]	1.93±0.11[a]	0.000
FER	1.21±0.05[a]	0.79±0.04[b]	0.000	0.97±0.02[a]	0.53±0.03[b]	0.000
PER	0.62±0.03[a]	0.41±0.02[b]	0.000	0.68±0.01[a]	0.37±0.02[b]	0.000
SGR (%/day)	0.04±0.00[a]	0.03±0.00[b]	0.000	0.04±0.001[a]	0.03±0.00[b]	0.000
Average daily gain(g/animal/day)	0.25±0.01[a]	0.16±0.01[b]	0.000	0.18±0.00[a]	0.10±0.01[b]	0.000
Survival rate (%)	63.31±0.47[a]	41.16±0.23[b]	0.000	85.90±1.44[a]	68.83±0.84[b]	0.000

Values were expressed as mean ± standard error of mean (SEM). For each species, values in the same row with different superscripts are significantly different at $p< 0.05$. FCR, food converstion ratio; FER, feed efficency ratio; PER, protein efficiency ratio; SGR, specific growth rate.

Growth performance of shrimp and GIF tilapia

Growth parameters recorded in between the treatment groups are given in Table III, the study found significant difference in growth performance of shrimp and GIF tilapia reared in biofloc lined pond treatment compared to clear water. The present study found 35% and 45% of significantly higher weight gain in biofloc treatment lined pond in *P. vannamei* and GIF tilapia, respectively, compared to clear water system. Similarly, in polyculture of giant freshwater prawn and Nile tilapia reared in biofloc has reported 40% and 34%, respectively of higher weight gain compared to clear water system (Hisano *et al*., 2019). Contradictory to our results, Barbosa *et al*. (2022) have reported no significant difference in weight gain of Nile tilapia and freshwater shrimp in polyculture based biofloc technology. Similar to the present study, FCR values of *L. vannamei* and GIF tilapia reared in biofloc system has displayed a 1.45 (Xu and Pan, 2012) and 0.83 (Long *et al*., 2015), respectively, which was closer to the present study FCR. On the other hand, tilapia and prawn reared in biofloc system in polyculture model with complete feeding rate has displayed a FCR of 1.22-1.44 and 2.88-4.31, respectively, which was higher FCR than present study and it may be due to difference in feeding rate followed in the present study (50% feeding rate). Further it confirms that, *P. vannamei* and GIF tilapia reared in lined pond biofloc treatment does not have any effect on the culture, as the species utilize different niche in the culture system (Khan *et al*., 2016).

Histology

The histological observations of gill (Fig. 1A) of GIF tilapia exposed to biofloc has shown deformities such as tips of few primary lamellae were congested, whereas gill of GIF tilapia exposed to clear water has shown deformities such as tips of few primary and secondary lamellae were congested. On the other side, no abnormalities were observed in the gut of GIF tilapia in both the treatments (Fig. 1B). Interestingly, no deformities in hepatopancreas (Fig. 2A) and gut (Fig. 2B) of *P. vannamei* in biofloc treatment lined pond. Abnormalities were observed in clear water lined pond reared *P. vannamei* such as few scattered hepatopancreatic tubules showed hyperplastic changes in hepatopancreas and autolytic changes in gut.

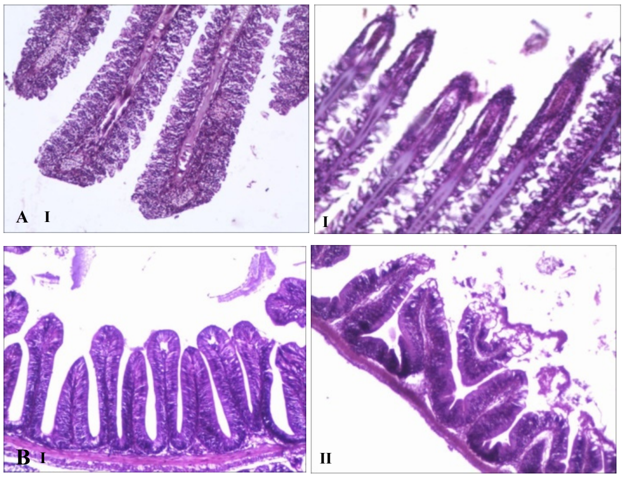

Fig. 1. A, Photomicrograph of gill of GIF tilapia. (I) GIF tilapia reared in biofloc– Tips of few primary lamellae were congested; (II) GIF tilapia reared in clear water- Tips of few primary and secondary lamellae were congested. B, photomicrograph of gut of GIF tilapia, (I) GIF tilapia reared in biofloc, No abnormalities; (II) GIF tilapia reared in clear water- No abnormalities.

In fish, gills are the main respiratory organs for the simple diffusion of gases (oxygen and carbon dioxide). Moderate alterations in gill were observed for the fish reared under biofloc and clear water lined pond systems,

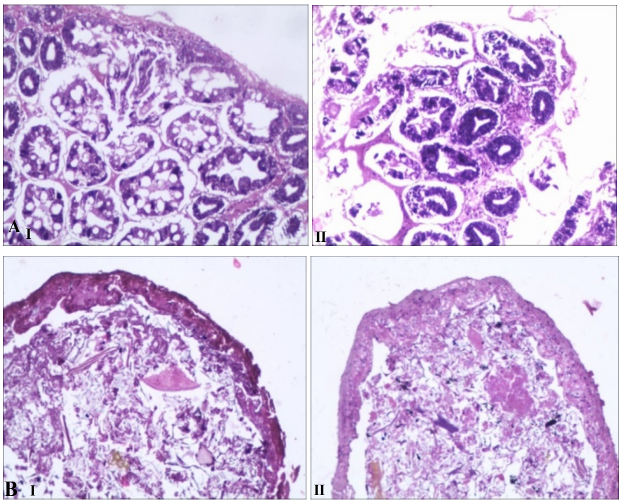

Fig. 2. A, Photomicrograph of hepatopancreas of *Penaeus vannamei.* (I) *P. vannamei* reared in biofloc– no abnormalities. (II) *P. vannamei* reared in clear water– a few scattered hepatopancreatic tubules showed hyperplasic changes. B, photomicrograph of gut of *P. vannamei.* (I) *P. vannamei* reared in biofloc– no abnormalities; (II) *P. vannamei* reared in clear water– autolytic changes.

tips of few primary lamellae were congested in biofloc treatment lined pond system and tips of few primary and secondary lamellae were congested in clear water lined pond system. Similar to the present study, zero water exchange system of Nile tilapia (Suloma, 2013) and tilapia raised in BFT and RAS system (Vincent, 2006) show alterations like congestion in the gill. On the other side, gill of *O. niloticus* (Azim and Little, 2008), *C. carpio* (Haghparast *et al*., 2020) and African cat fish (Romano *et al*., 2018) did not produce any potential gill damage when reared in biofloc system. The intestinal histology can be used to ascertain the gut condition (Khojasteh, 2012). The gut of GIF tilapia did not shown any damage in the presence of biofloc in culture water. Consistent with present study, Nile tilapia fed with biofloc meal did not cause any damage to the gut (Hersi *et al*., 2023). In present biofloc treatment study showed normal and healthy hepatopancreas, whereas the clear water shrimp has shown some deformities such as few scattered hepatopancreatic tubules showed hyperplasia changes. Similar to the present study, biofloc system did not show any histological changes in hepatopancreas of *L. vannamei* (Moss *et al*., 2001) and *M. monoceros* (Kaya *et al*., 2019). Presence of biofloc in the culture water did not cause any damage to the gut of shrimp. Similar to our results, Zheng *et al*. (2018) and Won *et al*. (2020) reported that shrimp grown in biofloc did not cause any damage to the gut. The study demonstrate that raising of GIF tilapia and shrimp in biofloc had fewer and less severe histopathological lesions in gill of GIF tilapia and hepatopancreas of *P. vannamei* and may consider as biofloc did not affect the normal physiological activity of GIF tilapia and shrimp.

CONCLUSION

The present polyculture study with *Penaeus vannamei* and GIF tilapia in a biofloc system has a potential to improve water quality, promoted the growth of shrimp and GIF tilapia and is beneficial for the growth of microbial biomass. Further, the present study has demonstrated that the *P. vannamei* and GIF tilapia polyculture is technically feasible, environmentally friendly and economically attractive with the appropriate feeding strategy.

DECLARATIONS

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IRB approval

The study was approved by Institutional Review Board of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.

Ethical approval

After the approval of the statutory authorities of the Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India, the research work was carried out in adherence with the current animal welfare laws in India. The care and treatment of the experimental animal was carried out by guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division, Govt. of India).

Statement of conflict of interest

The authors have declared no conflict of interest.

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