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Efficacy of probiotics and biofloc system in maintenance of water quality and growth performance in shrimp *Litopenaeus vannamei*

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Abstract
Using a variety of external carbon sources, including molasses, tapioca, maize flour and sucrose in combination for the development of bio flocs, an attempt has been made to investigate the effects of adding both probiotics *(Bacillus licheniformis* and *Lactobacillus rhamnosus*) and the Biofloc system on water quality parameters and the performance of Pacific shrimp, *Litopenaeus vannamei*, under a 15: 1 carbon to nitrogen ratio in the current experimental feeding trail. Feeding trail experiments were conducted with Seven Experimental diets including One Control, Probiotics added and Five Bioflocs developed with different external Carbon sources for a period of 60 days with *L. vannamei*. Due to the addition of combinations of both Probiotics and Bioflocs, maintain rigorous control of all the Water quality parameters and provide protection and resistance to the Candidate species of culture i.e. all the Water quality parameters were ideally maintained. Intense mechanical aeration also favours degradation of toxic nitrogen and other compounds by the microbial community that additionally serve as a food supplement for shrimp, thereby Productivity rates were substantially increased. Therefore, BFT is an efficient alternative against pathogens, maintenance of water quality at optimum levels and enhances survival and growth of shrimp *L. vannamei*. The combined use of both Probiotics and Bioflocs in the present study not only maintains a good Water quality parameters and also Growth performance in *L. vannamei*.

Keywords: Probiotics, Bioflocs, *L. vannamei*, water quality

1. Introduction
According to the State of World Fisheries and Aquaculture, these industries are among the fastest-growing food production sectors and have a major impact on lowering poverty, improving health, and reducing hunger [1]. The Indian Fisheries sector is important to the country's economy, accounting for 5.23% of the country's agricultural GDP and 0.91% of the country's overall GDP [1, 2]. About 14 million people in India depend on the food produced by the Indian fishing industry, which also helps with agricultural exports. Even though raising shrimp has been advantageous for many years, the shrimp culture sector still faces numerous obstacles [3, 4]. The intensification of production systems has been prompted by the growth of the prawn farming sector [5]. The higher-intensive Shrimp Culture system was developed in the 70's with higher stocking densities and large quantities of water exchange in confined areas and providing more balanced feeds were the important concepts introduced in Culture Operation of Candidate Species [6]. Although feed is a key component of aquaculture production, feed technology is one of the least developed areas of the industry, especially in Asian nations. Since feed increases production rates, it accounts for more than half of the investment in aquaculture, or between 50 and 70 percent of overall operating expenditures. Additionally, while addressing larger stocking densities and modified culture operations, it is crucial to take into account the impacts of feed on water quality and prawn growth rates [7]. Disease-related issues and various environmental circumstances frequently arise in large-scale production facilities where aquatic animals are subjected to structural conditions, leading to significant financial losses.

The disease issues brought on by Vibrio species have become one of the main obstacles to aquaculture output in recent years. The application of chemicals including Antibiotics became mandatory to protect the Candidate Species from pathogen invasion, thereby prone to diseases. In recent times to overcome the disease occurrence and simultaneously increase production rates, reducing feed costs via production of supplemental natural feeds, conservation of water usage were became the priority areas of Culture Operation. To meet the above said demands two important means, the introduction of use of Probiotics into the Culture system and incorporation of BioFloc Technology (BFT), were made a part of Culture Operation, Probiotics generally includes Bacteria, Cyanobacteria, Microalgae, Fungi etc., consequent upon its addition significantly improve the water quality of Culture Environment and inhibit the Pathogen productivity in water thereby increasing the Productivity rates [8, 9]. With a smaller area than outdoor ponds, the BFT has the ability to lessen the environmental effects of the intensification process by adopting limited or no water exchange practices [10, 11]. With increased stocking densities and Biofloc-dominated systems, aspects such as prawn growth and the impact of feed on water quality are crucial to take into account for a successful culture operation thus far. Feed can directly alter the suspended particles, pH, alkanity, and concentrations of various nitrogen species in the hyper-intensive, Biofloc-dominated system, necessitating careful monitoring and tuning to maximize production rates. It is necessary to assess how feed, water quality, and productivity interact with the unique features of each type of culture system in order to develop diets that are specifically engineered to improve prawn production rates.

Thus, the aim of the present study was to evaluate the efficacy of formulated experimental feeds with the addition of Probiotics and combinations of external Carbon sources as Bioflocs incorporated on selected Water quality indicators, Performance of feeds and Production rates of *L. vannamei*.

2. Materials & Methods

The present study was conducted at shrimp culture units located in Ramayapatnam (Latitude $15^{0}02^{1}$ 55^{11} N; Longitude 80^{0} 02^{1} 50^{11} E) Prakasam District of Andhra Pradesh, India.

Penaeid shrimp *L. vannamei* were obtained from local Aquaculture ponds and were transported in oxygenated double layered polythene bags. Selected shrimp of uniform size were tested for diseases occurrence and finally pathogen-free shrimp were moved into acclimatization tanks filled with sea water of desired salinity. Prior to the start of the experiment, all the shrimp were acclimatized to field conditions for one week and fed twice daily with formulated feed (35% CP) in the Experimental tanks (Square Tanks of 3000 lit capacity) with a salinity of 10±0.5 ppt. All the cement tanks sized 5x10 Mts, were kept in submerged earth crust and earthern bottom was provided with a water depth of 1 mt maintained throughout the present Experimental feeding trails. The completely randomized design method with independent variables were used in the present study, i.e. different external Carbon source mixtures for the preparation of Bioflocs in the present study.

All of the experimental feeding trails in the current investigation used water that was taken from the Buckingham Canal and treated in a designated pond. To ensure improved bioflocculation, blowers attached to electric compressors were used to aerate each experimental tank. According to Avnimelech [12], the Bioflocs were developed in various plastic containers with a 25-liter capacity, utilising various carbon sources and water from prawn culture ponds as an inocula growth. Two weeks were spent incubating the suspension to allow the microbial mass to grow. The approximate nutritional makeup of the experimental diets was calculated in accordance with Jackson [14] and the AOAC [13], and it is shown in Table 2.

Shrimp were raised according to a natural timetable (12:12 h Light: Dark). Sheets of black netlon plastic covered every tank. The water in the control tanks was replaced twice a week, while the BFT tanks were left empty for sixty days, with the exception of adding dechlorinated seawater to make up for evaporated water. A uniform size of 1.520.07 g of acclimated prawns was chosen, and they were randomly stocked in certain experimental tanks. At 10% of their starting weight, prawns were fed experimental diets; at the conclusion of the experiment, the weight was progressively reduced to 3-4%. The weekly sampled mean mass was estimated, and the daily feeding ration for each treatment was computed and modified accordingly. Two times a day, at 6:00 AM and 6:00 PM, the ration was divided and given out. Pre-weighed Carbon sources i.e. Molasses, Tapioca flour, Maize flour and Sucrose requirements of mixing were calculated through Excel programme and completely mixed and finally spread to the tank surfaces at 12.00 Noon once in a week. To stimulate bacterial growth, the C: N ratio in Biofloc Technology (BFT) treatments was kept at 15:1. This ratio was roughly determined using the daily feed input's carbon and nitrogen contents as well as the injection of carbon sources to the Biofloc tanks.

The experimental groups

Group-1: Control group fed with Experimental Feed formulated (Table. 1) (Control).

Group-2: Fed with Control di*et al*ong with Probiotics added (ED-1).

Group-3: Fed with Control feed along with Probiotics added + External source of Carbon Molasses & Tapioca for the formation of Bioflocs (ED-2).

Group-4: Fed with Control feed along with Probiotics added + External source of Carbon Molasses & Maize flour for the formation of Bioflocs (ED-3).

Group-5: Fed with Control feed along with Probiotics added + External source of Carbon Molasses & Sucrose for the formation of Bioflocs (ED-4).

Group-6: Fed with Control feed along with Probiotics added + External source of Carbon Molasses, Tapioca & Maize flour for the formation of Bioflocs (ED-5).

Group-7: Fed with Control feed along with Probiotics added + External source of Carbon Molasses, Maize flour & Sucrose for the formation of Bioflocs (ED-6).

Group-8: Fed with Control feed along with Probiotics added + External source of Carbon Molasses, Tapioca, Maize flour & Sucrose for the formation of Bioflocs (ED-7).

Water temperature was recorded daily at 12.00 noon with digital thermometer. pH and salinity were measured around 9.00 AM in the morning using Elico field water quality meter. Water samples from all Experimental tanks were collected every week and analysed Spectrophotometry for Total Ammonia Nitrogen (TAN), Nitrite-Nitrogen (NO_2-N) and Nitrate -Nitrogen (NO_3-N) by following standard method [15]. Biofloc Volume (BFV), was determined on site using Imhoff

cones every week, registering the volume taken in by the flocs in 1000 ml of tank water after 80 min of Sedimentation [16].

2.1 Probiotic Feed Preparation

Feeds enriched with probiotics were made following the procedure reported by Naresh [17]. The probiotic bacterial species *Lactobacillus rhamnosus* (MTCC: 1408) and *Bacillus licheniformis* (MTCC: 1520) were obtained from the Institute of Microbial Technology's Gene Bank and Microbial Type of Culture Collection in Chandigarh, India. After being kept alive in the nutrient broth, the bacterial cultures were harvested by centrifuging them for 10 to 12 minutes at 10,000 rpm. They were then cleaned with phosphate buffer and reconstituted in phosphate buffer saline (pH 7.4). Using a spraying technique, the re-suspended bacteria were evenly distributed throughout the meal pellets. The prepared probiotic blended feed was dried at 40 °C, sealed in airtight polythene covers, and refrigerated for storage. The probiotic-blend feed containing 10 billion CFU/kg of *Lactobacillus rhamnosus* and *Bacillus licheniformis* was made once in 10 days.

2.2 Preparation of Biofloc

After being chosen as a carbon source and found to have 36% carbon, 53% carbohydrates, and 24% moisture, sugarcane molasses was incubated for two days at 40 °C in warm water. The resulting mixture was then added to the culture medium in a 1:3 molasses to water ratio. In an aquaculture system, NH_4Cl, KH_2PO_4, and Na_2HPO_4 were added to each tank at 96, 31, and 64 mg/lit, respectively, to promote nitrogen loading [18]. Based on the assumption that 50% of the nitrogen consumed by the shrimp will be excreted into the water environment, the ratio of sugarcane molasses to feed to achieve the necessary Carbon: Nitrogen (C: N) ratio was calculated [12]. The weight ratio between the carbon supply and feed can be calculated using the aforementioned formula:

$$\frac{\Delta CH}{\Delta F} = \frac{((CN \times \%\ P\ (F) \times \%\ N\ (P)) - \%\ CF}{\%\ CCH}$$

Where

ΔCH	:	Weight of Carbon Source
ΔF	:	Weight of the Feed
CN	:	C: N ratio needs to be required
% P (F)	:	Protein content in Feed
% N (P)	:	Nitrogen content in Protein (15.5%)
%CF	:	Carbon content in the Feed (50%)
% CCH	:	Carbon content in the Carbon source

The carbon content was calculated using the Walkley and Black technique [19]. Using APHA-recommended protocols, the concentration of total ammonia nitrogen (TAN) and other water quality parameters were assessed [20]. The following growth metrics were tracked and recorded: feed conversion ratio, protein efficiency ratio, feed efficiency ratio, productivity rates, average body weights, average body growth rates, and specific growth rates. The following formulas were used to calculate each of the aforementioned parameters.

$$\text{Survival Percentage (\%)} = \frac{\text{Total number of live shrimp}}{\text{Total number of shrimps stocked}} \text{ X } 100$$

$$\text{Weight Gain (g)} = \frac{\text{Weight of the Shrimp (g)}}{\text{At the end of the Experiment}} - \frac{\text{Weight of the Shrimp (g)}}{\text{at the start of the Experiment}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total amount of Feed consumed (Kgs)}}{\text{Total Biomass of Shrimp (Kgs)}}$$

$$\text{Average Daily Growth Rates (ADGR)} = \frac{\text{Weight of the Shrimp (g) At the end of the Expt} \quad \text{Weight of the Shrimp (g) at the start of the Expt}}{\text{Total number of days of Experiment}}$$

$$\text{Specific Growth Rates (SGR)} = \frac{[\text{Log weight of the shrimp (g) At the end of the Experiment} - \text{Log weight of the shrimp (g)] at the start of the Experiment}}{\text{Total number of days of Experiment}} \text{ x } 100$$

$$\frac{(\text{Log W2} - \text{Log W1})}{\text{T}} \text{ x } 100$$

Where,
W1: Weight of the Shrimp at the start of the Experiment
W2: Weight of the Shrimp at the end of the Experiment
T: Total number of days of Experiment.

Biofloc Volume (FV): V Floc / V collection
Where,
V Floc: Biofloc Volume (ml)
V collection: Collected Sample Volume (ml)

The Experimental diets were examined for moisture, protein, fat, and ash using the AOAC standard procedures [21]. By drying the samples for two hours at 135 °C in an oven to maintain weight, the moisture content of the samples was determined. Following acid digestion, the crude protein content was calculated using Kjeldahl's technique (N X 6.25). The Soxhlet system's ether extraction method was used to calculate the crude lipid content. Dry samples were burned for six hours at 550 °C in a muffle furnace to estimate the amount of Ash.

2.3 Statistical Analysis

ANOVA (one-way analysis of variance; SPSS, 13.0) was employed to ascertain the presence of significant variation among the treatments. The DMRT test was used to compare and determine the difference between the means. $P<0.05$ was the significance threshold utilized in all of the tests. Standard deviations ± Means are used to report data.

3. Results & Discussions

Water Quality Parameters

Water samples were collected from both Control and Experimental Feeding trail tanks during morning hours around 08.00 AM in the morning for the whole experimental period of 60 days. The water management strategy adopted supports microbial development in all the Experimental tanks. Due to nutrient inputs in the form of Bioflocs lead to substantial increase of microbial populations. These microbes will colonize suspended particles i.e. Biofloc and then contribute to water quality maintenance and production of natural feed for Candidate species for culture operation. The colour of the Experimental tanks except Control and Probiotic added tanks, appears to be brownish green and stable for almost throughout the Experimental period of 60 days. Water quality management is crucial in Aquaculture operation, the health of the water quality is a necessary condition for the survival of Candidate species of culture including shrimp.

The lucrative *L. vannamei* prawn culture is becoming more and more significant because of its distinct flavour, excellent nutritional value, and enduring demand in both the home and foreign markets. Shrimp cultivation operations require careful management of water quality parameters to ensure the best possible development and sustainability of the shrimp. Beyond a certain point, variations in the water quality parameters will undoubtedly affect production rates and cause significant financial losses. Mean values and range values of Water quality parameters (Mean ± SD) from Control and Experimental feeding trails were analysed and presented in Table. 3.

Temperature

Temperature being a very important water quality parameter, in the present study, there was no significant variation in Temperature between both Control and Experimental Feeding trail tanks. Its values ranged between 27 to 28 °C, Control tank recorded a maximum of 28.5 °C, whereas all the Experimental tanks recorded around 27 °C. The temperature range, although small and insignificant, followed closely the changes in diurnal air temperatures. It is an important environmental factor for shrimp farming due to its influence on the metabolism of the crustaceans [22]. Temperature controls the solubility of gases, chemical reactions and toxicity of ammonia. The optimum range for growth of shrimp *L.vannamei* is from 27 to 32 °C [23-25]. The values of water temperature in the present study were within the desired range of 27 to 32 °C for normal growth and survival of aquatic organisms in tropical environments as well as favourable conditions for tropical aquaculture activities [26, 27].

pH

The pH values obtained in both Control and Experimental feeding trails recorded from 7.5 to 7.6 pH. In the present study Hydrated lime ($Ca(OH)_2$) is to be used to maintain both alkalinity and pH above 100 ppm and 7.5, respectively in the Biofloc system. pH reduction generally occurs due to alkalinity consumption during Ammonia-Nitrate-N conversion processes. The study revealed that the pH values are in optimum range for penaeid shrimp culture including *L.vannamei*. Several authors also reported that penaeid shrimp culture requires the pH value between 7.3 to 8.2 to show better growth rates and subsequently high production rates [11, 28].

pH is one of the factors that affects the levels of ionised and non-ionized ammonia in water. In a culture system, there are two types of ammonia: NH_3 (unionised ammonia) and NH_4+ (ionised ammonia). The total amount of the two forms of ammonia is called Total Ammonia Nitrogen (TAN). Shrimp that are stressed by extremely high or low pH have fragile shells and poor survival rates [29, 30] but in the present study the pH ranges between 7.3 to 8.2 are appropriate for shrimp aquaculture production. Salinity in the present study was recorded around 10 ppt throughout the Feeding experimental trails. The water volume in the present study was maintained in a constant way, the evaporated water volume was replaced with treated 10 ppt water from the stocking water pond.

Dissolved oxygen (DO)

The Dissolved Oxygen (DO) content were found to be in the range between 6.25 to 6.75 mg/lit in all the Experimental Feeding trail tanks. In the present study DO levels were maintained in a constant way with aeration through compressors, which also plays a vital role in supplying sufficient supply of oxygen, which is necessary for the formation of biofloc and also shrimp *L. vannamei*, is a euryhaline species and can tolerate a wide range of salinity fluctuations. But in the present study the salinity was observed ranged from 10-11 ppt, remained within the acceptable limits for *L. vannamei* culture operation. The level of DO in tank water depends on the production of phytoplankton and also weather conditions.

Due to introduction of Bioflocs into the Experimental tanks, has significantly enhanced the efficiency of waste removal, reduced the level of luminous *Vibrio* and stabilized the phytoplankton density. Water quality parameters represented by pH, DO and Temperature were found to be remained almost stable and are within the optimum range throughout the Experimental period of feeding trails. The DO levels were maintained in a constant way through proper aeration. In all the tanks, Biofloc formation was observed just in 3-4 days, which is characterized by low water transparency and high Biofloc volume index. In the present study, as the algae and bacteria have oxygen demand, DO levels were properly maintained around 6.5 mg/lit in all the Experimental tanks and ideal for the cultivation of Penaeid shrimp [29-31].

The continual aeration and high phytoplankton biomass in the experimental feeding trail tanks may have increased photosynthetic activity, as evidenced by the high concentrations of chlorophyll-a. This could account for the relatively higher levels of DO observed in this study. In this investigation, the parameters of temperature, salinity, and DO were likewise beneficial for the shrimp's growth and survival.

Total Ammonia Nitrogen (TAN)

The nitrogen molecules nitrite-N (NO_2-- N), nitrate-N (NO_3-- N), and total ammonia nitrogen (TAN) are crucial for the cultivation of aquatic species. The breakdown and excretion of organic matter produces TAN, which can have a detrimental effect on the performance of organisms in culture or even result in death in larger concentrations. The primary sources of TAN in culture operations were excretion products from faeces, urine, uneaten food, phytoplankton, and zooplankton. In contrast to ionised ammonia or ammonium ion (NH_4), ammonia or non-ionized ammonia (gaseous) is regarded as hazardous. High pH, high temperature, and low oxygen concentration all cause a rise in the unionised form (NH_3). In the present study the TAN levels were recorded relatively high i.e. 2.15 mg/lit in Control group of feeding

trails, compared to Probiotics added group i.e. 1.98 mg/lit, whereas all the Bioflocs added group of feeding trail tanks recorded relatively low concentrations of TAN ranging from 0.32 to 0.36 mg/lit clearly demonstrates the considerable reduction and is significant (p<0.05) compared to both Control and Probiotic added group of Feeding trail experiments. The TAN levels were reduced to around 85% in Biofloc added groups compared to Control and Probiotic added groups of feeding trails.

In crustaceans, the primary byproduct of protein catabolism is ammonia. Ammonia destroys gills, lowers blood's capacity to carry oxygen, and increases tissue oxygen use. Both ionised (NH_4+) and unionised (NH_3) forms of ammonia are found in water, but unionised ammonia is the most hazardous since it diffuses through cell membranes more easily [32]. In the present study the TAN levels were considered to be ideal and hence the growth rates and final productivity rates were considerably high in all the Biofloc added feeding trail groups compared to Control group. Furthermore, pH values are remained below, Temperature between 27-28 °C and Salinity of 10-11 ppt, which favours for speed of nitrification reactions and promote reduction of Ammonia toxicity [33]. From the results obtained it is very clear that, the inclusion of Probiotics had no significant effect on bioremediation. Hence the application of Probiotics alone unable to achieve complete maintenance of water quality in the culture operation, but along with the addition of Bioflocs, it is being worked to the fullest extent in maintaining the water quality in water tanks in the present study. Because the heterotrophic bacteria in Biofloc can absorb ammonia 40 times quicker than nitrification bacteria, Biofloc can balance the ammonia concentration in the culture system [33]. Furthermore, Biofloc produced in the Bioreactor can convert 98% ammonia to nitrate at a concentration of 110 mg/lit/day, according to De Schryver [34]. The ratio of organic carbon to nitrogen, or C/N ratio, in water affects the ability of heterotrophic bacteria to absorb ammonia; ammonia will be taken more quickly when the C/N ratio is higher. The earlier research, which showed that the C/N ratio (15:1) was found to be most suitable for the *L. vannamei*, also support the conclusions obtained in this investigation.

Nitrate-N (NO_3- N) & Nitrite-N (NO_2- N)

Nitrite-N and Nitrate-N, the main components of Nitrogenous substances also recorded in all the Experimental feeding trail groups. Nitrite-N recorded 1.3 mg/lit in the Control group compared to other Experimental feeding trails, recorded i.e. in Probiotic added Feeding trail group (1.14 mg/lit), whereas Bioflocs added groups recorded in the range 0.61 to 0.73 mg/lit. The values obtained for Biofloc added trails were found to be statistically significant (p<0.05) compared to Control group feeding trails. Nitrate-N values recorded higher in the case of control group 4.34 mg/lit, whereas the values were found to be significantly (p<0.05), decreased in all the Biofloc added feeding trails and the range of reduction was around 47%. In between the Nitrite-N and Nitrate-N, Nitrite-N values were found to be relatively lower 1.31 mg/lit in Control group, compared to Nitrate-N values recorded as 4.34 mg/lit in the Control group feeding trails. Both Nitrate-N and Nitrite-N levels were found to be significantly (p<0.05) decreased in all the Experimental feeding trail groups compared to Control group.

The transformation process of ammonia nitrogen to Nitrite-N and their toxicity form depends on the amount of Chlorides, Temperature and Oxygen concentrations in culture medium. Nitrite toxicity affects transport of oxygen, oxidation of important compounds and tissue damage. Nitrite-N concentrations less than 2 ppm found to be ideal for Biofloc shrimp culture [25]. In the present study all the Biofloc added Feeding trails were recorded range from 0.61 to 0.73 mg/lit clearly demonstrates that, the growth rates were found to be significantly (p<0.05) higher compared to Control feeding trail group. When ammonia is converted to nitrate, the intermediate state is represented by nitrite. It is commonly known that the farmed prawns were extremely poisonous to ammonia and nitrite. In the blood of crustaceans, high amounts of nitrite-N typically deactivate haemoglobin [36, 37]. However, because prawn blood lacks haemoglobin, oxygen binds to copper at the gills and is transported throughout the body, affecting aquatic organisms' immune and circulatory systems [38]. Nitrite-N, the end product of aerobic nitrification considered to be less toxic. The toxicity of these compounds is due to its effects on osmoregulation and oxygen transport. Nitrate-N is the end product was found to be showing range between 2.31 to 4.34 mg/lit, and these range values were found to be most ideal for shrimp culture, whereas concentrations above 200 mg/lit was found to be lethal [39]. The drastic reduction in the Nitrite-N in the present study may be attributed to the presence of phytoplankton in Biofloc and this algae needs Nitrate-N as a source of nutrients that are absorbed from the water and therefore the concentration of Nitrate-N were found to be significantly lower. The results obtained were found to draw the support of earlier reports [40, 41].

In Biofloc added groups, the TAN concentrations were found to be significantly (p<0.05) decreased compared to Control group of Feeding trail. In the present study all the Biofloc added groups, ammonia is being effectively taken by Heterotrophic bacteria, with the availability of Nitrate-N from the culture medium. Temperature and salinity, salinity and primary productivity, primary productivity and dissolved oxygen, dissolved oxygen and nitrate, and nitrate and total available phosphorus in culture ponds have all been shown to positively correlate, according to Pankaj Kumar *et al*.'s study [42].

Ammonia Nitrogen (TAN), Nitrite-N and Nitrate-N were considered to be important indicators in the culture operation, to avoid the toxic effects of high concentrations of Nitrite-N and Ammonia peaks in the cultured species in the process of using the Biofloc system. From the results obtained it is very clear that all the Nitrogen containing compounds were significantly decreased in all the Biofloc added, using BFT compared to Control or Probiotic added feeding trails. Balancing the concentration of ammonium in the Biofloc culture system by the addition of external Carbon sources is possible because the Heterotrophic bacteria in Biofloc can absorb ammonia more effectively for the production of proteinaceous materials. The microbial conversion coefficient and the C/N ratio in the microbial mass with the carbon content of the supplied material are the determining factors in this relationship. Avnimelech [43] showed that adding carbohydrates lowers the TAN level in the culture system and reduces the requirement for dietary protein concentration. In the present study the average levels of TAN observed was relatively higher in the Control group compared to other Experimental BFT treated Feeding trail groups (Table. 3). However, the mean value of a lower concentrations of NO_3-N was observed in the Biofloc added groups and this low level

may be attributed to NO_3-N uptake by microbes in the Biofloc added Feeding trail groups.
Overall, the study's Experimental Feeding group trails showed no detrimental effects of the various component concentrations on the shrimp's survival and zootechnical performance**.**

Phosphate-P
Phosphate-Phosphorus values recorded minimum of 0.65 (ppm) in the Control group and showed a progressive increase in all the Biofloc added feeding trail groups. Results showed that there was a significant variation in PO_4-P concentrations between Control and Experimental groups. Elevated PO_4-P concentrations in the experimental tanks may be related to the application of liming materials to raise the pH of bottom sediments since this increases the availability of phosphorus deposited in sediments in the water column [44]. While a buildup of phosphorus in the system does not directly impact shrimp development, it can create an environment that is conducive to the growth of filamentous cyanobacteria, which can clog shrimp gills and produce toxins that are toxic to penaeid shrimp [45]. Feed application and fertilizers are the principle routes of entry for phosphorous to the culture system [46]. Due to increased feed application results in increasing phosphorous loading into the culture pond or tank. Total Phosphorous is a measure of the total phosphorous in the water and does not discriminate between free and bound forms. Bound forms can be found as part of the Phytoplankton biomass.

Alkalinity
Alkalinity values obtained in the present study was recorded as 151 mg/lit with Control group, whereas the Experimental groups showed a significant (p<0.05) increase, and reaching a maximum of 518 mg/lit with Experimental Diet (EDT). The ability of water to buffer or withstand pH shifts brought about by the addition of acid and base is known as alkalinity. Because the nitrifying bacteria in nitrification continuously drain the alkalinity of the water in Biofloc systems, it is important to maintain sufficient reserves of alkalinity. When alkalinity is gone, pH can fall sharply, which stops bacteria - including vital nitrifying bacteria - from functioning. Ammonia builds up to the point that the feeding response of prawns deteriorates in this scenario. But in the present study the alkalinity levels were found to be maintained ideally thereby daily Feeding rates, Feed Conversion Efficiency and ultimately Production rates were significantly improved. The values obtained for alkalinity gains support from earlier reports [3].
By adding 10 to 20 percent of the feed provided in the experimental tanks on a regular basis, alkalinity and pH can be adjusted. This also boosts the inorganic uptake of the bacteria in the BFT system that are heterotrophic and nitrifying.

Total Suspended Solids (TSS)
Total Suspended Solids (TSS) recorded 254 mg/lit in the Control group and found to be increased significantly (p<0.05) in all the Experimental feeding trail groups, and maximum of 542 mg/lit recorded with ED-7. In Biofloc system, the TSS in the range 375-542 mg/lit ensures efficient bacterial activity and a good system to control Ammonia without excessive water respiration. Bacteria depend on suspended solids as a substrate for adhesion and as a source of energy from carbon. The colour attained with the addition of Biofloc. The experimental tanks were discovered to be brown to dark brown/brown greenish, and they were supposed to contain colonies of heterotrophic bacteria, microalgae, and protozoans in addition to suspended organic particles in the form of flocculated aggregates. Although TSS concentration is crucial to Biofloc systems, it is uncertain how controlling particle concentration can increase prawn production rates when the culture is in operation. TSS is also known to significantly alter the microbial communities and aid in the elimination of harmful species, which increases *L. vannamei's* productivity rates.

BOD and COD
The study revealed that there was a substantial (p<0.05) increase in both the Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values throughout all Experimental feeding trails when compared to the Control group. The water's oxygen demand is measured by BOD. The amount of dissolved oxygen (DO) that microorganisms, or aerobic bacteria, use while growing on the organic material in a water column at a particular temperature during a predetermined amount of time is measured analytically as BOD. Total Heterotrophic Bacteria (THB) numbers were significantly increased in all Experimental feeding trails in the current study, which may have contributed to the relatively higher BOD levels. Additionally, DO levels were maintained at a constant level through aeration. Because COD analysis assesses all organic matter that can be chemically oxidised rather than simply the amounts of biologically oxidised organic matter, it is less precise. Both BOD and COD were deemed to be within the permissible range for *L. vannamei* culture operation in the current investigation.
In the current investigation, it was discovered that the biofloc volume in every experimental feeding trail was significantly (p<0.05) higher than in the control group. While organic materials are being metabolised, inorganic nitrogen gets immobilised into bacterial cells when the ideal C: N ratio of 15:1 is maintained. The kind of carbon source used to create the flocs affects both the nutritional qualities of the flocs and the Biofloc system's ability to regulate the water quality in the culture system. The carbon sources used in this study are efficiently used to generate flocs, which are effectively taken up as food, meaning that the production rates were greatly increased. Heterotrophic bacteria control autotrophic microorganisms, immobilise ammonium ions for the synthesis of microbial protein, and keep the water's inorganic nitrogen content within permissible limits while maintaining a C: N ratio of 15:1. In all of the experimental feeding trail tanks, levels of total heterotrophic bacteria (THB) were observed to be significantly higher in the current study.
In all experimental feeding trails, there was a substantial (p<0.05) increase in the amounts of phytoplankton and chlorophyll-a when compared to the control group. Ammonia and nitrite concentrations are typically low under the Biofloc system because these chemicals are eliminated from microbial communities. Because there was less ammonia nitrogen available for oxidation by nitrifying bacteria, the nitrate concentration was too low. The main reason for the high quantities of chlorophyll in the current study is most likely phytoplankton's absorption of inorganic nitrogen in its reduced form. The current study's usage of certain carbon sources encourages bacterial succession and domination over microalgae. The most important indicator of phytoplankton

biomass is chlorophyll-a. The obtained data support a high correlation between the quantity of phytoplankton and chlorophyll-a. The observed results corroborated previous reports [47, 48] that indicated a high association (p<0.05) between the density of phytoplankton cells and chlorophyll-a.

Microbial Community

In the present study, an attempt was also made to analyse Total Heterotrophic Bacteria (THB) and Total Vibrio Bacteria (TVB) count weekly until the completion of the feeding trail experiment and presented in the Table. 4 and Fig. 1 & 2.

The THB count were found to be significantly increased with the progress of the culture operation both in Control and Experimental feeding trail tanks. Similarly, the THB count recorded were found to be significantly higher in all DOC groups compared to their respective control groups. The results of the bacteria showed that, regardless of the usage of probiotics and bioflocs, heterotrophic bacteria predominated in the bioremediation of water quality. In the current investigation, high densities of heterotrophic bacteria were discovered in all of the experimental feeding trails, as well as in the microbial populations in the control tanks with or without the addition of commercial probiotics. THB counts were shown to be significant in the current study when compared to the control group, while THB quantities were not significant when compared to other experimental groups.

Total *Vibrio* Bacteria (TVB) were found to be increased in the control group whereas significantly (p<0.05) decreased with the progress of the days of Culture Operation. TVB count found to significantly decrease with the progress of the days of Culture Operation in all the experimental feeding trail groups compared to their respective controls. The *Vibrio* load is one of the most important factors affecting the performance in both growth parameters and also production rates. THB generated due to Biofloc addition to the culture tanks were the main factor responsible for *Vibrio* control and but due to the addition of Probiotics, the *Vibrio* count appears to be relatively high in the Experimental feeding trail (ED-1) compared to other Biofloc added feeding trail groups. The dominance of heterotrophic and autotrophic bacteria over Vibrio in all experimental feeding trails, which undoubtedly exploited their antagonistic relationships to regulate the quantity of pathogenic Vibrio in the culture operation, may lend credence to this claim.

Several authors reported the benefits of addition of several commercial Probiotics including *Bacillus* species, *Nitrobacter* species, *Lactobacillus* species in Intensive / Semi-intensive system significantly reduced the *Vibrio* count in Culture water [49]. But in intensive biofloc cultures, the use of Probiotics remains controversion, as the main bacteria found are additionally present in the Biofloc. Therefore, the use of Probiotics would be interesting only in the case of system disturbances as dominance of undesirable or pathogenic microorganisms, disease outbreaks, damage to water quality, whereas dominance of beneficial native bacteria preferably isolated from the digestive tract of Shrimp seems to be an alternative to the re-establishment of Environmental conditions. On the other hand, due to the addition of combinations of both Probiotics and Bioflocs, maintains rigorous control of all the water quality parameters and provide protection and resistance to the Culture Organism.

Growth Performance Studies

In the present study, growth performance studies were conducted with *L. vannamei* through different feeding trails with the incorporation of probiotics and bioflocs into the culture operation. Results pertaining to growth patterns of *L. vannamei* under different feeding trails were presented in Table. 5 and Fig. 3. Growth patterns were monitored every week by obtaining the samples from all the experimental feeding trails. From the results obtained for growth patterns for *L. vannamei*, clearly indicates that both Probiotics and Bioflocs used in the present study induced relatively more growth rates compared to control feed fed feeding trail, clearly indicates that Bioflocs added experimental feeding trails induced the highest growth rates in *L. vannamei*.

Among the experimental feeding trails, ED-1 was formulated with the addition of Probiotics recorded relatively higher compared to control group and lower compared to remaining Biofloc added experimental trails in the present study. Due to the incorporation of both *Bacillus* and *Lactobacillus* as probiotics in the present study induces the growth potentials represented by Weight gain, Specific growth rates, Harvest size and Productivity rates.

Probiotics are seen to be a good substitute for antibiotics in aquaculture, especially when it comes to culturing of prawns since they increase growth rates and reduce excessive mortality. The current study unequivocally shows that feeding *L. vannamei* a diet including probiotics caused its growth patterns to increase noticeably. Probiotics such as bacteria, yeast, and microalgae have been researched for use in aquaculture of crustaceans, especially prawns. Bacillus species, which produce lactic acid bacteria, were used to enhance the aquatic environment in aquaculture. Strong antibacterial activity against pathogenic germs has been demonstrated by *Lactobacillus* species [49]. Probiotics have been shown to increase yields in aquaculture when used as water additives or as food supplements [50–52]. Probiotics in aquaculture have been demonstrated to have multiple mechanisms of action, including the competitive exclusion of pathogenic bacteria by producing compounds that inhibit them, improving the immune response of the host species, and improving the nutrition of the host species by producing additional digestive enzymes [50, 53-55]. In the present study two probiotic bacterial species were selected for incorporation into feed i.e. *Bacillus licheniformis* and *Lactobacillus rhamnosus* were used as feed probiotics during the culture operation induced the best growth potentials compared to control group of feeding trail experiment.

In the present study, both Probiotics and Bioflocs were added either into feed or into culture medium i.e. Feeding trail groups i.e. ED-2 to ED-7 recorded higher growth rates compared to either control group or experimental trail group incorporated only Probiotics through feed. There were significant (p<0.05) differences in growth patterns on variations between experimental and control groups. The performance of shrimp *L.vannamei* after feeding trail after 60 days was presented in Table. 4. In the present study 500 Nos of shrimp were stocked into respective feeding trail group of experimentation. The percent survival rates were recorded as 89% with the control group compared to more than 90% with all the experimental feeding trail groups. During the feeding trail experiments, the shrimp showed normal behaviour without any remarkable observations. Therefore, the Probiotics or Bioflocs added feeding trail group resulted in significantly higher survival rates compared to control feeding trail group.

Among the six groups of feeding trail experimental groups the

external source of carbon was varied keeping only molasses as a constant component to be added in addition to Tapioca (ED-2), Maize flour (ED-3), Sucrose (ED-4), Tapioca & Maize flour (ED-5), Maize flour & Sucrose (Ed-6) and Tapioca, Maize flour & Sucrose (ED-7) resulted in differential growth rates, though recorded higher growth rates compared to control & probiotic added feeding trail groups. In between the Experimental feeding trails ED-2, ED-3, and ED-4 resulted significantly lesser rates of growth compared to ED-5 and ED-6; ED-5 and ED-6, the Bioflocs were prepared using Molasses with two external carbon sources. But growth rates are significantly (p<0.05) higher among all the Biofloc experimental feeding trails i.e. recorded with ED-7, where in which four carbon sources were added for the production of biofloc in the present study.

The final weights and weight gain values recorded in the present study subjected to One way analysis of variance revealed that the variations in both final weights and weight gain of *L. vannamei* between control and experimental groups were statistically significant (p<0.05). Earlier reports also indicated that significant improvement in both final weights and weight gain in *L. vannamei* using probiotic bacteria i.e. *B. licheniformis* and *L. rhamnosus* and external carbon sources i.e. Sugarcane Molasses, Rice flour, Wheat flour for raising suitable Bioflocs added to the culture system [56] and also in fresh water prawn *M.rosenbergii* using *L. Sporogenes*, in *M. amazonicum* by supplementation of diets with *Saccharomyces cerevisiae* and yeast [57- 60].

Daily growth rates (DGR) were found to be significantly (p<0.05) increased in Experimental groups compared to control group after 60 days of feeding trail experimentation. Specific growth rates (SGR) though showed an increment, statistically not significant (p<0.01) with ED-1 to ED-4 feeding trail experimental groups but significant (p<0.05) with ED-5 to ED-7 feeding trail experimental groups compared to control group. Protein efficiency ratio (PER) values obtained in the present study clearly demonstrates that, there is a significant (p<0.05) increase in all the Experimental feeding trail groups compared to control group. The Feed conversion ratio (FCR) values recorded maximum of 2.53 with the control group compared to probiotic added group (2.22) and minimum values were recorded among all the Biofloc added group, least value of FCR recorded with ED-7, where in combination of 4 external carbon sources i.e. Molasses, Tapioca, Maize flour and Sucrose were added. One way ANOVA results clearly indicates that FCR values obtained with Experimental feeding trails are statistically significant (p<0.05) compared to control group with the addition of either Probiotics or combination of both Probiotics and Bioflocs significantly increased the growth patterns and reduced the FCR values in the present study. Similar kind of observations were also reported by several Authors, through the conduction of Feeding trail experiments with fresh water prawns *M. rosenbergii*, *M. amazonicum*, Penaeid Shrimp *P. monodon*, *L. vannamei*, where in which the diets were supplemented with probiotic bacteria including *L. bacillus*, *L. acidophillus*, *L. sporogenes*, *B. subtilis*, *B. licheniformis*, *Enterococcus faecium* [51, 61-64].

Feed Efficiency Ratio (FER) and Feed Conversion Efficiency (FCE) values obtained in the present study under different feeding trails showed an increase in all the Experimental feeding trail groups compared to control group and found to be significant (p<0.05). Similar kind of observations were reported with both fresh water prawns and Penaeid Shrimp after fed with both Probiotics, *Bacillus* and *Lactobacillus* and Bioflocs using Molasses, Rice flour and Wheat flour as external carbon sources [65] .

Protein Efficiency Ratio (PER) were recorded in all the feeding trails after 60 days found to be minimum recorded to be 5.42 with Control group, whereas maximum was recorded with ED-7, (8.54), Biofloc with Molasses, Tapioca, Maize flour and Sucrose as external Carbohydrate sources. The PER values recorded were found to be statistically significant (p<0.05) between Control and Experimental feeding trails in the present study. So, both Probiotics and Bioflocs capably inducing PER clearly indicates that the utilization of protein in the feed was substantially initiated. Moreover PER values obtained were also gains support from both Feeding, Absorption and Conversion rates of feeds, which showed a significant increase (p<0.05) in the present study. Similar kind of observations were also reported with Prawns *M. rosenbergii*, *L. vannamei*, *P. monodon*, fish *Clarius gariepinus*, *Oncorhynchus mykiss*, *Oreochromis niloticus* through the incorporation of probiotic bacteria with feeds and Bioflocs incorporation into culture media [50, 66-68]. Biofloc addition in the form of selected external Carbon sources facilitates the growth of Heterotrophic bacteria in the Culture Operation, which subsequently provides the supplementary feeding materials, thereby significantly increases the protein content in the Candidate Species of culture. In the present study both Harvest size and Productivity rates were found to significantly (p<0.05) increased in the Biofloc added Experimental feeding trails compared to both Control and Probiotic added feeding trail groups. Minimum harvest size of 16.32 g recorded with Control group, whereas maximum 33.12 g recorded with Experimental feeding trail in which both Probiotics and Bioflocs were added in the Culture Operation. In accordance with the Harvest size, the Productivity rates were also recorded highest and best with ED-7, in which both Probiotics and Bioflocs mixture of 4 external carbon sources were added.

Table 1: Ingredient composition of control experimental diet (Protein content 35%)

Feed Ingredient	(%)
Shrimp meal	15
Squilla meal	12
Soya bean meal	20
Wheat meal	20
Yeast meal	5
Groundnut oil cake	5
Cod liver oil	5
Vegetable oil	4
Ascorbic acid	2
Choline chloride	1
Vitamin mixture	1
Mineral mixture	1
Chromic oxide	1
Agar Agar	3
Gelatin	5
Total	100

Table 2: Proximate Composition of Experimental diets (% DM basis)

Parameter	Experimental diets							
	Control	ED-1	ED-2	ED-3	ED-4	ED-5	ED-6	ED-7

Organic matter (%)	82.87±2.54	82.38±2.48	81.88±2.37	82.47±2.52	82.38±2.46	82.19±2.44	82.23±2.51	83.12±2.58
Ash (%)	17.13±0.78	17.62±0.80	18.12±0.82	17.53±0.81	17.62±0.76	17.81±0.81	17.77±0.77	16.88±0.70
Crude Protein (%)	35.77±1.35	36.18±1.36	36.08±1.35	36.77±1.39	36.94±1.40	36.77±1.40	35.94±1.39	37.42±1.34
Crude Lipid (%)	7.05±0.73	6.92±0.72	7.14±0.74	7.05±0.71	7.14±0.72	7.08±0.75	7.18±0.71	7.28±0.75
Crude Fiber (%)	4.39±0.47	4.42±0.48	4.43±0.51	4.45±0.51	4.44±0.52	4.45±0.51	4.49±0.52	4.54±0.53
Nitrogen Free Extract (NFE) (%)	26.93±1.27	26.42±1.31	25.79±1.28	25.53±1.26	25.21±1.28	25.44±1.32	26.21±1.26	25.44±1.28
Moisture (%)	8.73±0.72	8.44±0.73	8.49±0.73	8.67±0.70	8.65±0.76	8.45±0.81	8.41±0.79	8.44±0.85
Gross Energy (Kcal/100g)	395	395	394	397	396	395	394	403

Organic Matter: 100 – Ash
NFE: 100 – (CP + CL + CF + Ash + Moisture)
Gross Energy: (CP x 5.6) + (CL x 9.44) + (CF x 4.1) + (NFE x 4.1) kcals/100 g
Control Diet (Composition in Table.1)
ED-1 Control Feed with Probiotics broad casted
ED-2 Control Feed + Probiotics added + Bioflocs (Molasses +Tapioca as carbon source)
ED-3 Control Feed + Probiotics added + Bioflocs (Molasses +Maize flour as carbon source)
ED-4 Control Feed + Probiotics added + Bioflocs (Molasses +Sucrose as carbon source)
ED-5 Control Feed + Probiotics added + Bioflocs (Molasses +Tapioca+ Maize flour as carbon source)
ED-6 Control Feed + Probiotics added + Bioflocs (Molasses +Maize flour + Sucrose as carbon source)
ED-7 Control Feed + Probiotics added + Bioflocs (Molasses +Tapioca+ Maize flour +Sucrose as carbon source)

Table 3: Water quality parameters under different Feeding trails for *L. vannamei*

	Control	**ED-1**	**ED-2**	**ED-3**	**ED-4**	**ED-5**	**ED-6**	**ED-7**
Temperature (°C)	$28±1.1^{a}$	$27±1.2^{a}$	$27±1.1^{a}$	$27±1.3^{a}$	$27±1.1^{a}$	$27±1.1^{a}$	$27±1.1^{a}$	$27±1.1^{a}$
pH	$7.5±0.64^{a}$	$7.5±0.67^{a}$	$7.6±0.62^{a}$	$7.6±0.60^{a}$	$7.6±0.65^{a}$	$7.6±0.61^{a}$	$7.6±0.62^{a}$	$7.6±0.66^{a}$
Salinity (ppt)	$10±0.76^{a}$	$10±0.77^{a}$	$10±0.79^{a}$	$10±0.77^{a}$	$10±0.78^{a}$	$10±0.77^{a}$	$10±0.76^{a}$	$10±0.77^{a}$
Dissolved oxygen (DO) (mg/lit)	$6.25±0.60^{a}$	$6.31±0.62^{a}$	$6.34±0.62^{a}$	$6.44±0.60^{a}$	$6.41±0.58^{a}$	$6.54±0.58^{a}$	$6.63±0.60^{a}$	$6.72±0.60^{a}$
	PDC	+0.96	+1.44	+3.04	+2.56	+4.64	+6.08	+7.52
Total Ammonia Nitrogen (TAN) (mg/lit)	$2.15±0.252^{a}$	$1.98±0.101^{a}$	$0.32±0.012^{b,c}$	$0.34±0.010^{b,c}$	$0.34±0.021^{b,c}$	$0.35±0.001^{b,c}$	$0.35±0.022^{b,c}$	$0.36±0.018^{b,c}$
	PDC	-7.91	-85.12	-84.19	-84.19	-83.72	-83.72	-83.26
Nitrite (NO_2-N) (mg/lit)	$1.31±0.20^{a}$	$1.14±0.15^{a}$	$0.73±0.05^{b,c}$	$0.71±0.06^{b,c}$	$0.71±0.06^{b,c}$	$0.68±0.04^{b,c}$	$0.67±0.04^{b,c}$	$0.61±0.03^{b,c}$
	PDC	-12.97	-44.27	-45.80	-45.80	-48.10	-48.85	-53.44
Nitrate (NO_3-N) (mg/lit)	$4.34±0.32^{a}$	$3.98±0.29^{a}$	$2.34±0.17^{b,c}$	$2.37±0.18^{b,c}$	$2.32±0.18^{b,c}$	$2.31±0.15^{b,c}$	$2.35±0.19^{b,c}$	$2.31±0.15^{b,c}$
	PDC	-8.30	-46.08	-45.39	-46.54	-46.77	-45.85	-46.77
Phosphate (ppm)	$0.65±0.04^{a}$	$0.79±0.04^{b}$	$0.83±0.05^{b}$	$0.85±0.06^{b}$	$0.88±0.04^{b}$	$0.94±0.07^{b,c}$	$0.98±0.07^{b,c}$	$1.12±0.12^{b,c}$
	PDC	+21.54	+27.69	+30.77	+35.38	+44.62	+50.77	+72.31
Alkalinity (mg/lit)	$151±8.36^{a}$	$173±9.69^{a}$	$417±34.38^{b,c}$	$424±35.84^{b,c}$	$435±34.61^{b,c}$	$448±34.38^{b,c}$	$462±37.58^{b,c}$	$518±40.48^{b,c}$
	PDC	+14.56	+176.16	+180.79	+188.08	+196.69	+205.96	+243.05
Total Suspended Solids (TSS) (mg/lit)	$254±13.94^{a}$	$273±14.46^{a}$	$375±17.92^{b,c}$	$384±20.74^{b}$	$416±26.11^{b}$	$452±33.17^{b}$	$475±37.17^{b}$	$542±41.28^{b}$
	PDC	+7.48	+47.64	+51.18	+63.78	+77.95	+87.01	+113.39
Biological Oxygen Demand (BOD) (mg/lit)	$15.12±0.75^{a}$	$18.14±0.80^{b}$	$22.19±0.83^{b,c}$	$28.14±0.99^{b,d}$	$46.77±2.55^{b,d}$	$56.42±2.72^{b,d}$	$63.45±2.95^{b,d}$	$73.42±3.12^{b,d}$
	PDC	+19.97	+46.76	+86.11	+209.33	+273.15	+319.64	+385.58
Chemical Oxygen Demand (COD) (ppm)	$28.44±1.06^{a}$	$39.38±1.13^{b}$	$41.43±1.18^{b}$	$42.44±1.26^{b}$	$43.04±1.35^{b}$	$43.77±1.37^{b}$	$43.99±1.28^{b}$	$48.74±1.30^{b,c}$
	PDC	+38.47	+45.68	+49.23	+51.34	+53.90	+54.68	+71.38
DO Reduction Rate (mg/lit/hr)	$0.45±0.010^{a}$	$0.56±0.017^{b}$	$1.12±0.13^{b,c}$	$1.18±0.18^{b,c}$	$1.22±0.17^{b,c}$	$1.28±0.19^{b,c}$	$1.31±0.20^{b,c}$	$1.38±0.20^{b,d}$
	PDC	+24.44	+148.89	+162.22	+171.11	+184.44	+191.11	+206.67
Biofloc Volume (mg/lit)	$12.45±0.81^{a}$	$17.34±0.86^{b}$	$20.13±1.07^{b,c}$	$23.16±1.20^{b,d}$	$28.75±1.24^{b,d}$	$30.49±1.35^{b,d}$	$32.33±1.43^{b,d}$	$34.45±1.46^{b,d}$
	PDC	+39.27	+61.69	+86.02	+130.92	+144.90	+159.68	+176.71
Chlorophyll-a	$49.77±1.57^{a}$	$61.12±1.61^{b}$	$69.71±1.64^{b,c}$	$73.41±1.66^{b,c}$	$78.43±1.74^{b,c}$	$83.33±2.03^{b,c}$	$84.18±2.06^{b,c}$	$89.79±2.09^{b,c}$
	PDC	+22.80	+40.06	+47.50	+57.58	+67.43	+69.14	+80.41
Phytoplankton density (x 10^5 cells/lit)	$2.31±0.23^{a}$	$2.83±0.25^{b}$	$7.18±0.50^{b,c}$	$8.03±0.51^{b,c}$	$8.77±0.51^{b,c}$	$10.72±0.58^{b,c}$	$11.12±0.61^{b,c}$	$13.78±0.65^{b,d}$
	PDC	+22.51	+211	+248	+280	+364	+381	+497

All Values are Mean ± SD of six individual observations
PDC: Percent Deviation over respective Control
Values with different superscripts are significantly different from each other @ $p < 0.05$.

Table 4: Total Heterotrophic Bacteria (THB) and Total Vibrio Bacteria (TVB) Count (10^6 cfu/ml) at different Days of Culture Operation

	DOC '0' Days	**DOC '30' Days**	**DOC '60' Days**
Total Heterotrophic Bacteria			
Control	6.88±0.21	$9.04±0.33^{a}$	$10.05±0.54^{a}$
ED-1	6.93±0.23	$12.77±0.54^{b}$	$16.18±0.68^{b}$
	PDC	+41.26	+61.00
ED-2	7.04±0.25	$14.12±0.56^{b}$	$21.35±0.80^{b,c}$
	PDC	+56.19	+112.43
ED-3	7.12±0.29	$16.39±0.70^{b,c}$	$24.04±0.84^{b,d}$
	PDC	+81.31	+139.20
ED-4	6.93±0.24	$18.43±0.78^{b,c}$	$27.13±0.85^{b,e}$

	PDC	+103.87	+169.95
ED-5	6.98±0.29	21.35±0.77^{b,d}	33.49±1.01^{b,f}
	PDC	+136.17	+233.23
ED-6	7.14±0.30	23.14±0.80^{b,e}	37.75±1.06^{b,g}
	PDC	+155.97	+275.62
ED-7	7.05±0.30	28.87±0.93^{b,f}	42.95±1.09^{b,h}
	PDC	+219.36	+327.36
Total Vibrio Bacteria			
Control	13.14±0.47	18.79±0.75^{a}	23.44±0.78^{a}
ED-1	13.19±0.48	16.08±0.57^{b}	20.31±0.77^{b}
	PDC	-14.42	-13.35
ED-2	13.74±0.47	12.13±0.45^{b,c}	17.28±0.72^{b,c}
	PDC	-35.44	-26.28
ED-3	13.42±0.46	10.12±0.37^{b,c}	14.15±0.48^{b,d}
	PDC	-46.14	-39.63
ED-4	13.08±0.44	9.14±0.29^{b,d}	12.04±0.41^{b,e}
	PDC	-51.36	-48.64
ED-5	13.41±0.46	7.38±0.23^{b,d}	10.04±0.34^{b,f}
	PDC	-60.72	-57.17
ED-6	13.43±0.42	7.45±0.24^{b,d}	7.15±0.23^{b,g}
	PDC	-60.35	-69.50
ED-7	13.41±0.43	5.33±0.13^{b,e}	5.24±0.12^{b,g}
	PDC	-71.64	-77.64

All Values are Mean ± SD of six individual observations
PDC: Percent Deviation over respective Control
Values with different superscripts are significantly different from each other @ $p< 0.05$

Table 5: Growth Performance details of *L. vannamei* in different Experimental feeding trails

	Control	**ED-1**	**ED-2**	**ED-3**	**ED-4**	**ED-5**	**ED-6**	**ED-7**
Number of shrimp stocked	500	500	500	500	500	500	500	500
Percent survival (%)	89	93	94	94	93	94	96	97
Final weight (g)	16.23±0.71^{a}	19.34±0.85^{b}	20.34±0.86^{b}	21.73±0.89^{b,c}	23.13±0.93^{b,d}	25.18±0.97^{b,e}	29.85±0.99^{b,f}	33.28±1.00^{b,g}
	PDC	+19.16	+25.32	+33.88	+42.51	+55.14	+83.92	+105.05
Weight gain (g)	14.71±0.67^{a}	17.82±0.78^{b}	18.82±0.82^{b}	20.21±0.85^{b}	21.61±0.88^{b}	23.66±0.92^{b,c}	28.33±0.96^{b,d}	31.76±0.97^{b,e}
	PDC	+21.14	+27.94	+37.39	+46.91	+60.84	+92.59	+115.91
Daily Growth rates (DGR) (g)	0.245±0.018^{a}	0.322±0.012^{b}	0.339±0.01^{b}	0.362±0.02^{b}	0.386±0.022^{b,c}	0.420±0.024^{b,d}	0.498±0.035^{b,e}	0.529±0.041^{b,f}
	PDC	+31.43	+38.37	+47.76	+57.55	+71.43	+103.27	+115.92
Specific Growth Rates (SGR)	1.72±0.14^{a}	1.84±0.15^{a}	1.88±0.14^{a}	1.93±0.16^{b}	1.97±0.16^{b}	2.03±0.19^{b}	2.16±0.21^{b}	2.29±0.25^{b,c}
	PDC	+6.98	+9.30	+12.21	+14.53	+18.02	+25.58	+33.14
Protein Efficiency Ratio (PER)	5.42±0.22^{a}	6.78±0.26^{b}	7.32±0.23^{b,c}	7.49±0.26^{b}	7.63±0.28^{b}	7.74±0.32^{b}	8.39±0.27^{b}	8.54±0.28^{b}
	PDC	+25.09	+35.06	+38.19	+40.77	+42.80	+54.80	+57.56
Feed Conversion Ratio (FCR)	2.53±0.20^{a}	2.22±0.12^{b}	2.13±0.14^{b}	1.78±0.12^{b,c}	1.65±0.10^{b}	1.59±0.09^{b}	1.41±0.09^{b}	1.23±0.07^{b,c}
	PDC	-12.25	-15.81	-29.64	-34.78	-37.15	-44.26	-51.38
Feed Conversion Efficiency (FCE) (%)	38.48±0.93^{a}	45.38±0.96^{b}	47.37±0.97^{b}	49.39±0.99^{b}	52.45±1.00^{b,c}	53.45±0.98^{b}	58.46±0.99^{b,c}	64.32±1.04^{b,d}
	PDC	+17.93	+23.10	+28.35	+36.30	+38.90	+51.92	+67.15
Feed Efficiency Ratio (FER)	0.389±0.012^{a}	0.441±0.025^{b}	0.445±0.02^{b}	0.452±0.02^{b}	0.472±0.028^{b}	0.483±0.025^{b}	0.525±0.026^{b,c}	0.538±0.031^{b}
	PDC	+13.36	+14.39	+16.19	+21.33	+24.16	+34.96	+38.30
Harvest Size (g)	16.32±0.68^{a}	19.36±0.73^{b}	20.38±0.75^{b}	21.79±0.76^{b,c}	23.22±0.74^{b,d}	25.24±0.78^{b,e}	29.88±0.90^{b,f}	33.12±0.86^{b,g}
	PDC	+18.62	+24.87	+33.51	+42.27	+54.65	+83.08	+102.94
Productivity (kgs)	7.26±0.29^{a}	9.01±0.34^{b}	9.58±0.39^{b}	10.24±0.50^{b,c}	10.80±0.54^{b,d}	11.86±0.56^{b,e}	14.34±0.63^{b,f}	16.06±0.59^{b,g}
	PDC	+24.10	+31.95	+41.04	+48.76	+63.36	+97.52	+121.21

Initial weight 1.52±0.07 g (60 days)
All Values are Mean ± SD of six individual observations
PDC: Percent Deviation over respective Control
Values with different superscripts are significantly different from each other @ $p < 0.05$.

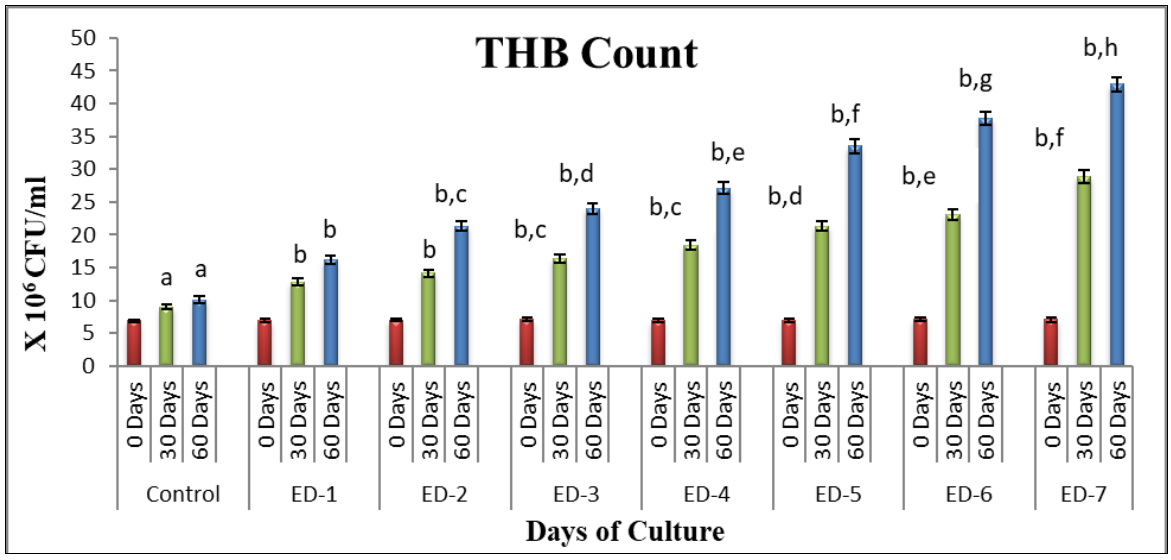

Fig 1: Total Heterotrophic Bacteria (THB) count at different Days of Culture operation

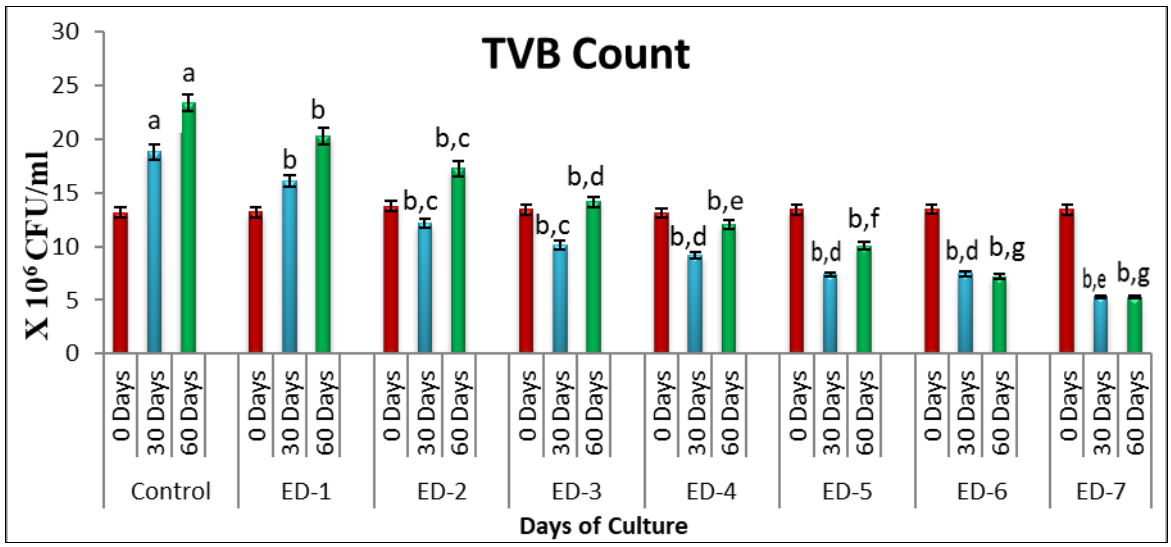

Fig 2: Total Vibrio Bacteria (TVB) count at different Days of Culture operation

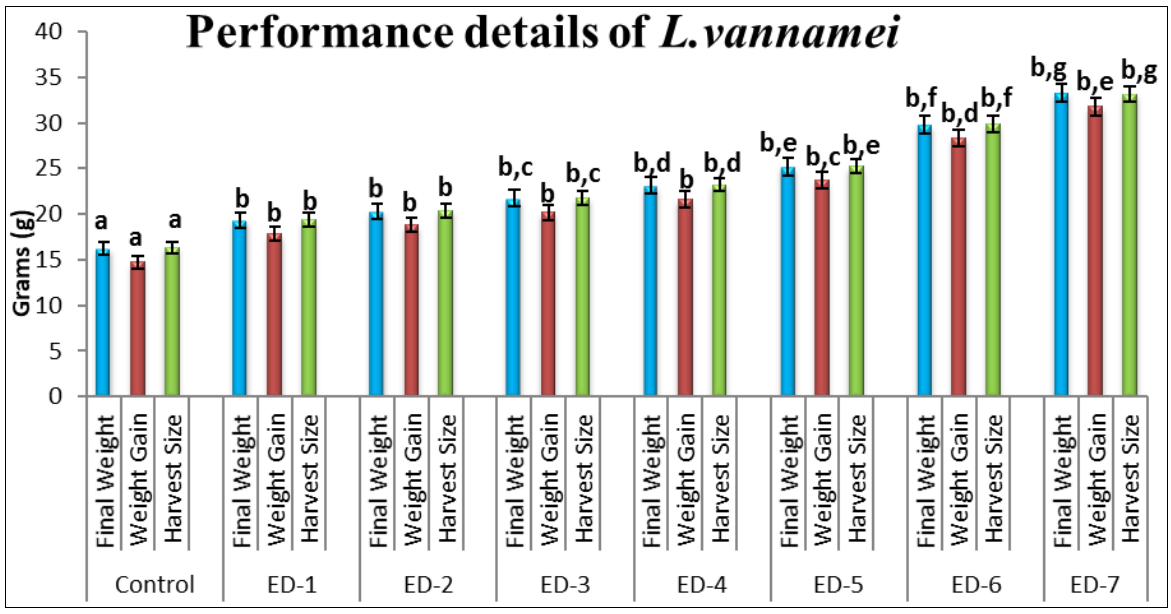

Fig 3: Growth Performance of *L. vannamei* under different Feeding trails

4. Conclusion

In summary, the addition of both probiotics and bioflocs resulted in the perfect maintenance of water quality metrics. High mechanical aeration encourages the microbial

community to break down harmful nitrogen and other substances, which also serves as a food supplement for prawns. As a result, productivity rates were significantly raised. Thus, BFT is a productive substitute for pathogens, maintains optimal water quality, and promotes prawn minimal water exchange system development and survival.

In addition to maintaining appropriate water quality parameters, the combined use of probiotics and bioflocs in the culture operation also improves *L. vannamei* growth performance. When culture management was correctly carried out by the culturists, the combined use was adequate to maintain the culture system and demonstrated to exert outstanding bioremediation and biocontrol.

5. References

1. NFDB (National Fisheries Development Board); 2020 March 15. Available from http://nfdb.gov.in/about-indian-fisheries
2. FAO (Food and Agriculture Organization of the United Nations). Global Aquaculture Production; c2020. p. 1950-2018. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en
3. Boyd CE. Environmental management of shrimp farms in Asia to promote healthy shrimp and reduce negative impacts. Asian Fisheries Science. 2010;23:463-481.
4. Mustafa A, Syah R, Paena M, Suguma K, Kontara EK, Muliawan I, *et al*. Strategy for Developing Whiteleg Shrimp (*Litopenaeus vannamei*) Culture Using Intensive/Super-Intensive Technology in Indonesia. Sustainability. 2023;15(3):1753. DOI: https://doi.org/10.3390/su15031753
5. FAO. Food and Agriculture Organization of the United Nations. In: The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome; c2012.
6. Andre Braga, Magalhaes V Hanson T, Morris T, Samocha TM. The effects of feeding commercial feed formulated for semi-intensive systems on *Litopenaeus vannamei* production and its profitability in a hyper-intensive biofloc-dominated system. Aquaculture Reports. 2016;3(2):172-177. DOI:10.1016/j.aqrep.2016.03.002
7. Tacon AGJ, Cody JJ, Conquest LD, Divakaran S, Forster IP, Decamp OE. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquaculture Nutrition. 2002;8(2):121-137. DOI: https://doi.org/10.1046/j.1365-2095.2002.00199.x
8. Ray AJ, Gloria S, Luis V, Browdy CL, Leffler JW. Effects of Biofloc Reduction on Microbial Dynamics in Minimal-exchange, Superintensive Shrimp, *Litopenaeus vannamei*, Culture Systems. Journal of World Aquaculture Society. 2012;43(6):790-801. DOI:10.1111/j.1749-7345.2012.00607.x
9. Krummenauer D, Peixoto S, Cavalli R, Poersch LH, Wasielesky W. Superintensive Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System in Southern Brazil at Different Stocking Densities. Journal of World Aquaculture Society. 2011;42(5):726-733. DOI:10.1111/j.1749-7345.2011.00507.x
10. Xu W, Wen G, Su H, Xu Y, Hu X, Cao Y. Effect of Input C/N Ratio on Bacterial Community of Water Biofloc and Shrimp Gut in a Commercial Zero-Exchange System with Intensive Production of *Penaeus vannamei*. Microorganisms. 2022;10(5):1060. DOI: https://doi.org/10.3390/microorganisms10051060
11. Ponce-Palafox J, Martine-Palacios CA, Ross LG. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture. 1997;157(1–2):107-115. DOI: https://doi.org/10.1016/S0044-8486(97)00148-8
12. Avnimelech Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture. 1999;176(3):227-235. DOI:10.1016/S0044-8486(99)00085-X
13. AOAC. Official methods of Analysis (ed. By K. Helrich) pp 1904.Association of Official Analytical Chemists. Virginia, Washington DC, USA; c1995.
14. Jackson ML. Soil Chemical Analysis. Prentice Hall of India Pvt Ltd, New Delhi. 1967;498:111-203.
15. Parsons TR, Maita Y, Lalli CM. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York; c1985.
16. Avnimelech Y, Kochba M. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks using 15N tracing. Aqua. 2009;287:163-168.
17. Naresh. Efficacy of selected Probiotics and Prebiotics in larval Rearing and culture of Penaeid prawns *Penaeus monodon* and *Penaeus semisulcatus*. Ph.D. Thesis, Sri Venkateswara University, Tirupati; c2016.
18. Ekasari J, Crab R, Verstraete W. Primary nutritional content of Bioflocs cultured with different organic sources and salinity. Hayati Journal of Biosciences. 2010;17(3):125-130. DOI: https://doi.org/10.4308/hjb.17.3.125
19. Walkley A, Black IA. An examination of the Degrjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science. 1934;37:29-38. DOI: http://dx.doi.org/10.1097/00010694-193401000-00003
20. APHA. Standard Methods for the examination of Water and Waste water, 19th ed. American Public Health Association. New York. Petrusewicz K and Macfadyen A (1970). Blackwell, Oxford, 2005.
21. AOAC. Official Methods of Analysis. 18th edn. Association of Official Analytical Chemists; Arlington, VA, USA; c2005.
22. Ferreira NC, Bonetti C, Seiffert WQ. Hydrological and Water Quality Indices as management tools in marine shrimp culture. Aquaculture. 2011;318(3-4):425-433. DOI: https://doi.org/10.1016/j.aquaculture.2011.05.045
23. Boyd CE. Chemistry and efficacy of amendments used to treat water and soil quality imbalances in shrimp ponds. In: Swimming through troubled water. Proceedings of the special session of Shrimp Farming Aquaculture 1995. Eds. CL Browdy and JS Hopkins p. World Aquaculture Society, Baton Rouse, USA; c1995. p. 166-188.
24. Carbajal Hernandez JJ, Fernandez LPS, Pogrebnyak O. Assessment and prediction of water quality in shrimp culture using signal processing techniques. Aquaculture International. 2011;19(6):1083-1104. DOI:10.1007/s10499-011-9426-z
25. Lalitha Kumari Ch. The Effect of Bioflocs on the Aquaculture production with special emphasis to Pacific White shrimp *Litopenaeus vannamei*. Ph.D. Thesis S.V University, Tirupati, India; c2019.

26. Boyd CE, Tucker CS. Pond Aquaculture Water Quality Management. Kluwer Academic publishers. Boston, USA; c1998.
27. Abdelrahman HA, Abebe A, Boyd CE. Influence of variation in water temperature on survival, growth and yield of Pacific white shrimp Litopenaeus vannamei in inland ponds for low-salinity culture. Aquaculture Research. 2019;50:658-672. DOI: 10.1111/are.13943
28. Yassien MH, Khoreba HM, Mohamed M, Ashry OA. Effect of biofloc system on the water quality of the white leg shrimp *Litopenaeus vannamei* reared in zero water exchange culture tanks. Egyptian Journal of Aquatic Biology and Fisheries. 2019;23(2):133-144. DOI:10.21608/ejabf.2019.33952
29. Zafar MA, Haque MM, Aziz MSB, Alam MM. Study on water and soil quality parameters of shrimp and prawn farming in the southwest region of Bangladesh. Journal of the Bangladesh Agricultural University. 2016;13(1):153-160. DOI:10.3329/jbau.v13i1.28732
30. Itala GSS, Glauber PCS, Carlos YBO, Clarissa VESC, Luis OB, Alfredo OG. Can shrimp farming waste water negatively affect water quality and zooplankton community structure of a Neotropical estuary? A case study during a productive cycle of *Litopenaeus vannamei*. International Aquatic Research. 2021;13:209-217. DOI: https://doi.org/10.22034/IAR.2021.1935831.1172
31. Lavanya C, Neeraja T. Rearing of White Leg Shrimp *Litopenaeus vannamei* (Boone, 1931) in Biofloc and Substrate Systems: Microbial Community of Water, Growth and Immune Response of Shrimp. Turkish Journal of Fisheries & Aquatic Sciences. 2021;22(3):TRJFAS20130. DOI: http://doi.org/10.4194/TRJFAS20130
32. Carbajal Hernandez JJ, Fernandez LPS, Villa LA, Ochoa JA, Trinidad JFM. Water quality assessment in shrimp culture using an analytical hierarchical process. Ecological Indiators. 2013;29:148-158. DOI: 10.1016/j.ecolind.2012.12.017
33. Ebeling JM, Timmons MB, Bisogni JJ. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture. 2006;257:346-358.
34. De Schryver P, Crab R, Defoirdt T, Boon N. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture. 2008;277(3-4):125-137. DOI:10.1016/j.aquaculture.2008.02.019
35. Krishna Naik M, Srinivasulu Reddy. Effect of Biofloc system on growth performance in shrimp *Litopenaeus vannamei* under different C: N ratios with sugarcane molasses. International Journal of Scientific & Engineering Research. 2020;11(5):243-262.
36. Jose JCH, Luis PSF, Luis AVV, Jesus ACO, Jose FMT. Water quality assessment in shrimp culture using an analytical hierarchical process. Ecological indicators. 2013;29:148-158.
37. Alexander C, Akhtar MS. Nitrite implications and its management strategies in aquaculture: A review. Reviews in Aquaculture. 2020;12(2):878-908. DOI: https://doi.org/10.1111/raq.12354
38. Cheng Z, Qianqian Z, Erchao L, Daxian Z, Shengming S. Role of hypoxia in the behaviour, physiology, immunity and response mechanisms of Crustaceans: A Review. Reviews in Aquaculture. 2021;14 (2):676-687. DOI: https://doi.org/10.1111/raq.12618
39. Gladys VC, Martin GFE, Cecilia V, Cristina CS. Toxicity of Ammonia, nitrite and nitrate to *Litopenaeus vannamei* juveniles in low-salinity water in single and ternary exposure experiments and their environmental implications. Environmental Toxicology and Pharmacology. 2019, 70. DOI:10.1016/j.etap.2019.05.002
40. Guozhi L, Xu J, Meng H. Nitrate accumulation in Biofloc systems. Aquaculture. 2019;520(1):734675. DOI:10.1016/j.aquaculture.2019.734675
41. Glen RRP, Teresa G, Luis RMC, Anselmo MB, Francisco VA, Marcel MP. The Nitrification process for nitrogen removal in Biofloc system aquaculture. Reviews in Aquaculture. 2020;12(4):12431. DOI:10.1111/raq.12431
42. Pankaj Kumar, Jetani KL, Yusuzai SI, Sayani AN, Dar SA, Rather MA. Effect of sediment and water quality parameters on the productivity of coastal shrimp farm. Advances in Applied Science Research. 2012;3(4):2033-2041.
43. Avnimelech Y. Biofloc Technology. A Practical Guide Book. World Aquaculture Society. LA, USA; c2015.
44. Boyd CE, Thunjai T, Boonyaratpalin M. Dissolved salts in water for inland low salinity shrimp culture. Global Aquaculture Advocate. 2002;5:40-45.
45. Wu JXU, Lu QP, Xiao HS, Jie H. Effects of Bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research, 2013, 44(7). DOI:10.1111/j.1365-2109.2012.03115.x
46. Rouf MA, Islam MMM, Hambrey J, Telfer T. Nutrient budget of Shrimp Culture Ponds in Southwest Coastal Region of Bangladesh. Journal of Aquaculture in the Tropics. 2012;27(1-2):41-51.
47. Bambang W, Rifqi M, Mashar A, Nazar F, Wardiatno Y. The contribution of phytoplankton in the carbon adsorption and stock during shrimp culture in Brackish water ponds. Biodiversitas Journal of Biological Diversity. 2020;21(11):5170-5177. DOI:10.13057/biodiv/d211123
48. Hassan AH, Rashid T, Magreth K. Study on the water quality parameters in semi-intensive coastal shrimp culture system in Mafia Island, Tanzania. Journal of Environment and Earth Science. 2015;5(1):142-150.
49. Goh JXH, Tan LTH, Law JWF, Khaw KY, Zengin G, Chan KG, Letchumanan V, Lee LH, Goh BH. Probiotics: Comprehensive exploration of the growth promotion mechanisms in shrimps. Progress in Microbes and Molecular Biology. 2023;6(1):1-86. DOI: https://doi.org/10.36877/pmmb.a0000324
50. He S, Liu W, Zhou Z, Mao W, Ren P, Marubashi T, Ringe E. Evaluation of Probiotic strain *Bacillus subtilis* C-3102 as a feed supplement for koi carp (*Cyprinus carpio*). Journal of Aquaculture Research & Development. 2011;S1:005. DOI: 10.4172/2155-9546.S1-005
51. Hasan KN, Banerjee G. Recent studies on Probiotics as beneficial mediator in Aquaculture: A review. The Journal of Basic and Applied Zoology. 2020;81:53-69. DOI: https://doi.org/10.1186/s41936-020-00190-y
52. Einar Ringo. Probiotics in shell fish aquaculture. Aquaculture and Fisheries. 2020;5(1):1-27.

DOI: https://doi.org/10.1016/j.aaf.2019.12.00
53. Chaudhary A, Hussain Z, Akram AM, Alorabi M, Sarwar N, Rehman RA, *et al*. Impact of *Bacillus subtilis* supplemented feed on growth and biochemical constituents in *Labeo rohita* fingerlings. Journal of King Saud University. 2021;33(8):101668. DOI: https://doi.org/10.1016/j.jksus.2021.101668
54. Soto JO. Feed intake improvement, gut microbiota modulation and pathogens control by using *Bacillus* species in shrimp aquaculture. World Journal of Microbiology and Biotechnology. 2021;37(2):28. DOI: 10.1007/s11274-020-02987-z.
55. Shoaibe HTS. Probiotic strains used in Aquaculture. International Research journal of Microbiology. 2018;7(2):43-55. DOI: 10.14303/irjm.2018.023
56. Krishna Naik M. Monitoring the effects of Probiotics and Bioflocs on growth performance and immune responses in Pacific White shrimp *Litopenaeus vannamei*. PhD Thesis, Sri Venkateswara University, Tirupati; c2021.
57. Seenivasan C, Bhavan PS, Radhakrishnan S. Effect of Probiotics (Binifit TM) on survival, growth, biochemical constituents and energy budget of the fresh water prawn *Macrobrachium rosenbergii* post-larvae. Aquaculture. 2011;41:5919-5927.
58. Seenivasan C, Bhavan PS, Radhakrishnan S, Muralisankar T. Effects of Probiotics on survival, growth and Biochemical constituents of Fresh water prawn *Macrobrachium rosenbergii* post Larvae. Turkish journal of Fisheries and Aquatic Sciences. 2012;12:331-338. DOI:10.4194/1303-2712-v12_1_04
59. Seenivasan C, Bhavan, PS, Radhakrishnan S, Shanthi R. Erichment of *Artemia nauplii* with *Lactobacillus sporogenes* for enhancing the survival, growth, and levels of biochemical of constituents in the post- larvae of freshwater prawn *Macrobrachium rosenbergii*. Turkish journal of Fisheries and Aquatic Sciences. 2012a;12:23-31. DOI:10.4194/1303-2712-v12_1_04
60. Seenivasan C, Radhakrishnan S, Shanthi R, Muralisankar T, Bhuvan PS. Effect of *Lactobacillus sporogenes* on survival growth, biochemical constituents and energy utilization of fresh water prawn *Macrobrachium rosenbergii* post larvae. The Journal of Basic & Applied Zoology. 2014;67(2):19-24. DOI: https://doi.org/10.1016/j.jobaz.2013.12.002
61. Hai NV. The use of Probiotics in Aquaculture. Journal of Applied Microbiology. 2015;119(4):917-935. DOI: https://doi.org/10.1111/jam.12886
62. Michael SE, Abarike ED, Cai J. A Review on the probiotic effects on Haematological parameters in fish. Journal of Fisheries Sciences. 2019;13(3):25-31.
63. Puvanasundram P, Chong CM, Sabri S, Sabri MY, Karim M. Multistrain probiotics: Functions, effectiveness and formulations for Aquaculture operations. Aquaculture Reports. 2021;21(81):100905. DOI: 10.1016/j.aqrep.2021.100905
64. Wuertz S, Schroeder A, Wanka KM. Probiotics in Fish Nutrition – Long-standing household Remedy or Native Nutraceuticals. Water. 2021;13(10):1348. DOI: https://doi.org/10.3390/w13101348
65. Aisyah, Ayi Y, Yuli A. Evaluation of various sources of bacteria on Biofloc productivity. Asian Journal of Fisheries and Aquatic Research. 2021;12(2):30-39. DOI: 10.9734/AJFAR/2021/v12i230231
66. Vincent MS, Amoah K, Cai J. Effect of *Bacillus* Probiotics on the Immunological Responses of Nile Tilapia (*Oreochromis niloticus*): A Review. Fishes. 2023;8(7):366. DOI: https://doi.org/10.3390/fishes8070366
67. Kuebutornye FKA, Abarike ED, Sakyi ME, Lu Y, Wang Z. Modulation of nutrient utilization, growth, and immunity of Nile Tilapia, *Oreochromis niloticus*: The role of Probiotics. Aquaculture International. 2020;28(1):277-291. DOI: 10.1007/s10499-019-00463-6
68. Sundaram M, Panigrahi A, Ganesh J, Nila rekha P, Sivagnanam S, Rajamanickam S. Evaluation of different Probiotic strains for growth performance and immuno modulation in Pacific white shrimp *Penaeus vannamei* Boone, 1931. Indian Journal of Fisheries. 2017;64:89-96. DOI: 10.21077/ijf.2017.64.special-issue.76210-13.