



Study on Biofloc Characteristics, Digestive Enzyme Activity and Physiological Responses in Polyculture Model *Penaeus vannamei* and GIF Tilapia (*Oreochromis niloticus*) Culture System-BFT Aquaculture System

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ABSTRACT

Background: The present study was undertaken to investigate the efficiency of biofloc on the floc characteristics, digestive enzyme activity, immunological responses and haemato-biochemical parameters for the production of *Penaeus vannamei* and Genetically Improved Farmed Tilapia in a polyculture model.

Methods: Four lined pond were used following completely randomized design using biofloc and clear water culture systems. Each lined pond (12 m × 10 m × 1.5 m) was stocked with 60 shrimp/m³ of *P. vannamei* (1.06±0.08 g) and 5 no's/m³ of GIF tilapia (0.42±0.01 g) and reared for 90 days. Biofloc was developed and maintained using soya pellet powder as carbon source and vigorous aeration was provided to keep floc in suspension.

Result: The study found significantly higher TS (1167.50±78.50 mg/L), TSS (434.05±62.90 mg/L) and TDS (733.45±108.22 mg/L) were recorded in lined pond based biofloc system. Significantly higher digestive enzymes of protease (7.98±0.36 and 3.62±0.16 U/ mg protein/min), lipase (3.43±0.20 and 8.65±0.20 U/ mg protein/min) and amylase (1.35±0.10 and 3.25±0.17 U/mg protein/min) were recorded in *P. vannamei* and GIF tilapia in lined pond based biofloc culture system, respectively. The study found significantly improved immunological responses of prophenol oxidase activity (71.25±1.28 µmol.min⁻¹.ml⁻¹), total haemocyte count (4.75±0.12 × 10⁶ cells/ml) and catalase activity (3.56±0.03 Units/ml) in biofloc cultured *P. vannamei*. Higher values of RBC (1.95±0.05 million/cu mm), WBC (49.65±1.56 1000/cu mm), hemoglobin (9.21±0.15 g/dl), hematocrit (33.42±0.89%), albumin (1.18±0.04 g/dl), globulin (4.39±0.16 g/dl), total protein (5.58±0.14 g/dl) and total cholesterol (9.32±0.41 g/dl) were recorded in GIF tilapia reared in biofloc culture system. Therefore, the study suggests that polyculture model of *P. vannamei* and GIF tilapia in biofloc culture system improves the physiological performance and production augmentation.

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

INTRODUCTION

To the best of our knowledge, around 35% of the nitrogen produced from the diet of fish is retained, while the remaining was being lost as nitrogen and ammonia in feed residues and feces (Samocha *et al.*, 2004). On the other side, alternative primary sources such as fish meal and fish oil are constraints in aquaculture formulated diet (Zhu *et al.*, 2010). Therefore alternative strategies should be investigated to make aquaculture industry more sustainable. Biofloc technology acts as live food, which converts the nitrogenous waste (uneaten feed and fecal matter) into microbial protein through heterotrophic microbial assimilation with the addition of carbon source and vigorous aeration, thus a proteinaceous feed is available for cultured organisms (Crab *et al.*, 2010). Thus biofloc technology is an eco-friendly technique adopted in zero-water culture system.

Polyculture is a traditional fish farming practice, where compatible species with different feeding habits were stocked in a single pond for grow-out practice (Jhingran, 1975). Polyculture of river prawn with tilapia has shown

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best productive performance under biofloc based polyculture system. Similarly, polyculture of shrimp and mullet in earthen pond favors the production of mullet and shrimp (Costa *et al.*, 2013). An another polyculture study using Nile tilapia and shrimp found enhanced pond

productivity at different stocking densities. In polyculture, shrimps consume leftover fish food and waste and fishes filter the phytoplankton, which lowers the danger of low dissolved oxygen levels at night times (Santos and Valenti, 2002). Moreover, shrimp bioturbation at the pond bottom recycles nutrients into the water column, which increase the phytoplankton production, a natural food for fish. Therefore, interlinking of biofloc and polyculture model techniques may enhance the food availability for species and increase the yield per unit area which in turn improves the economic return of the hybrid fish production system.

Among the various shrimp species, *Penaeus vannamei* has a consolidated and expanding production chain, due to its adaptability, rapid growth and adaptability to polyculture with fish (Hossain and Islam, 2006). Similarly, in the farmed fish species, GIF tilapia is emerging as an important cultivable fish after carps. GIF tilapia strain has better growth, meat quality and good market value than normal tilapia strain (Sgnaulin *et al.*, 2020). Farming of these species is generally performed to meet the growing market demand. The positive effect of biofloc can enhance the water quality (De Schryver *et al.*, 2008); digestive enzyme activity (Xu *et al.*, 2013); immune responses (Smith *et al.*, 2003) and enhance biosecurity (Crab *et al.*, 2010) of shrimp farming. However, no published information is so far available on the feasibility of biofloc for culture of shrimp and GIF tilapia in polyculture model. The present study was designed to evaluate the floc parameters, digestive enzymes, immunology, hematology and histology of shrimp and GIF tilapia in biofloc based polyculture model.

MATERIALS AND METHODS

Experimental setup

Experimental trial was carried out in High Density Polyethylene (HDPE) lined ponds of 12 m × 10 m × 1.5 m (L × W × H), filled with 5ppt salinity water were used for the experiment conducted at Pulicat Research Farm Facility (PRFF), Pazhaverkadu, Tamil Nadu. Dr. J. Jayalalithaa Fisheries University, Chennai, Tamil Nadu, India. The experiment consists of two treatments, biofloc treatment and clear water treatment with zero water exchange, replicate was maintained for each treatment. Healthy *P. vannamei* and GIF tilapia were procured from a farm at Pazhaverkadu, Chennai, Tamil Nadu. Prior to the stocking, the shrimp and GIF tilapia, acclimatized in 2 ton FRP tank for a period of three weeks. *P. vannamei* and GIF tilapia having average body weight of 1.06±0.08 g and 0.42±0.01 g were distributed randomly to all four ponds at a stocking density of 60shrimp/m³ and 5 fish/m³, respectively. A week prior to stocking, biofloc was developed, aspirator aerator were placed in lined pond to keep floc in suspension and C: N ratio of 15:1 was maintained in biofloc treatment with soyahull pellet powder as carbon source. Shrimp and fish were fed with pellet feed having 36% and 24% crude protein, fed four times per day.

Floc characteristics

Water sampling was done with the help of plankton net (100 µm) and the microorganisms were counted with Sedgewick-Rafter cell and viewed under light binocular microscope (Lawrence and Mayo Microscopes, Tamil Nadu) with magnification of 40 × and plankton was identified using standard reference (Ruppert, 1996).

Total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS) were analyzed at the end of the experiment in both systems using standard procedure (APHA, 2005). Settled particles of floc in imhoff cone were measured as floc volume (Magara *et al.*, 1976), floc concentration, floc density, floc volume index, floc density index and porosity of the biofloc system were determined by following the methods of Mohlman (1934), Mueller *et al.*, 1967, WHO international reference center (1978) and Smith and Coakley (1984), respectively.

Tissue sampling and digestive enzyme analysis

At the end of the experiment, shrimp and fish (10 no's/tank) were collected and anaesthetized using clove oil (10 ppm), for tissue sampling. Gut samples were collected aseptically and mixed with 0.25 M chilled sucrose using a handheld homogenizer. The mixture was centrifuged at 3,070g for 5 min and supernatant was collected and used for digestive enzyme analysis. Protease, lipase and amylase were determined by the standard procedures of Sarath *et al.* (1989); Cherry and Crandall (1932) and Clark (1964), respectively. One unit of protease, lipase and amylase activities were expressed as 1 µg of tyrosine, fatty acid and maltose released per minute, respectively.

Estimation of Immune parameters

Hemolymph (0.15 ml) was collected from ventral sinus cavity of shrimp using 22-gauge needle with 1 ml syringe of each treatment mixed with 1.35 ml of pre-cooled anticoagulant was transferred to a Neubauer hemocytometer and observed under Olympus light microscope (CX21i, LED) at 400× magnification for observing total haemocyte count (Raja *et al.*, 2012). Prophenol oxidase activity and catalase activity were determined by standard procedures of Cheng and Chen (2000) and Takahara *et al.* (1960), respectively.

Hematological profiles

Fresh blood (2 ml) was collected from caudal vein of GIF tilapia using 2.5 ml syringe and expelled into heparinized and non-heparinized tubes. The RBC and WBC were counted by Neubauer hemocytometer by following the method Stoskopf, (2015). Hemoglobin, hematocrit and erythrocyte indices (MCV, MCH and MCHC) were determined by following standard procedures of Drabkin, (1946); Nelson and Morris (1989) and Wintrobe (1934), respectively. Albumin, total protein and serum cholesterol levels were determined by standard procedures of Reinhold (1953); Dumas *et al.* (1971) and Parekh and Jung (1970),

respectively. Whereas, Globulin and A/G ratio were calculated using standard formulas.

$$\text{Globulin} = \text{Total protein} - \text{Albumin}$$

$$\text{A/G ratio} = \text{Albumin value} / \text{Globulin value}$$

Histology

Gut of shrimp and GIF tilapia were collected and preserved in neutral buffered formalin for 48 h. To summarize, samples were dehydrated using ethanol, cleared with xylene, saturated with paraffin wax, implanted in paraffin box (58°C), sectioned with rotary microtome (Leica RM2255, India), stained with Hematoxylin and Eosin (Microm HMS7) and microscopically examined at 400× magnification and photographed for further examination.

Statistical analysis

The experimental results were statistically analyzed using SPSS software version 20.0 at 5% level of significance. The data on floc parameters, digestive enzyme analysis, immune parameters and hematological profile were analyzed by student's t-test to test difference between the treatments. Histology analysis of gut of shrimp and GIF tilapia were analyzed descriptively.

RESULTS AND DISCUSSION

Floc characteristics

Plankton density was significantly dominated (Fig 1) in biofloc system compared to clear water system. Initially, biofloc system was thrived with green colour floc due to the presence of phytoplankton dominance, particularly class of *Chlorophyceae* and *Cyanophyceae* and later turned into brown colour due to dominance of zooplankton. Similar to the present study, Rajkumar *et al.*, 2016 in biofloc based shrimp culture, Choo and Caipang, 2015 in biofloc based

tilapia culture and Nie Wei *et al.* (2018) in biofloc system has reported that plankton density was dominated in biofloc system. Among the plankton diversity recorded in biofloc system, class of *Cyanophyceae* and *Chlorophyceae* were the most dominant (Table 1 and Fig 2). The findings were matching with the earlier results of Ju *et al.* (2008) reported that Class of *Chlorophyceae* was dominant in biofloc aquaculture system. The mean $\pm$ SEM of biofloc parameters were presented in Table 2. TS, TSS and TDS were significantly ($p < 0.05$) higher in biofloc system compared with clear water system due to supplementation of carbon source and vigorous aeration, uneaten feed waste and feces are converted into microbial particles. Similar range of TS, TSS and TDS was previously reported by Luo *et al.*

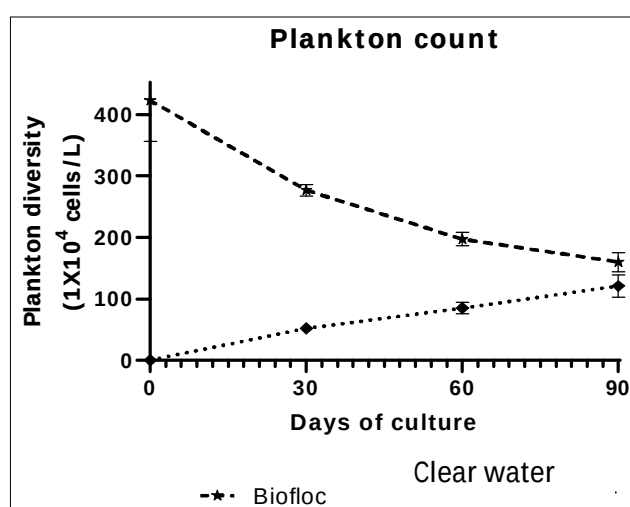


Fig 1: Plankton count in biofloc and clear water systems in lined pond (Mean $\pm$ SEM, n=2).

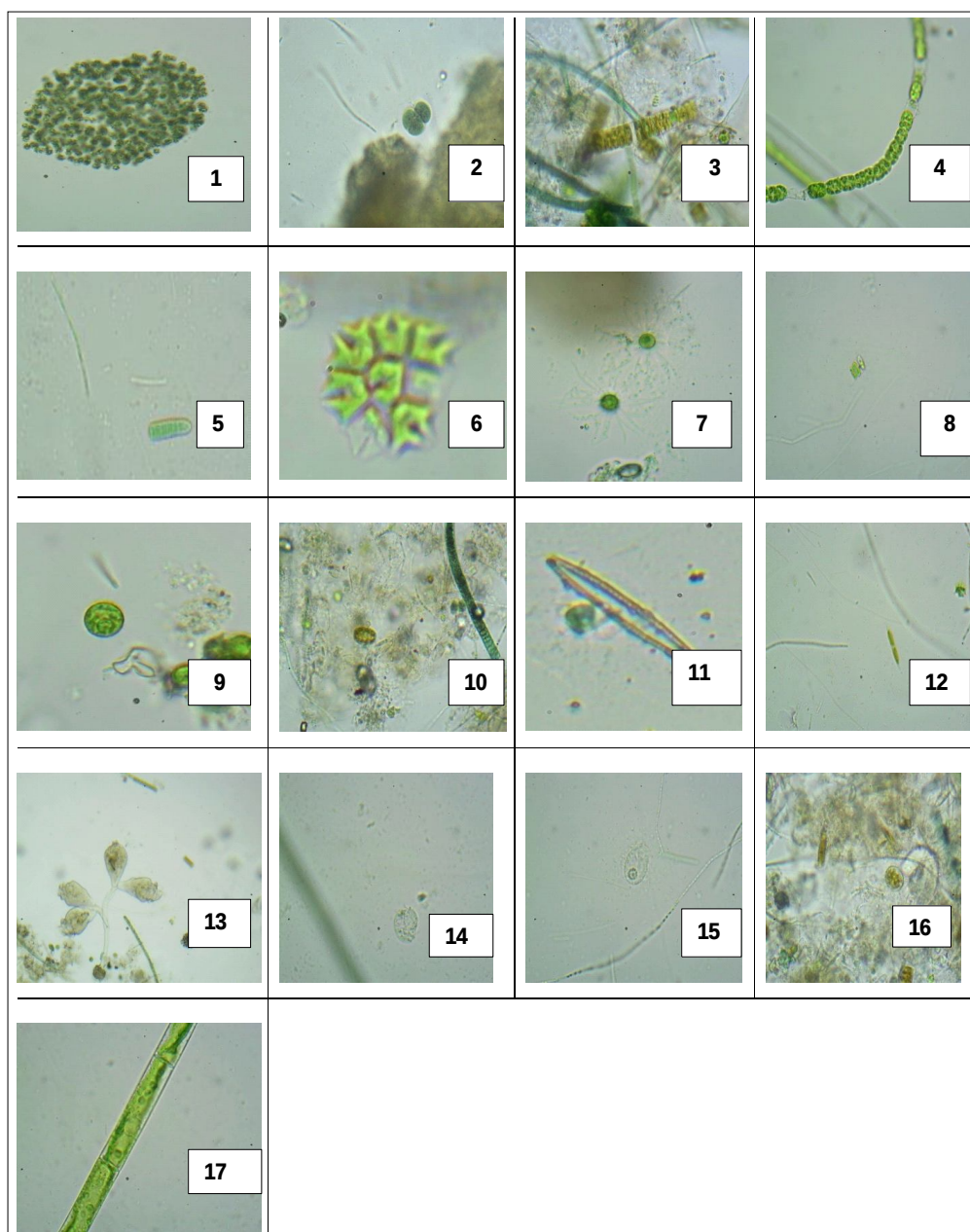
Table 1: Plankton diversity observed in polyculture of *Penaeus vannamei* and GIF tilapia using biofloc system in lined pond.

Class	Order	Family	Genus	No.
<i>Cyanophyceae</i>	Chroococcales	Microcystaceae	<i>Microcystis</i>	1.
		Chroococcaceae	<i>Chroococcus</i>	2.
	Nostocales	Rivulariaceae	<i>Calothrix</i>	3.
		Nostocaceae	<i>Anabena</i>	4.
<i>Chlorophyceae</i>	Oscillatoriales	Oscillatoriaceae	<i>Oscillatoria</i>	5.
	Sphaeropleales	Hydrodictyaceae	<i>Pediastrum</i>	6.
		Neochloridaceae	<i>Golenikinia</i>	7.
		Scenedesmaceae	<i>Scenedesmus</i>	8.
<i>Bacillariophyceae</i>	Chlorellales	Chlorellaceae	<i>Chlorella</i>	9.
	Thalassiosirales	Stephanodiscaceae	<i>Cyclotella</i>	10.
		Fragilariaceae	<i>Synedra</i>	11.
	Naviculales	Naviculaceae	<i>Navicula</i>	12.
<i>Chrysophyceae</i>	Chromulinales	Dinobryaceae	<i>Dinobryon</i>	13.
<i>Euglenoidea</i>	Euglenida	Phacaceae	<i>Phacus</i>	14.
<i>Oligohymenophorea</i>	Peniculida	Parameciidae	<i>Paramecium</i>	15.
<i>Trebouxioiphycea</i>	Chlorellales	Oocystaceae	<i>Oocystis</i>	16.
<i>Zygnematophyceae</i>	Desmidiaceae	Gonatozygaceae	<i>Gonatozygon</i>	17.

Table 2: Characteristics of biofloc and clear water system in polyculture model in lined pond.

Floc parameters	Biofloc	Clear water	p-value
Floc volume (ml/L)	46.50±3.50	ND	-
Floc concentration (g/L)	8.85±1.15	ND	-
Floc density (mg/cm ³)	0.19±0.10	ND	-
Floc volume index (ml/g)	5.29±0.29	ND	-
Floc density index (g/100 ml)	1.90±0.10	ND	-
Porosity (%)	95.35±0.35	ND	-
Total solids (mg/L)	1167.50±78.50 ^a	158.50±5.50 ^b	0.05
Total dissolved solids (mg/L)	434.05±62.90 ^a	76.00±4.04 ^b	0.01
Total suspended solids (mg/L)	733.45±108.22 ^a	82.50±0.87 ^b	0.01

Values expressed in mean ± SEM of two samples at the end of the experiment.

**Fig 2:** Plankton diversity observed in the biofloc system under light binocular microscope with magnification of 40 X.

2014 and Martins *et al.*, 2020. Interestingly, FV, FC, FD, FVI, FDI and porosity were matching with the earlier findings of Yuvarajan, 2021 in biofloc based tilapia culture system in lined pond.

Digestive enzyme analysis

At the end of the experimental trial, the digestive enzyme levels indicated significantly higher activity ($p < 0.05$) in shrimp and GIF tilapia under biofloc based system compared to clear water system (Table 3). Biofloc may consist of additional enzymes and stimulate the secretion of digestive enzymes in the gut and promotes the breakdown of nutrients into simple molecules, which are further transformed as body building blocks (Dong *et al.*, 2018). In present study, significantly higher protease, lipase and amylase enzyme activities of 32%, 28% and 25%, respectively, were recorded in *P. vannamei* gut reared in biofloc treatment lined pond compared to clear water treatment lined pond. Similarly, 56%, 79% and 57% of significantly higher protease, lipase and amylase enzyme activities, respectively, were recorded in gut samples of GIF tilapia reared in biofloc treatment of lined pond. The increased digestive enzyme activity in shrimp and GIF tilapia could be due to microbial enzymes in biofloc that helps in the breakdown of nutritional ingredients of protein, carbohydrates and others into smaller units in the animal gut. The results are in line with previous findings of *P. vannamei* (Xu and Pan, 2012), *O. niloticus* (Long *et al.*, 2015), when reared under biofloc system had increased digestive enzyme activity. Moreover, development, consumption and regeneration of biofloc results in recycling and reutilization of feed and eventually increases feed utilization efficiency (Hargreaves, 2006).

Immune parameters

In *Penaeus vannamei*, total haemocyte count, prophenol oxidase and catalase activity was significantly higher

($p < 0.05$) in biofloc system compared to clear water system (Table 4). The present study found 26%, 33% and 41% of increased Prophenoloxidase activity, total haemocyte count and catalase activity were observed in biofloc treatment compared to clean water treatment, which may be due to unidentified microbial components in biofloc. Similarly, increased total haemocyte count and prophenol oxidase activity of 40% and 21%, respectively was reported in *L. vannamei* (Xu and Pan, 2013), *P. monodon* (Kumar *et al.*, 2017) in biofloc based systems. On the other hand, Ju *et al.*, 2008 reported that shrimp reared in biofloc has increased level of catalase activity. Increased level of immune responses in shrimp may be due to presence of beneficial bacteria in the biofloc which might improve their colonization in the gut leading to better immune mechanism.

Hematological profiles

Biofloc had apparent effect on RBC, WBC, hemoglobin, hematocrit and MCV indicating that the biofloc system had positive effect on the physical condition of GIF tilapia. Albumin, Globulin, total protein and total cholesterol were significantly differed in between the treatments (Table 5). Similar to the present study, increased hemoglobin and hematocrit was observed in *O. niloticus* (Mansour and Esteban, 2017) which might be due to assimilation of dietary bioactive compounds from the biofloc and then excreted an immune-stimulating effect of the fish. The hematology profile of the present study was in the acceptable limit of the teleost fish (Satheeshkumar *et al.*, 2012). On the other hand, no significant variation in hematology profile of Nile tilapia reared in biofloc (Mabroke *et al.*, 2018). The biochemical parameter of the blood serum was significantly differed among the treatments and similar results were observed in *O. niloticus* in biofloc system (Martins *et al.*, 2020). Increased level of hemato-biochemical values were observed in biofloc treatment may

Table 3: Digestive enzyme activity of *P. vannamei* and GIF tilapia reared using lined pond systems maintained with biofloc and clear water in polyculture model.

Digestive enzymes (U/mg protein/min)	<i>Penaeus vannamei</i>			GIFT		
	Biofloc	Clear water	p-value	Biofloc	Clear water	p-value
Protease	7.98±0.36 ^a	5.39±0.32 ^b	0.007	3.62±0.16 ^a	1.60±0.05 ^b	0.000
Lipase	3.43±0.20 ^a	2.46±0.09 ^b	0.002	8.65±0.20 ^a	1.81±0.08 ^b	0.000
Amylase	1.35±0.10 ^a	1.01±0.08 ^b	0.002	3.25±0.17 ^a	1.41±0.07 ^b	0.000

For each species, values in the same row with different superscripts are significantly different at $p < 0.05$.

Table 4: Immune responses of *Penaeus vannamei* reared in biofloc and clear water in Lined pond (Mean ± SEM, n=8).

	Biofloc	Clear water	p-value
Prophenol oxidase (μmol.min ⁻¹ .ml ⁻¹)	71.25±1.28 ^a	52.38±0.75 ^b	0.000
Total haemocyte count (X 10 ⁶ cells/ml)	4.75±.12 ^a	3.20±0.24 ^b	0.001
Catalase activity (Units/ml)	3.56±0.06 ^a	2.10±0.03 ^b	0.000

For each species, values in the same row with different superscripts are significantly different at $p < 0.05$.

due to increase in amino acid intake and thus modifying the elevated metabolic parameters (Teodósio *et al.*, 2020).

Histology

The histological observations of gut of shrimp (Fig 3b) exposed to clear water system has shown deformities such

as autolytic changes. 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. On the other side, no abnormalities were observed in the gut of GIF tilapia in both treatments (Fig 3a). Consistent with present study, Nile tilapia fed with biofloc meal did not cause any

Table 5: Hematological and biochemical parameters of GIFT reared in biofloc and clear water in Lined pond (Mean $\pm$ SEM, n=8).

	Biofloc	Clear water	p-value
Hematological parameters			
RBC (million/ cu mm)	1.95 $\pm$ 0.05 ^a	1.40 $\pm$ 0.07 ^b	0.001
WBC (1000/cu mm)	49.65 $\pm$ 1.56 ^a	41.85 $\pm$ 0.77 ^b	0.008
Hb (g/dl)	9.21 $\pm$ 0.15 ^a	7.36 $\pm$ 0.15 ^b	0.000
Ht (%)	33.42 $\pm$ 0.89 ^a	22.15 $\pm$ 1.47 ^b	0.000
MCV (fl)	172.85 $\pm$ 8.80 ^a	158.21 $\pm$ 7.36 ^b	0.001
MCH (pg)	47.35 $\pm$ 0.88	53.29 $\pm$ 1.94	0.070
MCHC (g/dl)	27.74 $\pm$ 1.04 ^b	34.24 $\pm$ 2.16 ^a	0.004
Biochemical parameters			
Albumin (g/dl)	1.18 $\pm$ 0.04 ^a	1.01 $\pm$ 0.02 ^b	0.000
Globulin (g/dl)	4.39 $\pm$ 0.16 ^a	2.71 $\pm$ 0.04 ^b	0.000
Total protein (g/dl)	5.58 $\pm$ 0.14 ^a	3.73 $\pm$ 0.06 ^b	0.000
A/G	0.35 $\pm$ 0.02	0.38 $\pm$ 0.02	0.084
Total cholesterol (g/dl)	9.32 $\pm$ 0.41 ^a	4.29 $\pm$ 0.31 ^b	0.000

For each species, values in the same row with different superscripts are significantly different at $p < 0.05$.

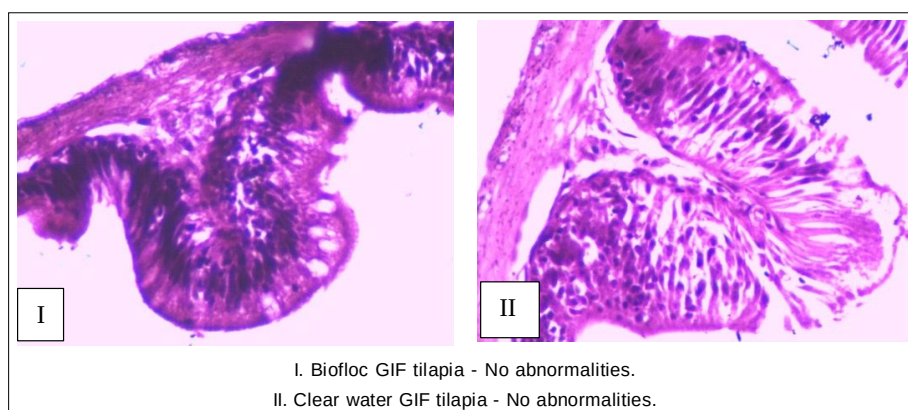


Fig 3a: Micrographs of gut of GIF tilapia.

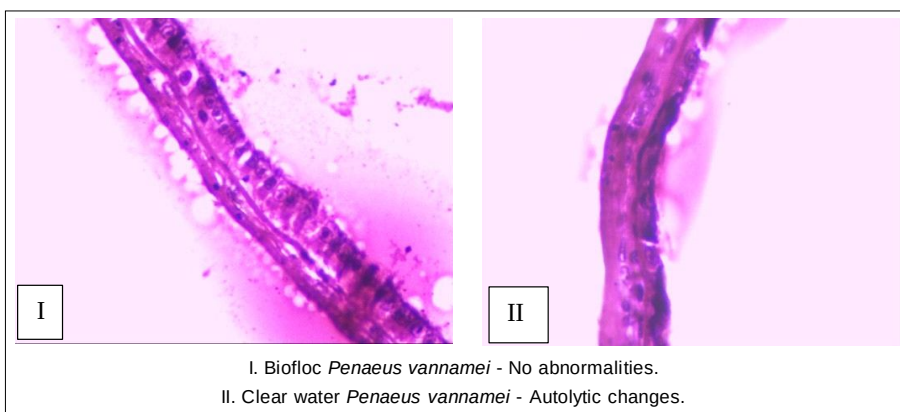


Fig 3b: Micrographs of gut of *Penaeus vannamei*.

damage to the gut (Hersi *et al.*, 2023). The study demonstrate that raising of GIF tilapia and shrimp in biofloc had no abnormalities in gut of GIF tilapia and *P. vannamei* and may consider as biofloc did not affect the normal physiological activity of GIF tilapia and shrimp.

CONCLUSION

This study reveals that the polyculture biofloc based system is more sustainable for *Penaeus vannamei* and GIF tilapia for better immune status with enhanced water quality. This would help in expanding zero-water exchange culture system to achieve maximum production. Further, polyculture model has synergistic effect on environment safety and sustainability.

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Data availability statement

The data that support the findings of this study are available within the article.

Conflict of interest

The authors declare that they have no conflict of interest.

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