Pakistan J. Zool., pp 1-8, 2024. DOI: https://dx.doi.org/10.17582/journal.pjz/20240202051952

Influenceof Biofloc on Haemato-Biochemical and Immunological Responses in GIF Tilapia and *Penaeus vannamei* in Polyculture Model

Joshna Malreddy[1]*, B. Ahilan[2], Cheryl Antony[3], K. Ravaneswaran[4], P. Chidambaram[5], A. Uma[6] and P. Ruby[1]

[1]Department of Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Dr. M.G.R Fisheries College and Research Institute, Ponneri – 601 204, Tamil Nadu, India.
[2]Dean, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Fisheries College and Research Institute, Thoothukudi – 628 008, Tamil Nadu, India.
[3]Director of Research, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam – 611 002, Tamil Nadu, India.
[4]Director, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Directorate of Incubation and Vocational Training in Aquaculture (DIVA), ECR Muttukadu, Chennai – 603 112, Tamil Nadu, India.
[5]Controller of Examination, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam – 611 002, Tamil Nadu, India.
[6]Department of Aquatic Animal Health Management, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Dr. M.G.R Fisheries College and Research Institute, Ponneri – 601204, Tamil Nadu, India.

Article Information
Received 02 February 2024
Revised 05 March 2024
Accepted 17 March 2024
Available online 16 July 2024 (early access)

Authors' Contribution
MJ: Conducted the trial and analysis, analyzed the data and drafted the manuscript. BA, KR, PC: Conceptualized and designed the study and corrected the manuscript. CA: Carried out the digestive enzyme analysis and corrected the manuscript. AU: conceptualized the design. PR: carried out the statistical analysis part

Key words
Biofloc, GIF tilapia, Hematology, Immunology, *Penaeus vannamei*, Polyculture

ABSTRACT

A 90-day, culture trial was conducted to investigate the effect of biofloc on the haemato-biochemical responses in Genetically Improved Farmed Tilapia and immunological responses in *Penaeus vannamei*. Six tanks were used following the completely randomized design to discover the haemato-biochemical parameters and immunological responses using biofloc and clear water culture systems. The tank was stocked with 60 shrimp/m^3 of *P. vannamei* and 5 fish/m^3 of GIF tilapia. Biofloc was developed using the soyahull pellet powder, with C: N of 15:1. The study found significantly improved immunological responses of prophenol oxidase activity (42.25±0.59 µmol.min^{-1}.ml^{-1}), total haemocyte count (2.17±0.06 X10^6 cells/ml) and catalase activity (2.19±0.01 Units/ml) in biofloc cultured *P. vannamei*. Higher values of RBC (22.28±0.05 million/cu mm), hemoglobin (8.70±0.09 g/dl), hematocrit (32.36±0.19%), albumin (2.46±0.04 g/dl), total protein (5.98±0.02 g/dl) and total cholestrol (9.00±0.14 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.

INTRODUCTION

In aquaculture, biofloc technology is seen as a new blue revolution since it is eco-friendly. The manufacturing method known as biofloc technology not only maintains water quality for intensive animal raising while using the least amount of water possible, but it also supplies an additional food supply in the form of biofloc (Burford *et al*., 2004). However, there are certain disadvantages to biofloc technology, including resource waste and the absorption of dissolved compounds and organic matter buildup in the culture unit (Ebeling *et al*., 2006). By using polyculture, the aforementioned issue can be mitigated.

Farmers in traditional polyculture stocked five to eight species and were frequently disappointed by the extended culture time and low yield (Rahman *et al*., 2008).Farmers prefer to employ fewer species these days. There have been extensive, semi-intensive, and intensive systems used for shrimp-tilapia polyculture. To reuse shrimp feed wastes and enhance water quality, tilapias were cultivated as the secondary species in the majority of shrimp-tilapia

* Corresponding author: rubyfcri@gmail.com
0030-9923/2024/0001-0001 $ 9.00/0

polyculture systems, whereas shrimp were cultured as the primary species (Saelee, 2002). The food provided is used more effectively in polyculture than in monoculture when the chosen species inhabit distinct niches and have complementary but diverse eating patterns (Yuan *et al*., 2010). Therefore, by permitting a greater variety of feeding behaviours, stocking two complimentary species can raise the maximum standing crop.

Penaeid shrimp farmers need to maintain water quality within a certain range in order to raise healthy, fast-growing shrimp (Chen, 1993). Typically, decapod crustaceans are opportunistic omnivores that obtain their sustenance from benthos or animals related to submerged and coastal plants in aquatic environments (Marte, 1989). Shrimp producers might therefore save money on artificial food if they manage water quality to ensure that there is an adequate supply of natural food (Laokiatsophon *et al*., 2006). The ability of tilapia to efficiently convert organic wastes into high-quality protein, their general hardiness, adaptation to both fresh and brackish water environments, resistance to disease, and ranking second only to carps among the world's most important farmed fish (Suresh and Lin, 1992). As a species in shrimp polyculture, tilapia can filter sediment and result in a top-down effect that raises the rates of the nitrogenous and phosphorus cycles, decreases zooplankton abundance, and increases phytoplankton biomass (Yuan *et al*., 1993).

The direct and indirect effects of eutrophication and anoxic sediment conditions brought on by shrimp farm effluents are other important concerns. One significant consequence has been the increase of pathogenic and opportunistic vibrios. Using fish culture, pond water appears to have lower incidence of bacterial infections from luminous vibriosis in prawn ponds (Yap, 2001). But because animals are more likely to get illnesses than people, maintaining the health of the animals is also crucial to optimizing polyculture (Lin *et al*., 2018). Therefore, the study's objective was to assess, using immunological and haemato-biochemical studies, the health condition of *Penaeus vannamei* raised in biofloc polyculture with GIF tilapia.

MATERIALS AND METHODS

Experimental setup

Experimental trial was carried out for 90 days (June to August) in tanks (6 no's) of 33 tons capacity at TNJFU-Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu, Chennai, India. The experiment consists of two treatments, biofloc and clean water system, and replicated in completely randomized design (CRD). Prior to the stocking, biofloc was developed using soya hull pellet powder (carbon source) in biofloc treatment and maintained as per Avnimelech (1999) with minor modifications. Aeration was provided continuously to keep the floc in suspension. For every 1 g of total ammonia nitrogen (TAN), 15 g of carbon source (soyahull pellet powder) were added. The shrimp (*Penaeus vannamei*) and tilapia strain (GIF tilapia) were polycultured in the tank and GIF tilapia was restricted in the hapa. A mean body weight of shrimp 0.93±0.09 g (60 shrimp/m^3) and 0.42±0.01 g (5 fish/m^3) of uniformed sized were distributed randomly, with replicates per treatment. The shrimp and fish were fed with commercial diets of 36% and 24% of crude protein until apparent satiation four times per day (06.00, 10.00, 14.00 and 18.00 h).

Plankton count and biodiversity analysis

Water samples were collected with the help of plankton net (100µm mesh), and the microorganisms were counted on fortnight basis using Sedgewick-Rafter cell and viewed under a light binocular microscope with magnification of 40X in biofloc treatment (Lawrence and Mayo Microscopes, Tamilnadu). Plankton were identified using standard references (Patterson and Hedley, 1996) up to the genus level.

Floc characteristics

Floc volume was analyzed at weekly interval in biofloc treatment (Avnimelech and Kochba, 2009). According to Mohlman (1934), floc concentration and floc volume index (FVI) were calculated. Floc density, floc density index (FDI) and porosity were determined by following the methods of Muller *et al*. (1967) and WHO International Reference Centre (1978), respectively. Total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) were analyzed by following standard protocols (APHA, 2005).

Immunological parameters in shrimp

At the end of the experiment, shrimp (10/treatment) were collected and anaesthetized using clove oil (Sigma-Aldrich), at 10 ppm concentration. Around 50 µl hemolymph was collected from the ventral sinus cavity of shrimp using 1 ml syringe fitted with 22-gauge needle. The collected hemolymph was used to analyze the total haemocyte count using the Olympus light microscope (CX21i, LED) at 400X magnification (Raja *et al*., 2012). Prophenol oxidase (ProPO) activity and catalase activity were determined by following the methods of Gollas-Galvan *et al*. (1999) and Takahara *et al*. (1960), respectively (Table II).

Haemato- biochemical assay

At the end of the trial, fish (10/treatment) were collected and anaesthetized using clove oil (10 ppm) to analyze the hematological and serum biochemical parameters using 1 ml syringe at caudal vein puncture. The blood samples were collected and expelled into heparinized and non-heparinized tubes and kept on ice, immediately. According to Stoskopf (2015), total red blood cells (RBCs) and white blood cells (WBCs) counts were counted using Neubauer hemocytometer. Hemoglobin (Hb) concentration and hematocrit (Ht) value were determined by following the methods of cyanmethemoglobin (Drabkin, 1946) and microhematocrit method (Nelson and Morris, 1989), respectively. MCH, MCV and MCHC of erythrocyte indices were calculated, according to Wintrobe (1934). Non-heparinized tubes were kept in slant position for 2 h at 4°C. Then it was centrifuged at 3500 g for 25 min in a refrigerated centrifuge at 4°C (Eppendorf Centrifuge 5804R). The supernatant was collected and stored as serum. The biuret method (Reinhold, 1953) was used to analyze the total serum protein and bromocresol green binding method (Doumas *et al*., 1971) was used to analyze the albumin content of the serum. The globulin value and A/G ratio were calculated using standard formulae. Serum cholestrol (CHO) levels were estimated using Parekh and Jung (1970).

Statistical analysis

All of the data was provided as the average of three replicates with standard error of the mean (SEM). SPSS version 20.0 for windows (SPSS Inc., Chicago, IL, USA) was used statistical analysis on the data, which included student's t-test. Floc parameters, plankton count, immune responses and haemato-biochemical profile was performed using SPSS software version 20.0 at 5% level of significance.

RESULTS AND DISCUSSION

Plankton count and biodiversity analysis

Plankton count was significantly decreasing in biofloc treatment from 0th day to 90th day (Fig. 1). The plankton diversity recorded in the biofloc treatment was dominated from the class of Chlorophyceae (22%) and Cyanophyceae (22%) (Table I, Fig. 2).

Generally, biofloc technology comprehends more bacterial communities, according to the carbon source, C/N ratio and floc characteristics compared to clean water aquaculture system (Qiao *et al*., 2020). Till the end of the experiment, Plankton count was decreasing, which might be due to formation of floc are held together in a loose matrix of mucus (Ahmad *et al*., 2017). Similar results were obtained by Yuvarajan (2021) in biofloc based GIF tilapia culture, used distillery spent wash (DSW) as carbon source and found 350x10^4 cells/L to 180x10^4 plankton cells/L in biofloc treatment. Copious research in the biofloc systems have shown that biofloc technology endorses nitrogenous wastes in to functional microbial protein, in the presence of bacterial growth in the biofloc and acts as food source for other organisms (Padeniya *et al*., 2022). According to the Tipsrisukond *et al*. (2020), the choice of carbon source plays a crucial role in shaping the microbial composition and nutritional characteristics of the floc in the system. Predominant group of microorganisms in the present study were Class of *Chlorophyceae* and *Cyanophyceae*, each of 22% in the biofloc treatment. Similarly, 75% of the class Chlorophyceae was reported by the Ju *et al*. (2008) in jaggery used carbon source biofloc aquaculture system. The present study found lower number of microorganisms which might be due to different carbohydrate content, algal community and heterotrophic bacterial load in the biofloc culture (Choo and Caipang, 2015).

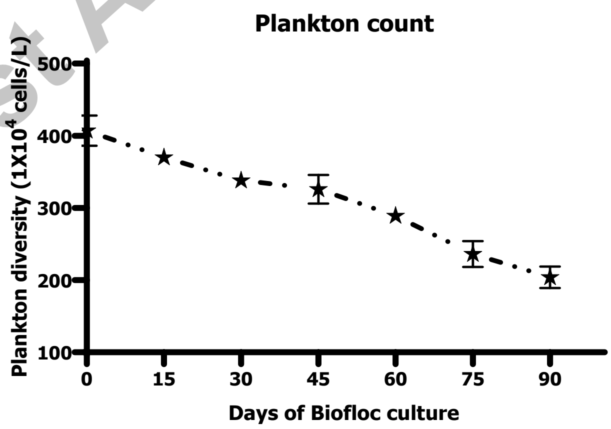

Fig. 1. Plankton count in biofloc system. Data represents mean ± standard error of mean (SEM) of two replications.

Floc characteristics

The mean±SEM of biofloc parameters were recorded at the end of the experiment, floc volume, floc concentration, floc density, floc volume index, floc density index and porosity were 15.50±0.50 ml/L, 2.85±0.05 g/L, 0.18±0.01 mg/cm^3, 5.44±0.27 ml/g, 1.84±0.09 g/100ml and 98.45±0.05%, respectively. Whereas, total solids, total suspended solids and total dissolved solids were 336.53±14.78 mg/L, 292.43±13.38 mg/L and 44.10±1.40 mg/L, respectively.

To understand the structure and composition of biofloc - associated microorganisms, porosity, floc volume index and floc density index were determined. The desirable range of floc volume for finfish and shellfish culture was 10 to 25 ml/L (Hargreaves, 2013) and similar to our present study, Haridas *et al.* (2021) and Menaga *et al.* (2019) reported 15 ml/L and 3 g/L of floc volume and floc concentration, respectively were desirable for the intake of shrimp and GIF tilapia. Density of floc may be indorsed with the density of bacteria in the floc and was in the range of 0.18 mg/cm^3, which showed the higher settleability of floc and also shows inverse relationship with floc volume index (Barbusinki and Koscielniak, 1995). Good settling and compaction characteristic were determined through floc with low floc volume index. Yuvarajan *et al.* (2018) observed a FDI, TS, TSS and TDS of 1.43 ml/g, 1340 mg/L, 589 mg/L and 751 mg/L which are higher than the present study, which may be due to higher floc volume and settleability. In general, type of carbon source and percentage of carbohydrate in the carbon source, can significantly influence the structure, composition (Li *et al.*, 2018) and floc characteristics (Du *et al.*, 2018).

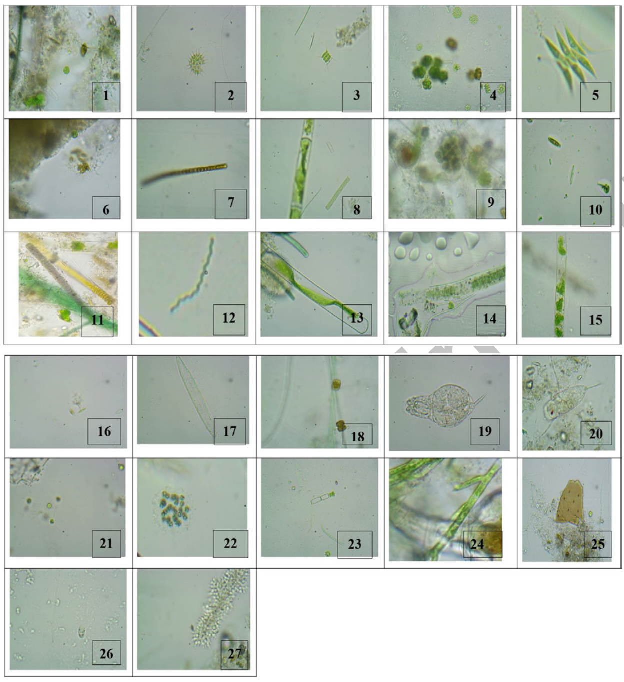

Fig. 2. Plankton diversity observed in the biofloc system under light binocular microscope with magnification of 40x.

Immunological parameters in shrimp

The present study found 12%, 21% and 20% of significantly higher level of 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. Prophenol oxidase is an enzyme in shrimp which mediated the immune responses to activate components leading to melanin synthesis (Amparyup *et al.*, 2013). Similar to the present study, Chiu *et al.* (2007) and Li *et al.* (2009) have reported that better immune responses can be elicited by shrimp when reared in biofloc system. Shrimp reared in biofloc treatment had a significantly higher number of haemocyte; these findings show the role of biofloc in activating the immune responses and were

Table I. Plankton diversity observed in *Penaeus vannamei* in polyculture with GIF tilapia using biofloc culture treatment.

Class: Order	Family	Genus
Chlorophyceae		
Sphaeropleales	Neochloridaceae	*Golenikinia*
	Hydrodictyaceae	*Pediastrum*
	Scenedesmaceae	*Scenedesmus*
		Coelastrum
	Selenastraceae	*Ankistrodesmus*
Chlorellales	Chlorellaceae	*Chlorella*
Cyanophyceae		
Nostocales	Nostocaceae	*Anabena*
	Rivulariaceae	*Calothrix*
Chroococcales	Chroococcaceae	*Chroococcus*
	Microcystaceae	*Microcystis*
Oscillatoriales	Oscillatoriaceae	*Oscillatoria*
Spirulinales	Spirulinaceae	*Spirulina*
Zygnematophyceae		
Desmidiales	Gonatozygaceae	*Gonatozygon*
	Desmidiaceae	*Hyalotheca*
Zygnematales	Zygnemataceae	*Zygnema*
Bacillariophyceae		
Naviculales	Naviculaceae	*Navicula*
Fragilariales	Fragilariaceae	*Synedra*
Thalassiosirales	Stephanodiscaceae	*Cyclotella*
Monogononta		
Ploima	Brachionidae	*Brachionus*
	Trichocercidae	*Trichocerca*
Trebouxiophyceae		
Chlorellales	Chlorellaceae	*Micractinium*
	Oocystaceae	*Oocystis*
Ulvophyceae		
Ulotrichales	Ulotrichaceae	*Ulothrix*
Cladophorales	Cladophoraceae	*Rhizoclonium*
Chrysophyceae		
Chromulinales	Dinobryaceae	*Dinobryon*
Oligohymenophorea		
Peniculida	Parameciidae	*Paramecium*
-	-	*Gastrotricha*

similar to the results of Ferreira *et al*. (2015). Xu and Pan (2013) also reported that improved haemocyte count in *L. vannamei* in biofloc based system. Contrast to the study, Xu and Pan (2014) and De Souza *et al*. (2014) reported that total haemocyte count did not vary significantly in shrimp between biofloc and control group. On the other hand, catalase protect the cells from oxidative damage and increased level of catalase activity has been noticed after rearing of shrimp in biofloc (Li *et al*., 2009) and similar results were observed in shrimp were reported by Ju *et al*. (2008). It is possible that presence of beneficial bacteria in the ingested biofloc might improve their colonization in the gastrointestinal tract leading to better immune mechanism (Xu and Pan, 2013).

Table II. Immune responses of *Penaeus vannamei* reared in biofloc and clean water in raceway system.

	Biofloc	Clean water	p value
Prophenol oxidase ($\mu mol.min^{-1}.ml^{-1}$)	42.25±0.59[a]	37.00±0.33[b]	0.000
Total haemocyte count (X 10^6 cells/ml)	2.17±0.06[a]	1.72±0.02[b]	0.001
Catalase activity (Units/ml)	2.19±0.01[a]	1.75±0.03[b]	0.000

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. Hematological and biochemical parameters of GIF tilapia reared in biofloc and clean water in raceway system.

	Biofloc	Clean water	p-value
Hematologicalparameters			
RBC (million/ cu mm)	2.28±0.05[a]	1.60±0.02[b]	0.000
WBC (1000/cu mm)	29.19±0.31[b]	33.44±0.22[a]	0.000
Hb (g/dl)	8.70±0.09[a]	7.58±0.01[b]	0.000
Ht (%)	32.36±0.19[a]	23.93±0.06[b]	0.000
MCV (fl)	142.90±0.17[b]	149.54±1.10[a]	0.000
MCH (pg)	38.75±0.19[b]	47.44±0.14[a]	0.000
MCHC (g/dl)	27.19±0.25[b]	31.77±0.12[a]	0.000
Biochemical parameters			
Albumin (g/dl)	2.46±0.04[a]	2.15±0.01[b]	0.000
Globulin (g/dl)	3.45±0.04[b]	3.52±0.03[a]	0.000
Total protein (g/dl)	5.98±0.02[a]	5.95±0.02[b]	0.000
A/G	0.73±0.01[a]	0.61±0.00[b]	0.000
Total cholestrol (g/dl)	9.00±0.14[a]	8.01±0.22[b]	0.000

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

Haemato- biochemical assay

RBC, WBC, Hb, Ht, MCV, MCH and MCHC levels were significantly differed among the treatments (Table III). Significantly higher values of RBC, Hb and Ht were 2.28±0.05 million/cu mm, 8.70±0.09 g/dl and 32.36±0.19 %, respectively in biofloc treatment.

The hematological values in the present study were within the acceptable limits of teleost fish (Satheesh Kumar *et al*., 2012). Significantly higher values of RBC, hemoglobin and hematocrit was observed in the GIF tilapia reared in biofloc treatment compared to clean water treatment, which might be due to assimilation of dietary bioactive compounds from the biofloc and then excreted an immune-stimulating effect of the fish and the interrelationship of RBC and anemia were observed in invertebrates, including fishes. Similar to present findings, Mansour and Esteban (2017), has reported that *O. niloticus* reared in biofloc culture has increased hemoglobin and hematocrit value. Contrast to our present study, no significant variation in the hematology profile of Nile tilapia reared in biofloc (Azim and Little, 2008; Mabroke, 2018). In the present study, albumin level in the biofloc treatment was significantly increased by 13%, which might be due to multifunctional of albumin plays a crucial role in the transportation of enzymes, vitamins and hormones and indicates the healthy functioning of the immune system (Punitha *et al*., 2008). Slightly higher levels of total protein in biofloc treatment may be due to depletion in the levels of liver glycogen (Ojolick *et al*., 1995). Increased level of hemato-biochemical values was observed in biofloc treatment as GIF tilapia meet part of their protein requirement from microorganisms present in the system and from the feed (Durigon *et al*., 2020) and the diet may cause an increase in amino acid intake, modifying the elevated metabolic parameters (Teodósio *et al*., 2020).

CONCLUSION

Biofloc technology is an environmentally benign and trash is managed in the tank, because waste is a protein source, the desired yield of *P. vannamei* and GIF tilapia can be achieved with modest amount of feed. As a result, polyculture with biofloc technology is projected as improved physiological responses and beneficial technology.

ACKNOWLEDGEMENT

The authors sincerely thank the SERB, CII and Murugappa Fish Feeds, India and Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India for grants and facilities provided.

Funding

Prime Minister's Fellowship for Doctoral Research, a joint initiative of Confederation of Indian Industry (CII) and Science and Engineering Research Board (SERB) and Murugappa Fish Feeds for sustainable and maximum profit from unit area (17th Batch).

IRB approval

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

Ethics statement

The study was performed in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU, 2021), Nagapattinam, Tamil Nadu, India, has also approved the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ahmad, I., Babitha, R.A.M., Verma, A.K. and Maqsood, M., 2017. Biofloc technology: An emerging avenue in aquatic animal healthcare and nutrition. *Aquacult. Int.*, **25**: 1215-1226. https://doi.org/10.1007/s10499-016-0108-8

Amparyup, P., Charoensapsri, W. and Tassanakajon, A., 2013. Prophenoloxidase system and its role in shrimp immune responses against major pathogens. *Fish Shellf. Immunol.*, **34**: 990-1001. https://doi.org/10.1016/j.fsi.2012.08.019

Anand, T., Srinivasan, A., Padmavathy, P., Jawahar, P. and Sampathkumar, J.S., 2022. Nursery rearing of *Penaeus vannamei* in biofloc systems with different salinities and organic carbon sources. *Indian J. Anim. Res.*, **56**: 392-399. https://doi.org/10.18805/IJAR.B-4753

APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Environment Federation), 2005. *Standard methods for examination of water and waste water,* 20th edition, Port city press, Baltimore, Maryland, USA.

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, **176**: 227-235. https://doi.org/10.1016/S0044-8486(99)00085-X

Avnimelech, Y. and Kochba, M., 2009. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using ^{15}N tracing. *Aquaculture*, **287**: 163-168. https://doi.org/10.1016/j.aquaculture.2008.10.009

Azim, M.E. and Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **283**: 29-35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Bani, A. and Haghi-Vayghan, A., 2011. Temporal variations in haematological and biochemical indices of the *Caspian kutum*, Rutilusfrisiikutum. *Ichthyol. Res.*, **58**: 126-133. https://doi.org/10.1007/s10228-010-0199-6

Barbusiński, K. and Kościelniak, H., 1995. Influence of substrate loading intensity on floc size in activated sludge process. *Water Res.*, **29**: 1703-1710. https://doi.org/10.1016/0043-1354(94)00326-3

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture*, **232**: 525-537. https://doi.org/10.1016/S0044-8486(03)00541-6

Chiu, C.H., Guu, Y.K., Liu, C.H., Pan, T.M. and Cheng, W., 2007. Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum*. *Fish Shellf. Immunol.*, **23**: 364-377. https://doi.org/10.1016/j.fsi.2006.11.010

Choo, H.X. and Caipang, C.M.A., 2015. Biofloc technology (BFT) and its application towards improved production in freshwater tilapia culture. *Aquac. Aquarium, Conserv. Legislat.*, **8**: 362-366.

De Souza, D.M., Suita, S.M., Romano, L.A., Wasielesky Jr, W. and Ballester, E.L.C., 2014. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system. *Aquacult. Res.*, **45**: 270-277. https://doi.org/10.1111/j.1365-2109.2012.03223.x

Doumas, B.T., Watson, W.A. and Biggs, H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta*, **31**: 87-96. https://doi.org/10.1016/0009-8981(71)90365-2

Drabkin, D.L., 1946. Spectrophotometric studies: XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. *J. Biol. Chem.*, **164**: 703-723. https://doi.org/10.1016/S0021-9258(17)41272-5

Du, X., Almeida, D., Song, D., Zhao, Z., Luo, L., Li, J. and Xu, Q., 2018. Effects of organic carbon addition on water quality and phytoplankton assemblages in biofloc technology ponds. *Aquaculture*, **497**: 155-163. https://doi.org/10.1016/j.aquaculture.2018.07.058

Durigon, E.G., Lazzari, R., Uczay, J., de Alcântara Lopes, D.L., Jerônimo, G.T., Sgnaulin, T. and Emerenciano, M.G.C., 2020. Biofloc technology (BFT): Adjusting the levels of digestible protein and digestible energy in diets of Nile tilapia juveniles raised in brackish water. *Aquacult. Fish*., **5**: 42-51. https://doi.org/10.1016/j.aaf.2019.07.001

Ebeling, J.M., Timmons, M.B. and Bisogni, J.J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. *Aquaculture*, **257**: 346-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Ferreira, G.S., Bolivar, N.C., Pereira, S.A., Guertler, C., do Nascimento Vieira, F., Mouriño, J.L.P. and Seiffert, W.Q., 2015. Microbial biofloc as source of probiotic bacteria for the culture of *Litopenaeus vannamei*. *Aquaculture*, **448**: 273-279. https://doi.org/10.1016/j.aquaculture.2015.06.006

Gollas-Galván, T., Hernández-López, J. and Vargas-Albores, F., 1999. Prophenoloxidase from brown shrimp *(Penaeus californiensis*) hemocytes. *Comp. Biochem. Physiol. B: Biochem. mol. Biol*., **122**: 77-82. https://doi.org/10.1016/S0305-0491(98)10143-8

Gustilatov, M., Widanarni, W., Ekasari, J., Julyantoro, P.G.S. and Waturangi, D.E., 2023. Biofloc system supplemented by *Pseudoalteromonas piscicida* 1Ub protects the Pacific white shrimp *Penaeus vannamei* from *Vibrio parahaemolyticus* infection. *Aquacult. Fish*., https://doi.org/10.1016/j.aaf.2023.05.003

Hargreaves, J.A., 2013. *Biofloc production systems for aquaculture*. Stoneville, MS: Southern Regional Aquaculture Center. 4503: 1-11.

Haridas, H., Chadha, N.K., Sawant, P.B., Deo, A.D., Ande, M.P., Syamala, K. and Lingam, S.S., 2021. Different carbon sources influences the growth and digestive enzyme activity of grey mullet (*Mugil cephalus)* in biofloc based nursery rearing system. *J. environ. Biol*., **42**: 1249-1256.

Holanda, M., Ravagnan, E., Lara, G., Santana, G., Furtado, P., Cardozo, A. and Poersch, L.H., 2023. Integrated multitrophic culture of shrimp *Litopenaeus vannamei* and tilapia *Oreochromis niloticus* in biofloc system: A pilot scale study. *Front. Mar. Sci*., **10**: 1060846. https://doi.org/10.3389/fmars.2023.1060846

Hued, A.C. and de losÁngelesBistoni, M., 2002. Effects of water quality variations on fish communities in the Central Part of Argentina, South America. *Int. Vereinigung fürtheoretische und angewandte Limnologie: Verhandlungen*, **28**: 1476-1481. https://ui.adsabs.harvard.edu/link_gateway/2002SILP...28.1476H, https://doi.org/10.1080/03680770.2001.11902702

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., Kuo, W.C. and David, H.F., 2008. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquacult. Res*., **39**: 118-133. https://doi.org/10.1111/j.1365-2109.2007.01856.x

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

Laokiatsophon, P., Limsuwan, C., Teparhudee, W. and Chuchird, N., 2006. Study on the species composition and abundance of plankton, water quality and stomach contents of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) reared in low salinity conditions. *J. Fish. Environ*., **29**: 14-22.

Li, J., Liu, G., Li, C., Deng, Y., Tadda, M.A., Lan, L. and Liu, D., 2018. Effects of different solid carbon sources on water quality, biofloc quality and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture*, **495**: 919-931. https://doi.org/10.1016/j.aquaculture.2018.06.078

Li, J., Tan, B. and Mai, K., 2009. Dietary probiotic Bacillus OJ and is omaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp *(Litopenaeus vannamei*). *Aquaculture*, **291**: 35-40. https://doi.org/10.1016/j.aquaculture.2009.03.005

Lin, W., Li, L., Chen, J., Li, D., Hou, J., Guo, H. and Shen, J., 2018. Long-term crowding stress causes compromised nonspecific immunity and increases apoptosis of spleen in grass carp (*Ctenopharyngodonidella*). *Fish Shellf. Immunol*., **80**: 540-545. https://doi.org/10.1016/j.fsi.2018.06.050

Mabroke, R.S., 2018. Complexity of carbon sources and the impact on biofloc integrity and quality in tilapia (*Oreochromis niloticus*) tanks. *AACL Bioflux*, **11**. http://www.bioflux.com.ro/aacl

Mansour, A.T. and Esteban, M.Á., 2017. Effects of carbon sources and plant protein levels in a biofloc system on growth performance, and the immune and antioxidant status of Nile tilapia (*Oreochromis niloticus*). *Fish Shellf. Immunol*., **64**: 202-209. https://doi.org/10.1016/j.fsi.2017.03.025

Marte, C.L., 1980. The food and feeding habit of Penaeus monodon Fabricius collected from Makatoriver, Aklan, Philippines (DecapodaNatantia). *Crustaceana*, pp. 225-236. https://doi.org/10.1163/156854080X00139

Menaga, M., Felix, S., Charulatha, M., Gopalakannan, A. and Panigrahi, A., 2019. Effect of in-situ and ex-situ biofloc on immune response of genetically improved farmed Tilapia. *Fish Shellf. Immunol*., **92**: 698-705. https://doi.org/10.1016/j.fsi.2019.06.031

Mohlman, F.W., 1934. The sludge index. Sewage Works J.,119-122.

Mueller, J.A., Morand, J. and Boyle, W.C., 1967. Floc sizing techniques. *Appl. Microbiol*., **15**: 125-134. https://doi.org/10.1128/am.15.1.125-134.1967

Nelson, D.A. and Morris, M.W., 1989. Basic methodology: Hematology and coagulation, part IV. In: *Clinical diagnosis, management by laboratory methods seventeenth* (eds. D.A. Nelson and J.B. Henry). W.B. Saunder Company, Philadelphia, PA. pp. 578– 625.

Ojolick, E.J., Cusack, R., Benfey, T.J. and Kerr, S.R., 1995. Survival and growth of all-female diploid and triploid rainbow trout (*Oncorhynchus mykiss*) reared at chronic high temperature. *Aquaculture*, **131**: 177-187. https://doi.org/10.1016/0044-8486(94)00338-O

Padeniya, U., Davis, D.A., Wells, D.E. and Bruce, T.J., 2022. Microbial interactions, growth, and health of aquatic species in biofloc systems. *Water*, **14**: 4019. https://doi.org/10.3390/w14244019

Parekh, A.C. and Jung, D.H., 1970. Cholesterol determination with ferric acetate-uranium acetate and sulfuric acid-ferrous sulfate reagents. *Anal. Chem*., **42**: 1423-1427. https://doi.org/10.1021/ac60294a044

Patterson, D.J. and Hedley, S., 1996. *Free living freshwater Protozoa*. CRC Press. https://doi.org/10.1201/9781840765847

Punitha, S.M.J., Babu, M.M., Sivaram, V., Shankar, V.S., Dhas, S.A., Mahesh, T.C. and Citarasu, T., 2008. Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinephelustauvina juvenile* against *Vibrio harveyi* infection. *Aquacult. Int*., **16**: 511-523. https://doi.org/10.1007/s10499-007-9162-6

Qiao, G., Chen, P., Sun, Q., Zhang, M., Zhang, J., Li, Z., and Li, Q., 2020. Poly-β-hydroxybutyrate (PHB) in bioflocs alters intestinal microbial community structure, immune-related gene expression and early Cyprinid herpesvirus 2 replication in gibel carp (*Carassiusauratusgibelio*). *Fish Shellf. Immunol*., **97**: 72-82. https://doi.org/10.1016/j.fsi.2019.12.045

Rahman, M.M., Nagelkerke, L.A., Verdegem, M.C., Wahab, M.A. and Verreth, J.A., 2008. Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. *Aquaculture*, **275**: 108-115. https://doi.org/10.1016/j.aquaculture.2008.01.027

Raja, R.A., Kumar, S., Sundaray, J.K., De, D., Biswas, G. and Ghoshal, T.K., 2012. *Hematological parameters in relation to sex, morphometric characters and incidence of white spot syndrome virus in tiger shrimp,* Penaeus monodon *Fabricius, 1798 from Sunderban, West Bengal*.

Reinhold, J.G., 1953. Total protein, albumin, and globulin. *Stand. Methods Clin. Chem*., **1**: 88-97. https://doi.org/10.1016/B978-0-12-609101-4.50019-8

Ríos, L.D.M., Monteagudo, E.B., Barrios, Y.C., González, L.L., Vaillant, Y.D.L.C.V., Bossier, P. and Arenal, A., 2023. Biofloc technology and immune response of penaeid shrimp: A meta-analysis and meta-regression. *Fish Shellf. Immunol*., pp. 108805 https://doi.org/10.1016/j.fsi.2023.108805.

Saelee, W., 2002. *Shrimp-tilapia polyculture in low salinity water*. Unpublished M. Sc. thesis, Asian Institute of Technology, Thailand.

Satheeshkumar, P., Ananthan, G., Kumar, D.S. and Jagadeesan, L., 2012. Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. *Comp. clin. Pathol*., **21**: 1187-1191. https://doi.org/10.1007/s00580-011-1259-7

Silva, V.F., Pereira, S.A., Martins, M.A., Rezende, P.C., Owatari, M.S., Martins, M.L. and Vieira, F.D.N., 2023. Hemato-immunological parameters can be influenced by microalgae addition and fish feed supplementation in the integrated rearing of Pacific white shrimp and juvenile Nile tilapia using biofloc technology. *Aquaculture*, **574**: 739622. https://doi.org/10.1016/j.aquaculture.2023.739622

Stoskopf, M.K., 2015. Biology and management of laboratory fishes. In: *Laboratory animal medicine*. Academic Press. pp. 1063-1086. https://doi.org/10.1016/B978-0-12-409527-4.00021-3

Suresh, A.V. and Lin, C.K., 1992. Tilapia culture in saline waters: A review. *Aquaculture*, **106**: 201-226. https://doi.org/10.1016/0044-8486(92)90253-H

Takahara, S., Hamilton, H.B., Neel, J.V., Kobara, T.Y., Ogura, Y. and Nishimura, E.T., 1960. Hypocatalasemia: A new genetic carrier state. *J. clin. Invest*., **39**: 610-619. https://doi.org/10.1172/JCI104075

Teodósio, R., Engrola, S., Colen, R., Masagounder, K. and Aragão, C., 2020. Optimizing diets to decrease environmental impact of Nile tilapia (*Oreochromis niloticus*) production. *Aquacult. Nutr*., **26**: 422-431. https://doi.org/10.1111/anu.13004

Tipsrisukond, N., Kramer, A. and Li, D., 2020. *U.S. Patent Application No. 16/958,515.*

Wintrobe, M.M., 1934. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. *Folia Haematol*., **51**: 32-49.

World Health organization, 1978. *Methods of analysis of sewage sludge, solid wastes and compost*. World Health Organization. International reference center for wastes disposal.

Xu, W.J. and Pan, L.Q., 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture*, **412**: 117-124. https://doi.org/10.1016/j.aquaculture.2013.07.017

Xu, W.J. and Pan, L.Q., 2014. Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. *Aquaculture*, **426**: 181-188. https://doi.org/10.1016/j.aquaculture.2014.02.003

Yap, W.G., 2001. The lowdown on world shrimp culture-II. *Infofish Int*., **3**: 20-23.

Yuan, D., Yi, Y., Yakupitiyage, A., Fitzimmons, K. and Diana, J.S., 2010. Effects of addition of red tilapia (*Oreochromis* spp.) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. *Aquaculture*, **298**: 226-238. https://doi.org/10.1016/j.aquaculture.2009.11.011

Yuan, J., Rong, K., Wang, S. and Liu, Q., 1993. Effect of Nile tilapia on plankton community and primary productivity of freshwater microcosms. *China J. appl. Ecol*., **41**: 65–73.

Yuvarajan, P., 2021. Study on floc characteristics and bacterial count from biofloc-based genetically improved farmed tilapia culture system. *Aquacult. Res*., **52**: 1743-1756. https://doi.org/10.1111/are.15030

Yuvarajan, P., Felix, S., Antony, C., Gopalakannan, A., Menaga, M. and Ezhilmathi, S., 2018. Nursery intensive rearing of GIFT tilapia in outdoor lined pond utilizing aerobic microbial floc technology (AMFT). *J. Ent. Zool. Stud*., **6**: 705-709.