Monoculture and Mixed culture of Pacific white shrimp *Litopenaeusvannamei* and Tiger shrimp *Penaeus monodon* in Biofloc system – A comparative study

ABSTRACT

The microbial modification method known as Biofloc Technology (BFT) has been used a regularly in prawn culture operations lately. The primary component of BFT culture systems, microbial Biofloc, is responsible for improving water quality, supplementing natural nutrition, and encouraging the growth and welfare of grown prawns. An attempt has been made to investigate the effects of adding probiotics (*Bacillus licheniformis* and *Lactobacillus rhamnosus*) and the Biofloc system on water quality parameters and the performance of shrimp *Litopenaeusvannamei*and *Penaeus monodon*. Various external carbon sources, such as molasses and tapioca flour combinations, were used for the development of Bioflocs in the present study both under monoculture and mixed culture operation. Experimental diets comprising one control, one supplemented with probiotics and bioflocs (combinations of tapioca flour and molasses developed with varying external carbon sources), and both monoculture and mixed culture operations of *Litopenaeusvannamei* and *Penaeus monodon* were used in feeding trail experiments for about 120 days. Because mixtures of both Probiotics and Bioflocs were added, all the water quality indicators remained in optimal condition and provided resistance and protection against Candidate species of cultivation. Along with acting as a food supplement for prawns, intense mechanical aeration also promotes the microbial community's breakdown of toxic nitrogen and other pollutants. Productivity rates increased dramatically as a result. As a result, BFT is an effective pathogen replacement, preserves ideal water quality, and boosts the growth and survival of prawns from *L. vannamei* and *P. monodon*. Probiotics and bioflocs are used in combination in this work to maintain good water quality metrics and *P. monodon* and *L. vannamei* growth performance.

Key words:*L. vannamei*, *P. monodon*, Bioflocs, C : N ratios

1. INTRODUCTION

To enhance productivity and satisfy the growing need for protein in the population, aquaculture is still at the forefront of the development of intensive farming technologies. Most prawns are raised in many regions of the world, including India, through intense culturing. India rose to prominence as the world's top producer of prawns. Disease outbreaks and more severe environmental issues have been linked to the proliferation of prawn growing techniques [1, 2, 3]. The design and development of alternative farming systems for the integrated culture of aquatic creatures has been the focus of several studies [4].Aquaculturists have started using a variety of techniques to address these problems, such as Integrated Multi-Trophic Aquaculture and Polyculture, which is a better model than Monoculture because it uses resources more effectively, generates less waste, and yields a wider range of products [3]. In a perfect Polyculture pond, the stocking species have complimentary and dissimilar eating patterns and

occupy distinct niches. Major issues confront prawns raised in dense monocultures. First, a high metabolite content degrades the water quality; second, the pond system experiences excessive water exchange and low nutrient utilisation [5].One workable solution to the aforementioned issues is the application of Biofloc technology (BFT) and probiotics, which allow for better nutrient cycling than traditional farming systems, increasing the yield of candidate species and possibly preventing disease outbreaks [6]. BFT, a novel kind of water treatment technology, is frequently mentioned as a way to lower feed costs and environmental issues related to waste product discharge [7]. BFT entails accelerating the heterotrophic bacteria's uptake of inorganic nitrogen. These bacteria produce new cells by consuming carbohydrates and ammonium in addition to organic nitrogenous wastes. At the same time, they make Bioflocs by utilising suspended particles, organic materials, and microorganisms [8].Apart from decreasing the production of wastewater, the microorganisms found in bioflocs can be used as food [9,10]. This allows for a decrease in the amount of protein in commercial feeds or even as a source of protein supplementation for species that need larger amounts. Penaeid shrimp's capacity to ingest microorganisms can contribute to the high success rate of this kind of feeding and culture system. The zootechnical performance of native species in a BFT system has not been thoroughly studied. Examining the viability of raising Pacific white prawns (*L. vannamei* and *P. monodon*) in a BFT system during the Grow out phase under both Monoculture and Mixed culture operation was the study's main goal.

2. MATERIALS AND METHODS

The present Experiments was conducted in the field ponds at Allur (Latitude 14.7021° N and Longitude 80.0758° E) located at a distance of about 20 kms from Nellore, Andhra Pradesh, India.

2.1 Experimental ponds and cultivable species

Five ponds of 0.5 ha i.e. one each pond devoted for Control, two ponds were allocated for the Monoculture of *L.vannamei* and *P.monodon*, whereas the fourth pond was used for the mixed culture of both *L.vannamei* and *P.monodon*under Biofloc treatments. The ponds were prepped by carrying out all of the standard pre-stocking procedures prior to stocking. The ponds were entirely emptied, and any undesired or predatory fish were manually eliminated. Following drying, the cultured ponds were kept at 1.5 metres throughout the duration of the experimental feeding trail, or roughly 120 days, to make up for water lost to evaporation and seepage.Cow dung was applied to all of the cultured ponds at a rate of 10 tons/Ha/Crop, divided into five doses and applied every two weeks. Split doses of additional inorganic fertilizers, such as phosphates and nitrogen, were administered every two weeks. Calcium carbonate, or $CaCO_3$, was limed at irregular intervals of one month at a rate of 80 kg per hectare to preserve pond sanitation and the appropriate pH of the water.

Local aquaculture farms provided the penaeid shrimp, *L. vannamei* and *P. monodon*, which were subsequently transferred to the shrimp field units in oxygenated polythene bags. After being chosen, prawns weighing 0.67 ± 0.05 g were allowed to acclimatize in their designated ponds for a week. They were then fed twice a day with specially prepared feed (see the table for the meal's composition). Intermittent sampling allowed for the weekly adjustments of the feeding quantity

based on the rise in biomass. Molasses and tapioca are employed as external carbon sources for the manufacture of Bioflocs in both Monoculture and Mixed culture of *L. vannamei* and *P. monodon.* This study used the completely randomised design method with independent variables. Using specific carbon sources and water taken from nearby shrimp culture ponds for innocula growth [7], bioflocs were created in a variety of plastic containers with a 25-liter capacity. The microbial mass was allowed to grow to its maximum volume for two weeks. Once a week at noon, pre-weighed carbon sources with molasses and tapioca flour were well combined and released into the cultured ponds. The calculations for this process were made using an Excel programme. The Carbon:Nitrogen (C/N) ratio in Biofloc treatments (BFT) was maintained at 15:1 to promote bacterial growth. The carbon and nitrogen contents of the daily feed intake as well as the addition of carbon sources to the culture ponds were used to approximate this ratio.

2.2 Probiotic Feed Preparation

Probiotic-enriched feeds were made in accordance with Naresh's recommendations [11]. Probiotic bacteria *Lactobacillus rhamnosus* and *Bacillus licheniformis* were procured from the Gene Bank and Microbial Type of Culture Collection of the Institute of Microbial Technology located in Chandigarh, India. The bacterial cultures were harvested by centrifuging them for 10 to 12 minutes at 10,000 rpm after being maintained alive in the nutrient broth. After being washed with phosphate buffer, they were reconstituted in pH 7.4 phosphate buffer saline. The re-suspended bacteria were dispersed uniformly throughout the food pellets by spraying. After the probiotic blended feed was created, it was dried at 400 degrees Celsius, covered with an airtight polythene bag, and stored in the refrigerator. Once every ten days, a probiotic-blend meal containing 10 billion CFU/kg of *Bacillus licheniformis* and *Lactobacillus rhamnosus* was prepared.

2.3 Preparation of Bioflocs

The levels of feed Carbon: Nitrogen (C:N) ratio were calculated by dividing the total carbon intake by the total nitrogen input used in prawn cultures [7,12,13, 14]. Using a carbon to nitrogen (C:N) ratio of 15:1 and two sources of carbohydrates (Tapioca flour and Molasses mixed), a completely randomized experimental design with three replications was used. The amount of pre-weighed carbon sources, like molasses and tapioca flour, that required to be well mixed before being applied to the tank surfaces once a week at 12:00 Noon was calculated using the Excel software.A C:N ratio of 15:1 is used in Biofloc Technology (BFT) treatments to stimulate bacterial growth. This ratio is roughly determined using the daily feed input's carbon and nitrogen contents as well as the injection of carbon sources to the Biofloc tanks. Feed was the primary source of nitrogen in the shrimp culture process, while external carbon sources such molasses and tapioca flour provided the majority of the carbon. Moreover, the 30% protein feed with 4.65% nitrogen content assumption was used to calculate the meal's nitrogen content [7]. Since the feed and all external carbon sources employed in this study had a carbon content greater than 30%, the nitrogen content of the feed with more than 30% protein was determined by converting that assumption.

To encourage nitrogen loading in an aquaculture system, NH_4Cl, KH_2PO_4, and Na_2HPO_4 were added at 96, 31, and 64 mg/lit, respectively, to each tank [15]. The ratio of molasses to feed was

estimated to obtain the required Carbon: Nitrogen (C:N) ratio, assuming that 50% of the nitrogen from the feed taken by the shrimp would be excreted into the aquatic environment [7]. The weight ratio between the carbon source and feed can be calculated using the formula below, which is based on the previously provided data:

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

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%)
% C_{CH} :		Carbon content in the Carbon source

$$\textbf{C:N} = \frac{\text{C = Approximately 50\% of total feed amount}}{\text{N = \% of protein in feed x 0.155}}$$

The Walkley and Black method was applied to determine the carbon content [16]. Total ammonia nitrogen (TAN) concentration and other water quality indicators were measured using APHA-recommended methods [17]. The feed protein/nitrogen and input carbon utilized in the prawn culture were taken into account when calculating the C:N ratio [18]. The addition of different levels of carbon and the assumption of feed protein (35% protein feed) were used to compute the concentrations of carbon and nitrogen. One gram of feed cannot be added to an external carbon source until the nitrogen in the system has been assimilated and assumed.

2.4 Growth Performance Parameters

The feed conversion ratio, protein efficiency ratio, feed efficiency ratio, productivity rates, average body weights, average body growth rates, and particular growth rates were all monitored and documented as growth parameters. All of the above parameters were computed using the following formulas.

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

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

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

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

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

$$\frac{(\text{Log } W_2 - \text{Log } W_1)}{T} \times 100$$

Where,
W_1 : Weight of the Shrimp at the start of the Experiment
W_2 : Weight of the Shrimp at the end of the Experiment
T : Total number of days of Experiment.

Using the AOAC standard techniques [19], the moisture, protein, fat, and ash content of the chosen experimental diets were assessed. The samples' moisture content was ascertained by drying them in an oven for two hours at 135^0C in order to preserve weight. Using Kjeldahl's method (N X 6.25), the crude protein concentration was determined after acid digestion. The crude lipid content was determined by ether extraction using the Soxhlet technique. To measure the amount of Ash, dry samples were burned in a muffle furnace for six hours at 550^0C.

2.5 Statistical Analysis

The one-way analysis of variance (ANOVA; SPSS, 13.0) was utilized to determine whether there was a significant difference between the treatments. The means were compared and the difference between them was ascertained using the DMRT test. Every test had a significance level of $p<0.05$. Data are reported using standard deviations ± Means.

3. RESULTS AND DISCUSSION

The goal of the current study is to examine the growth performance and feed utilisation of *L. vannamei* and *P. monodon* in both monoculture and mixed shrimp cultures. The shrimp cultures are maintained for 120 days in rectangular culture ponds within a meticulously maintained shrimp culture facility, with the addition of probiotics and bioflocs. Ten thousand 0.67±0.05 g prawns were supplied for feeding experimental trails in order to undertake monoculture or mixed culture operations using either *P. monodon* or *L. vannamei*.Five thousand *L. vannamei* and five thousand *P. monodon* were selected in the mixed culture ponds for the purpose of culture

operation. The control diet wasformulated using feed items that were readily available in the area, such as animal protein from prawn meal and squilla meat, as well as plant protein from soy bean meal and wheat flour, in addition to basic formulation elements with a protein level of 35%. Two experimental feeds, one for control and the other for broad casting into the culture operation, were developed for this work.Probiotics, specifically *B. licheniformis* and *L. rhamnosus* (@10 billion cfu/kg feed), were added to the experimental feed along with Tapioca flour and molasses, which served as external carbon sources to promote the growth of Bioflocs in the culture ponds. Table 2 presents an analysis of the proximate composition of the control and experimental diets that were formulated. In both the control and Probiotic and Biofloc added experimental feeds, which are formulated to be iso-energetic and have 395 Kcal/100 g feed, the organic matter values were found to be in the range of 82-83%, the ash content values were found to be around 16-17%, the crude protein content values were found to be between 35-36%, the crude lipid content values were 6-7%, the crude fiber content values were approximately 4.5%, and the nitrogen free extract (NFE) values were found to be between 26-27%.

Water Quality Parameters:

Every water quality measure was tracked, examined, and shown in Table 3 (Figures.1-3). Throughout the 120-day feeding trail experiment, 100 ml of water from the Control and Experimental ponds were taken early in the morning, at roughly 7:00 AM. Every day, visual measurements of the water's turbidity, flocculation, and colour were made. Every day, twice in the morning and again in the evening, measurements of temperature, pH, salinity, and Floc volume were made. Biological oxygen demand (BOD), DO Reduction Rate (DORR), Total Alkalinity, Total Ammonia Nitrogen (TAN), Dissolved Oxygen (DO), Total Suspended Solids (TSS), Chlorophyll-a, and Phytoplankton density were among the other parameters that were measured twice a week.

In the current study, the temperature ranged between 22 and 29°C, with a low of 22°C and a maximum of 29°C recorded in culture pond water. Temperature is an important water quality measure. However, the measured average temperatures range from 25.5 to 26.5°C, and neither the Control nor the Biofloc supplemented culture ponds ever shown any appreciable fluctuations in temperature. The temperature range, while still regarded as noteworthy, is mostly influenced by variations in the daily air temperature. Since temperature has a major impact on crustacean metabolism, it is thought to be one of the most critical environmental elements that affect prawn farming [20].The study's temperature readings fell between the ideal and desirable ranges for prawns to allow for appropriate growth and survival of aquatic life. The pH values that were obtained in this investigation ranged from 7.2 to 7.4. In the current study, the Biofloc system was kept at an alkalinity of 100 ppm and a pH of around 7.5 ppm using hydrated lime. The pH levels observed in this investigation were determined to be within the appropriate range, making them perfect for the monoculture or mixed culture operations of both *L. vannamei* and *P. monodon*.According to earlier findings, Penaeid prawn cultures grow better at pH values between 7.3 and 8.2, which results in higher production rates [21, 22]. One of the main variables influencing the concentrations of ionized and non-ionized ammonia in water is pH. Ammonia is present in the pond culture system in two distinct forms: NH3 (unionized ammonia) and NH_4+ (ionized ammonia), which when combined are referred to as total ammonia nitrogen (TAN). It is well known that fragile shells and low survival rates are caused by extreme low and high pH

levels [18]. However, the pH ranges of 6.8 to 7.9 in the current study are suitable and perfect for a higher rate of prawn production.During the feeding trail in the current investigation, the salinity was kept constant at 10±1 ppt, the water volume was kept constant, and the evaporated water volume was refilled with freshly treated water. The current study concurs with previous publications in stating that raising the C:N ratios in the Biofloc ponds/tanks over 15:1 has a substantial impact on the pH values during the culture period by maintaining them relatively constant throughout the culture operation. This may be connected to the existence of heterotrophic bacteria, which eat organic debris and raise the concentration of carbon dioxide (CO_2) in the water while lowering pH levels. Because of microbial activity, pH typically decreases as the redox potential does [23].The current investigation also found that adding Biofloc to the culture ponds increased the rate of microbial activity. Previous researchers also found that alkalinity is consumed by nitrogen uptake by heterotrophic processes, which are likely to predominate in BFT systems, by half compared to nitrification (3.57 g alkalinity/of NH_4+-N). They also came to the conclusion that, since alkalinity concentration is related to water's buffering capacity, the high concentration of CO_2 resulting from cultured organisms and microbial respiration on water pH could be adequately buffered in BFT systems.When compared to the control pond, the dissolved oxygen (DO) content of the ponds treated with Biofloc was significantly ($p<0.05$) lower. While the Biofloc-added ponds registered 3.89 to 4.94 mg/lit, indicating a decrease of approximately 25%, the control ponds recorded a range of 5.28-6.43 mg/lit ($p<0.01$). When compared to the control ponds, the Biofloc culture system's characteristics—high respiration rates, bacterial metabolic activity, and organic matter accumulation—may be the cause of the DO decrease in the ponds with Biofloc added [24]. Low microbial and plankton densities could be the cause of the increased DO levels in the control ponds. In addition, the control pond's plankton density was found to be lower in this study than in ponds with Biofloc added.

The total ammonia nitrogen (TAN) values recorded were between 1.88 and 2.48 (2.18 mg/lit) in the control pond and 0.28 to 0.49 mg/lit (Average 0.39-0.41 mg/lit) in the ponds with Biofloc added. On the other hand, the values of nitrite (NO_2-N) were between 1.20 and 1.54 (Average 1.37 mg/lit) in the control pond and 0.55 to 0.84 (Average 0.68-0.71 mg/lit) in the Biofloc added ponds, and the values of nitrate (NO_3-N) were between 3.72 and 5..99 (Average 4.86 mg/lit) in the control pond and 1.51-2.78 (Average 2.07-2.25 mg/lit) in the ponds with Biofloc added. In comparison to control pond values, all three water quality parameters—TAN, Nitrite-N, and Nitrate-N—were found to be considerably ($p<0.05$) lower in all ponds that had Biofloc injected. All of the ponds with Biofloc added maintained water chemical quality parameters within an optimal range for the operation of prawn production.TAN, Nitrite, Nitrate, and Phosphate were among the chemical water quality indicators that varied considerably throughout the treatments. These variations were largely attributed to the type of aquatic organisms cultivated, particularly in polyculture mixed culture systems. In comparison to ponds that had Biofloc added, the phosphate content in control ponds was found to be lower. When comparing the phosphate content of all the Biofloc-added ponds to the control pond, a substantial ($p<0.05$) increase was observed. The use of liming materials to raise the pH of bottom sediments may be the cause of the elevated PO_4-P concentrations in all of the experimental ponds, since this enhances the availability of phosphorous deposits in sediments of the water column for culture operation [18].

Total Ammonia Nitrogen (TAN):

For the aquaculture of aquatic species, the nitrogen molecules nitrite-N (NO_2--N), nitrate-N (NO_3--N), and total ammonia nitrogen (TAN) are essential. TAN is liberated during the decomposition and excretion of organic materials. In higher concentrations, TAN can even be fatal to organisms and negatively impact their performance in culture. In culture operations, excretion products from faeces, urine, uneaten food, phytoplankton, and zooplankton were the main sources of TAN. Ammonia or non-ionized ammonia (gaseous) is considered harmful, in contrast to ionized ammonia or ammonium ion (NH_4).Low oxygen content, high temperature, and high pH all contribute to an increase in the unionised form (NH_3). The TAN levels in the feeding trail control group in this study were relatively high, 1.88 mg/lit, in comparison to all the feeding trail ponds in the Biofloc added group, which recorded relatively low TAN concentrations, ranging from 0.28 to 0.32 mg/lit. This indicates a significant ($p<0.05$) reduction in TAN levels when compared to both feeding trail control groups. In comparison to the Control and Probiotic addition groups of feeding trails, the TAN levels were lowered to almost 85% in the Biofloc added groups.Ammonia is the main consequence of protein catabolism in crustaceans. Ammonia increases tissue oxygen use, damages gills, and decreases blood's oxygen-carrying ability. Water contains both unionised (NH_3) and ionised (NH_4+) forms of ammonia, but the latter is more dangerous because it diffuses through cell membranes more readily [25]. Because the TAN levels in this study were deemed optimal, all of the Biofloc supplemented feeding trail groups had significantly higher growth and final production rates than the Control group.Additionally, the pH is kept below, the temperature is maintained between 27 and 28^0C, and the salinity is between 10 and 11 ppt, all of which encourage nitrification reactions and the reduction of ammonia toxicity [25]. It is abundantly evident from the data that probiotic addition had no discernible impact on bioremediation. Therefore, in the current study, the use of Probiotics in conjunction with the inclusion of Bioflocs is being fully utilised to preserve the water quality in water tanks.Biofloc can balance the ammonia concentration in the culture system because its heterotrophic bacteria can absorb ammonia 40 times faster than nitrification bacteria [26]. Additionally, according to De Schryver [27], Biofloc generated in the Bioreactor can convert 98% ammonia to nitrate at a concentration of 110 mg/lit/day. Heterotrophic bacteria's capacity to absorb ammonia is influenced by the C/N ratio, or the proportion of organic carbon to nitrogen, in the water. A higher C/N ratio will result in faster ammonia absorption. The results of this inquiry are corroborated by previous research, which demonstrated that the C/N ratio (15:1) was optimal for *L. vannamei* [28].

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

The primary nitrogenous material components, nitrite and nitrate, were also detected in every experimental feeding trail group. In contrast to the other experimental feeding trails, where groups that added Bioflocs recorded in the range of 0.68 to 0.71 mg/lit, the Control group's average Nitrite-N was 1.37 mg/lit. When comparing the Biofloc added trails to the Control group feeding trails, the results showed that the differences were statistically significant ($p<0.05$). The control group's nitrate-N values were determined to be 4.86 mg/lit, while all feeding trails with Biofloc added had considerably ($p<0.05$) lower values. The range of reduction was approximately 53-57%.

Between nitrite-N and nitrate-N, nitrite-N levels in the control group were 1.37 mg/lit, whereas nitrate-N values in the feeding trails of the control group were 4.86 mg/lit. It was discovered that

all Experimental feeding trail groups had considerably ($p<0.05$) lower levels of both nitrate-N and nitrite-N than the Control group. The number of chlorides, temperature, and oxygen concentrations in the growth media all affect how ammonia nitrogen transforms into nitrite-N and its hazardous form. The oxidation of significant chemicals, oxygen transport, and tissue damage are all impacted by nitrite toxicity.It was discovered that the optimal nitrite-N concentrations for Biofloc prawn production were less than 2 ppm [29]. The growth rates of the Biofloc-added feeding trails in the current study were found to be considerably ($p<0.05$) greater than those of the Control feeding trail group, as evidenced by the range of recorded values from 0.68 to 0.71 mg/lit.Nitrite is the intermediate stage that results from the conversion of ammonia to nitrate. It is well known that prawns raised in farms were incredibly toxic to nitrite and ammonia. High quantities of Nitrite-N usually deactivate haemoglobin in crustacean blood [30]. The immunological and circulatory systems of aquatic creatures are impacted by oxygen binding to copper at the gills and being carried throughout the body due to the lack of haemoglobin in prawn hemolymph [31].The byproduct of aerobic nitrification, nitrite-N, is thought to be less harmful. These chemicals' impacts on oxygen transport and osmoregulation are what make them hazardous.The final product, nitrate-N, was discovered to have a range of 2.31 to 4.34 mg/lit; concentrations over 200 mg/lit were found to be fatal, whereas these range values were determined to be most optimal for prawn culture [32]. Because phytoplankton in Biofloc requires nitrate-N to be taken from the water as a source of nutrients, the concentration of nitrate-N was found to be much lower in the current study, which may account for the sharp decrease in nitrite-N. The outcomes were discovered to corroborate previous reports [33].

The TAN concentrations in the Biofloc supplemented groups were found to be considerably ($p<0.05$) lower than those in the Feeding trail Control group. Heterotrophic bacteria in the current study are efficiently utilising ammonia, thanks to the availability of nitrate-N from the culture medium in all Biofloc supplemented groups. The study by Pankaj Kumar et al. [34] states that there is a positive correlation between salinity and temperature, salinity and primary productivity, salinity and dissolved oxygen, dissolved oxygen and nitrate, and nitrate and total available phosphorus at culture ponds.In order to prevent the harmful effects of high concentrations of Nitrite-N and Ammonia peaks in the cultured species during the process of employing the Biofloc system, Ammonia Nitrogen (TAN), Nitrite-N, and Nitrate-N were thought to be significant indicators in the culture operation. When comparing the results to feeding trails with either probiotics or controls, it is evident that all nitrogen-containing substances were greatly reduced in Biofloc added using BFT. Ammonia can be more efficiently absorbed by the heterotrophic bacteria in Biofloc for the production of proteinaceous components, hence adding external carbon sources can help balance the ammonium concentration in the Biofloc growth system.The governing parameters in this relationship are the microbial conversion coefficient and the C/N ratio in the microbial mass with the carbon content of the given material. According to Avnimelech [35], dietary protein concentration is not as necessary when carbs are added because doing so lowers the TAN level in the culture system. When compared to other Experimental BFT treated Feeding trail groups in the current investigation, the Control group's average levels of TAN were comparatively higher (Table 3). The Biofloc added groups did, however, show a mean value of lower NO3-N concentrations. This low level may be related to NO3-N uptake by microbes in the Biofloc added Feeding trail groups.

Overall, the varied component concentrations had no negative effects on the zootechnical performance or survival of the prawns, according to the study's Experimental Feeding group trails.

Phosphate-P:

The Control group's Phosphate-Phosphorus levels were at least 0.65 (ppm), and all of the Biofloc-added feeding trail groups had increasing values over time. The concentrations of PO4-P varied significantly between the Control and Experimental groups, according to the results. The use of liming materials to raise the pH of bottom sediments may be connected to elevated PO4-P concentrations in the experimental tanks because it enhances the availability of phosphorus deposited in sediments in the water column [36]. Phosphorus accumulation in the system can foster the formation of filamentous cyanobacteria, which can block shrimp gills and produce toxins lethal to penaeid shrimp, even though it has no direct effect on shrimp development [37]. Phosphorous enters the culture system primarily through fertilizers and feed application [38]. The culture pond or tank experiences a rise in phosphorus loading as a result of increased feed application. overall Phosphorous does not distinguish between bound and free forms; rather, it measures the overall amount of phosphorous in the water. Bound forms are present in the biomass of phytoplankton.

Alkalinity:

The control group in this study had alkalinity values of 151 mg/lit, while the experimental groups had significant ($p<0.05$) increases in alkalinity, with the experimental diet group reaching a maximum of 518 mg/lit (EDT).Alkalinity is the capacity of water to buffer or toleratepH changes caused by the addition of acid and base. It's critical to keep enough alkalinity reserves in Biofloc systems since the nitrifying bacteria in nitrification continuously deplete the water's alkalinity. Lack of alkalinity can cause a rapid drop in pH, which inhibits the growth of bacteria, especially essential nitrifying bacteria. In this case, ammonia builds up to the point that prawns' ability to feed declines. However, the current study indicated that the alkalinity levels were kept at an appropriate level, which led to considerable improvements in daily feeding rates, feed conversion efficiency, and production rates. Previous reports [39] provide support for the alkalinity levels found. 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):

The Control group recorded 259 mg/lit of total suspended solids (TSS), whereas all Experimental feeding trail groups showed a substantial ($p<0.05$) increase, with a maximum of 539 mg/lit recorded with Experimental Diet. TSS in the 375–542 mg/lit range in the Biofloc system guarantees effective bacterial activity and a good method to manage ammonia without causing excessive water respiration. Suspended particles provide bacteria both a surface on which to adhere and a carbon source for energy. The colour of the experimental tanks that had Biofloc added was discovered to be brown to dark brown/brown greenish. These tanks were assumed to include colonies of heterotrophic bacteria, microalgae, and protozoans, as well as suspended organic particles in the form of flocculated aggregates. While TSS concentration plays a critical

role in Biofloc systems, it is unclear how regulating particle concentration can boost prawn production rates in real-time. Additionally, TSS is known to drastically change the microbial communities and help get rid of dangerous species, which raises the productivity rates of *L. vannamei*.

BOD and COD:

Comparing the Experimental feeding trails to the Control group, the study found a significant ($p<0.05$) increase in the Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) values. BOD measures the oxygen requirement of the water. BOD is an analytical measure of how much dissolved oxygen (DO) aerobic bacteria, or microorganisms, utilize to develop on organic material in a water column at a specific temperature during a given period of time.

The present investigation found a considerable rise in the quantity of Total Heterotrophic Bacteria (THB) in all Experimental feeding trails. This finding could perhaps account for the comparatively elevated levels of BOD. Furthermore, aeration was used to keep the DO levels consistent. COD analysis is less accurate because it evaluates all organic matter that is capable of chemical oxidation rather than only the quantities of organic matter that has undergone biological oxidation. In the current experiment, both BOD and COD were found to be within the acceptable range for *L. vannamei* culture operation. 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 maintaining the ideal C:N ratio of 15:1, inorganic nitrogen becomes immobilised in bacterial cells.

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 biofloc volume in each experimental feeding trail was found to be considerably ($p<0.05$) larger in the current study than in the control group. When the optimal C:N ratio of 15:1 is maintained, inorganic nitrogen becomes immobilised within bacterial cells during the metabolism of organic substrates. The type of carbon source that is utilised to produce the flocs has an impact on the flocs' nutritional value as well as the Biofloc system's capacity to control the water quality in the culture system.Compared to the control group, there was a significant ($p<0.05$) increase in the numbers of phytoplankton and chlorophyll-a in all experimental feeding trails. Because ammonia and nitrite are removed from microbial communities by the Biofloc system, their concentrations are normally low. The nitrate concentration was too low because there was less ammonia nitrogen available for oxidation by nitrifying bacteria. The primary cause of the high levels of chlorophyll observed in this study is probably the reduced form of inorganic nitrogen that phytoplankton has absorbed. Because specific carbon sources are used in this study, bacterial succession and dominance over microalgae are encouraged. Chlorophyll-a is the primary indication of phytoplankton biomass. The collected findings are consistent with a strong relationship between phytoplankton abundance and chlorophyll-a. The obtained data supported earlier findings [40] that showed a strong correlation ($p<0.05$) between chlorophyll-a and phytoplankton cell density.

Growth and Feed Performance Studies:

Growth performance investigations using penaeid prawn *L. vannamei* and *P. monodon* were carried out in the current study using various feeding trails and the addition of Probiotics and Bioflocs in both monoculture and mixed culture operations. The acquired results were shown in Table 4 (Figures 4-6). Weekly growth rates were recorded in this investigation. The results show that, regardless of the type of culture—monoculture or mixed—both prawn species are growing more quickly in the Biofloc-added groups than in the control group. This is clearly attributable to the addition of both Probiotics and Bioflocs.The inclusion of both Probiotics and Bioflocs added feeding trail groups in monoculture or mixed culture operation considerably ($p<0.01$) boosted all growth related metrics, including weight gain, daily growth rates, specific growth rates, harvest size, and productivity rates.

Probiotics with both Lactobacillus and Bacillus are used in the current study to support growth potentials as measured by weight increase, particular growth rates, harvest size, and productivity rates. In aquaculture, probiotics are seen to be a good alternative to antibiotics, particularly when it comes to producing prawns because they boost growth rates and lower excessive mortality. The present study clearly demonstrates that providing probiotic-containing meals to *L. vannamei* resulted in a significant increase in the species' growth patterns.Research has been done on the use of probiotics, including bacteria, yeast, and microalgae, in the aquaculture of crustaceans, particularly prawns. Aquaculture has made use of Bacillus species, which generate lactic acid bacteria, to improve the wet environment [41]. Lactobacillus species exhibit potent antibacterial activity against pathogenic microorganisms [42]. It has been demonstrated that adding probiotics as food supplements or water additives can boost aquaculture production [40, 43, 44]. Probiotics have been shown to have a variety of effects in aquaculture, including the competitive exclusion of pathogenic bacteria by generating compounds that function as inhibitors, enhancing the host species' immune response, and enhancing nutrition by generating more digestive enzymes [43,45,46,47].Two probiotic bacterial species, *Bacillus licheniformis* and *Lactobacillus rhamnosus*, were selected for this study and introduced to the feed. In comparison to the control group in the feeding trail experiment, these probiotics generated the best growth potentials during the culture operation.

10,000 prawns were put into each feeding trail group for experimentation in the current study. The control group's percent survival rate was 82%, while all experimental feeding trail groups had survival rates of over 90%. The feeding trail experiments revealed that the prawns exhibited typical behavior; no noteworthy observations were made. As a result, compared to the control feeding trail group, the Probiotics and Bioflocs supplemented feeding trail group had noticeably greater survival rates.The present study's final weights and weight gain values were subjected to One Way Analysis of Variance, which demonstrated statistical significance ($p<0.05$) in the differences between the control and experimental groups' final weights and weight gain of *L. vannamei* and *P. monodon*, whether in monoculture or mixed culture operation. According to earlier reports, probiotic bacteria like *B. licheniformis* and *L. rhamnosus*, as well as external carbon sources like sugarcane molasses, rice flour and wheat flour for raising suitable Bioflocs added to the culture system, significantly improved both final weights and weight gain in *L. vannamei*and *P. monodon* [5,48]. Additionally, fresh water prawn *M. rosenbergii* using *L. Sporogenes*, in *M. amazonicum* by supplementing diets with *Saccharomyces cerevisiae* and yeast [49,50].After 120 days of culture operation, it was discovered that daily growth rates (DGR) in

both monoculture and mixed cultures were considerably ($p<0.05$) higher in the experimental groups than in the control group. Nonetheless, feeding trail experimental groups exhibited a statistically significant ($p<0.05$) increase in specific growth rates (SGR) when compared to the control group. The study's protein efficiency ratio (PER) statistics amply reveal that, as compared to the control group, all experimental feeding trail groups saw a significant ($p<0.05$) increase. In comparison to probiotic &Biofloc supplemented groups, which range from 1.21-1.32, the feed conversion ratio (FCR) values recorded a maximum of 2.41 for *L. vannamei* and 2.14 for *P. monodon* with the control group.The study's FCR values obtained with experimental feeding trails are statistically significant ($p<0.05$) when compared to the control group, as demonstrated by the One Way ANOVA results. Adding a combination of Probiotics and Bioflocs significantly increased growth patterns while lowering FCR values. Similar findings were also documented by a number of authors via feeding trail experiments using freshwater prawns (*M. rosenbergii, M. amazonicum,* Penaeid Shrimp *P. monodon*, and *L. vannamei*), in which probiotic bacteria (*L. bacillus, L. acidophillus, L. sporogenes, B. subtilis, B. licheniformis*, and *Enterococcus faecium*) were added to the diets [51–55].

The study's feed efficiency ratio (FER) and feed conversion efficiency (FCE) values, measured under various feeding trails, were shown to be significantly higher ($p<0.05$) in all experimental feeding trail groups when compared to the control group. When probiotics, Bacillus and Lactobacillus, and Bioflocs were supplied to freshwater prawns and Penaeid shrimp utilising molasses, rice flour, and wheat flour as external carbon sources, similar kinds of observations were obtained [56]. After 120 days, Protein Efficiency Ratios (PER) were measured in each feeding trail. It was discovered that the Control group had the lowest PER, 5.39, while *P.monodon* had the highest PER, 8.58.In the current investigation, the recorded PER values between the Control and Experimental feeding trails were shown to be statistically significant ($p<0.05$). Therefore, it is evident from the probiotics' and bioflocs' ability to induce PER that the feed's protein utilisation was significantly accelerated. Furthermore, the feeding, absorption, and conversion rates of feeds all demonstrated a substantial increase ($p<0.05$) in the current investigation, supporting the PER values that were obtained. By incorporating probiotic bacteria with feeds and Bioflocs into culture medium, similar kinds of observations were also observed with prawns *M. rosenbergii, L. vannamei, P. monodon*, fish *Clariusgariepinus, Oncorhynchus mykiss*, and *Oreochromis niloticus* [43, 57-59].The addition of biofloc, in the form of specific external carbon sources, promotes the growth of heterotrophic bacteria in the culture operation, which in turn supplies additional feeding materials and raises the protein content of the Candidate Species of culture to a large degree.

In this investigation, it was discovered that the Experimental feeding trails supplemented with Probiotics and Biofloc had considerably ($p<0.05$) higher harvest sizes and productivity rates than the two Control feeding trail groups. The control group reported a minimum harvest size of 16.28 g for *L. vannamei* and a maximum of 44.72 g for *P. monodon* and 33.41 g for *L. vannamei*. The experimental feeding trail included both Probiotics and Bioflocs in the Culture Operation. Productivity rates were also shown to be highest and best in diets that included both probiotics and a combination of two external carbon sources (Bioflocs), in accordance with harvest size.

4. CONCLUSION

Based on the data collected, the current study can draw the conclusion that the addition of Probiotics and Bioflocs to the Monoculture or Mixed Culture operation of *L. vannamei* and *P. monodon* led to the flawless maintenance of all water quality parameters. This was followed by the microbial community breaking down harmful nitrogenous substances and converting them into natural feed, which prawns then ingested.So, the use of Biofloc technology in the Culture operation of shrimp plays a vital role in the elimination of pathogenic organisms from culture environment, through the maintenance of ideal water quality paves way for the promotion of enhancing the both feed performance followed by growth indices under minimal water exchange system in tropical regions of Andhra Pradesh.In addition to maintaining high water quality indices, the combined use of probiotics and bioflocs in the culture process also improves the growth performance of *L. vannamei* and *P. monodon*. When culture professionals effectively performed culture management, the combined use was adequate to maintain the culture system that demonstrated good bioremediation and biocontrol. Therefore, it is justified to utilise a mixed culture of *L. vannamei* and *P. monodon* to achieve maximum output and effective utilisation of both natural and supplemented feed that is formed by adding Bioflocs to a mixture of tapioca flour and molasses in this study.

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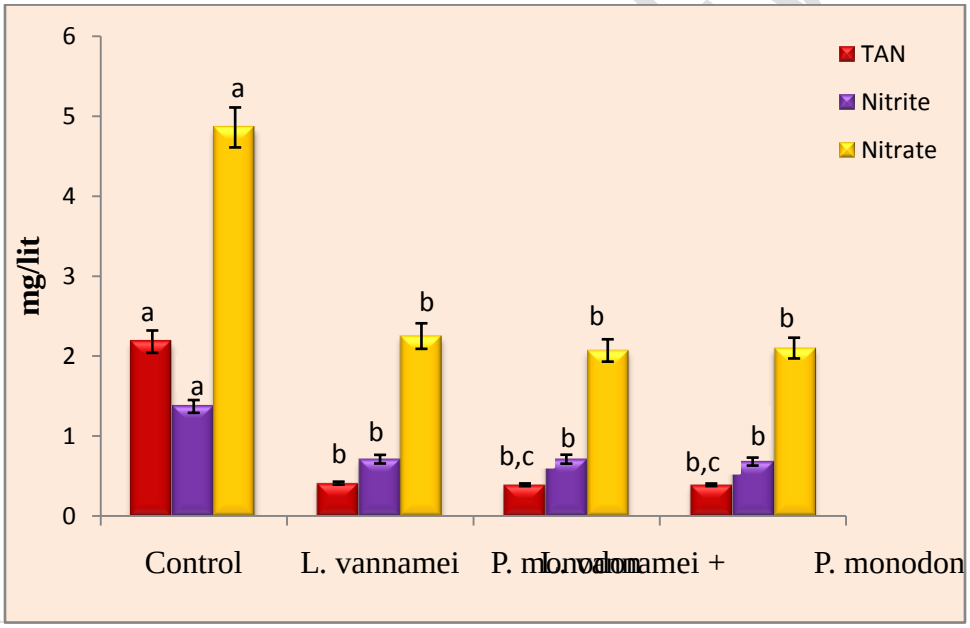

Figure. 1: Water quality variables (TAN, Nitrite and Nitrate) under Monoculture and Mixed culture of *L. vannamei*and *P. monodon*

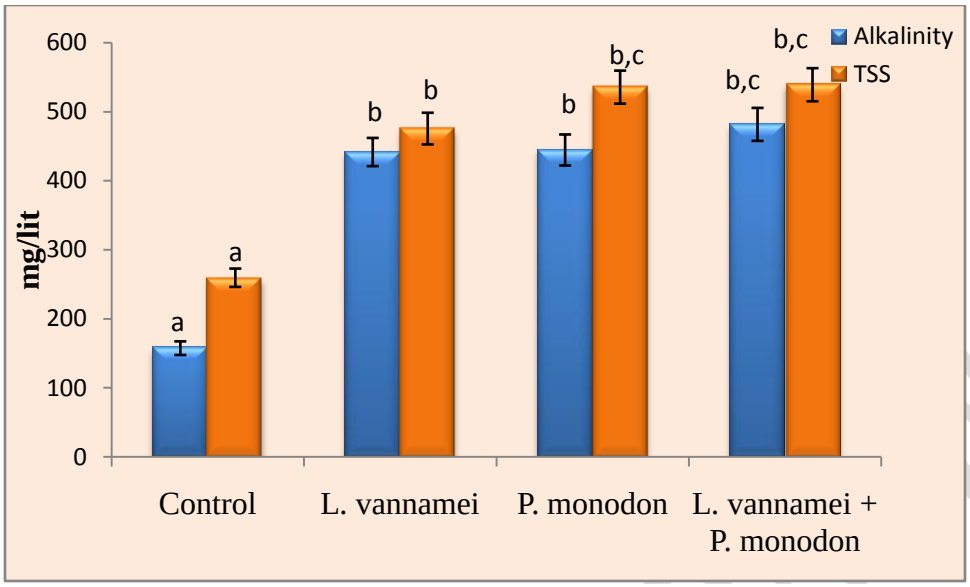

Figure. 2: Water quality variables (Alkalinity & Total Suspended Solids) under Monoculture and Mixed culture of *L. vannamei*and *P. monodon*

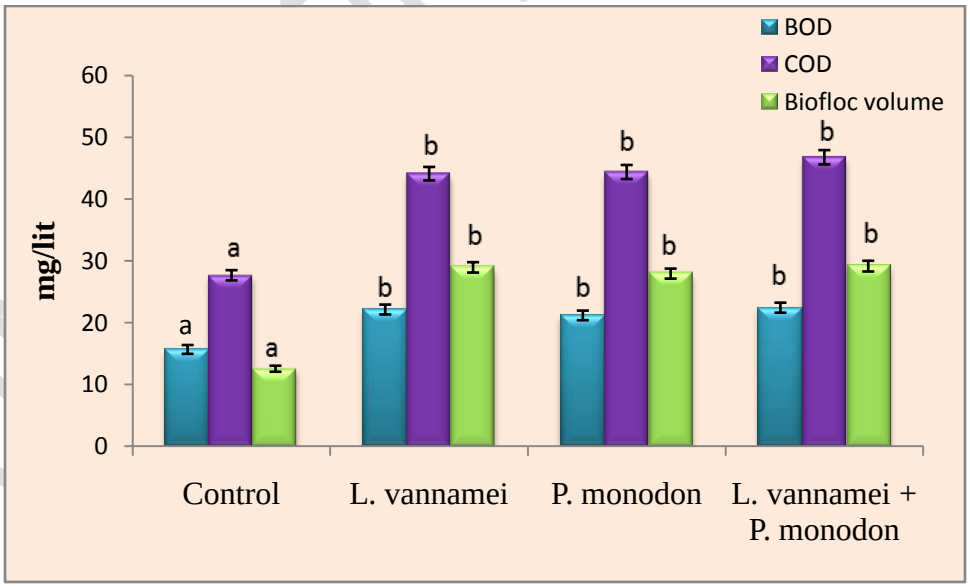

Figure. 3: Water quality variables (BOD, COD &Biofloc volume) under Monoculture and Mixed culture of *L. vannamei*and *P. monodon*

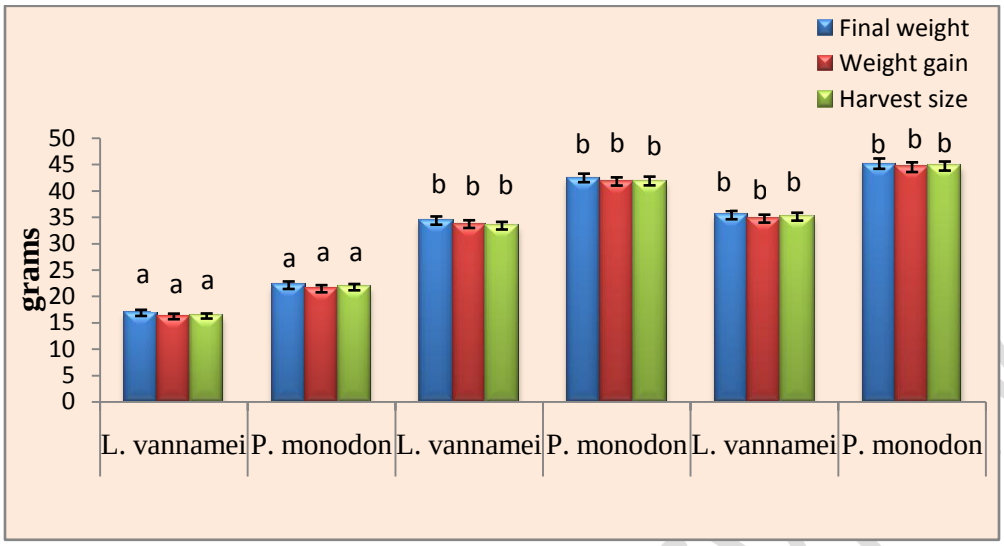

Figure. 4: Final weight, Weight gain and Harvest size of *L. vannamei*and *P. monodon* under Monoculture and Mixed culture

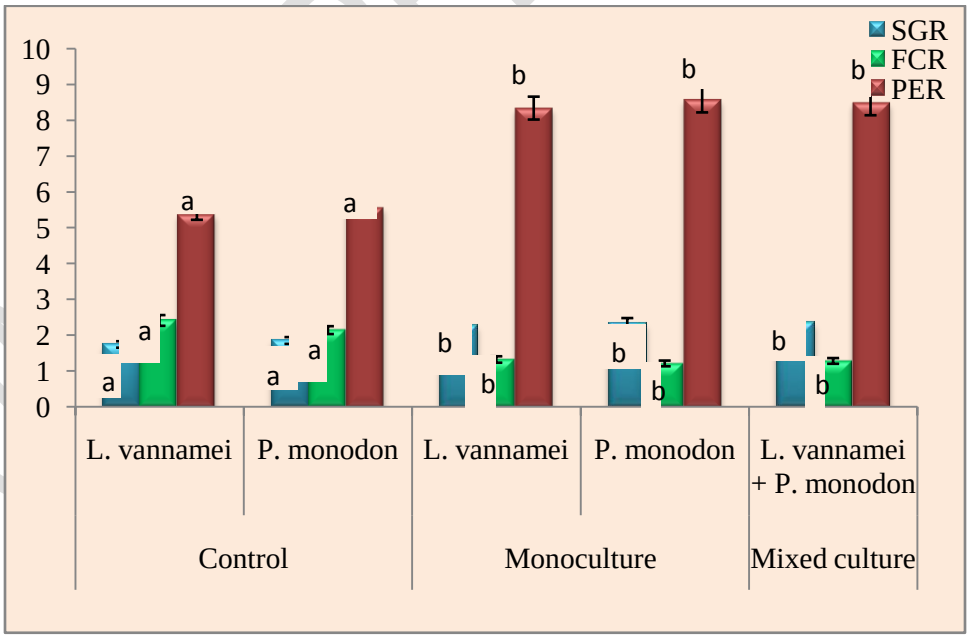

Figure. 5: SGR, FCR and PER of *L. vannamei*and *P. monodon* under Monoculture and Mixed culture

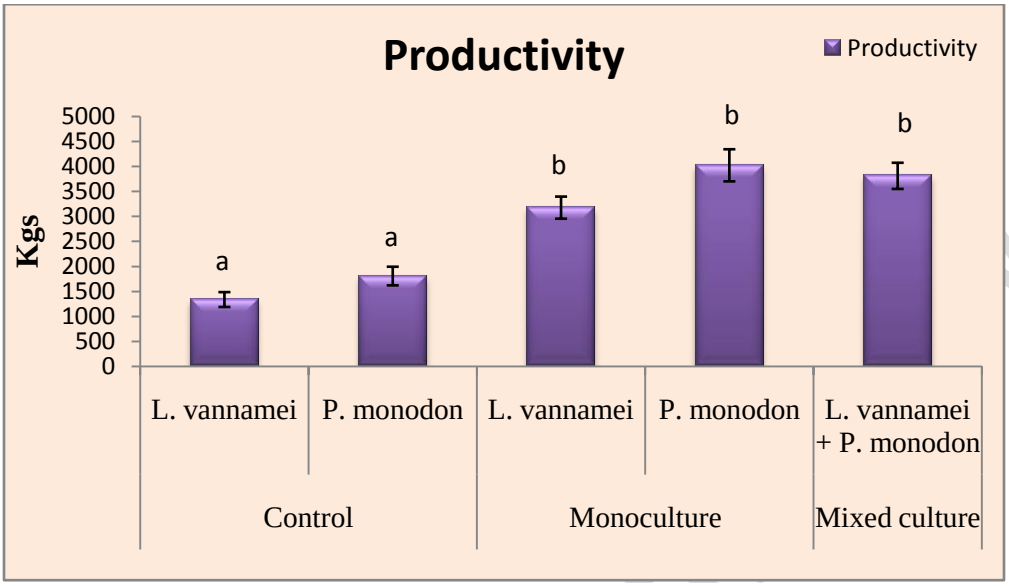

Figure. 6: Productivity rates of *L. vannamei*and *P. monodon* under Monoculture and Mixed culture

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).

	Control	Experimental Diet
Organic Matter (%)	83.18	82.69
Ash (%)	16.82	17.31
Crude Protein (%)	35.28	36.04
Crude Lipid (%)	6.82	7.04
Crude Fiber (%)	4.46	4.28
Nitrogen Free Extract (NFE) (%)	27.94	26.79
Moisture (%)	8.68	8.54
Gross Energy (Kcal/100 g)	395	395

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 presented in Table.1
Experimental Diet : Control Feed +Probiotics +Bioflocs (Molasses & Tapioca flour)

Table 3. Water Quality Variables under different Biofloc Feeding Trails for *L.vannamei* and *P. monodon* in Monoculture and Mixed culture operation

	Control		*L.vannamei*		*P.monodon*	
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximu**
Temperature(^{0}C)	24	29	22	29	23	28
Average	26.5		25.5		25.5	
pH	6.8	7.9	6.7	7.8	6.8	7.8
Average	7.35		7.25		7.3	
Salanity (ppt)	9	11	9	11	9	12
Average	10		10		10.5	
Dissolved Oxygen (DO) (mg/lit)	5.28	6.43	3.89	4.78	4.02	4.88
Average	5.86±0.23^a		4.34±0.20^b		4.45±0.21^b	
	PDC		-25.94		-24.06	
Total Ammonia Nitrogen (TAN) (mg/lit)	1.88	2.48	0.32	0.49	0.30	0.48
Average	2.18±0.14^a		0.41±0.019^b		0.39±0.017^{b,c}	
	PDC		-71.37		-84.27	
Nitrite (NO_2-N) (mg/lit)	1.20	1.54	0.58	0.84	0.57	0.84
Average	1.37±0.08^a		0.71±0.054^b		0.71±0.056^b	
	PDC		-40.83		-40.83	
Nitrate (NO_3-N) (mg/lit)	3.72	5.99	1.72	2.78	1.54	2.59
Average	4.86±0.25^a		2.25±0.16^b		2.07±0.14^b	
	PDC		-53.70		-57.41	
Phosphate (ppm)	0.51	0.72	0.62	0.99	0.78	1.24
Average	0.62±0.04^a		0.81±0.051^b		1.01±0.084^{b,c}	
	PDC		+30.65		+62.90	
Alkalinity (mg/lit)	125	190	342	541	324	565
Average	157.5±9.87^a		441.50±20.44^b		444.50±22.46^b	
	PDC		+180.32		+182.22	
Total Suspended Solids (TSS) (mg/lit)	214	305	372	575	402	669
Average	259.50 ±13.28^a		473.50±22.91^b		535.50 ±23.91^{b,c}	
	PDC		+82.47		+106.36	
Biological Oxygen Demand (BOD) (mg/lit)	11.18	20.17	15.17	29.11	14.19	28.19
Average	15.68±0.72^a		22.14±0.80^b		21.19±0.78^b	
	PDC		+41.20		+35.14	
Chemical Oxygen Demand (COD) (mg/lit)	24.18	31.18	38.45	49.78	37.33	51.44
Average	27.68±0.83^a		44.12 ±1.09^b		44.39±1.14^b	
	PDC		+59.39		+60.37	

DO Reduction Rate (mg/lit/hr)	0.41	0.49	01.02	1.29	1.05	1.38
Average	0.45±0.028[a]		1.16±0.08[b]		1.22±0.10[b,c]	
	PDC		+157.78		+171.11	
BioFloc Volume (mg/lit)	10.34	14.77	23.73	34.18	20.14	35.75
Average	12.56±0.49[a]		28.96 ±0.84[b]		27.95 ±0.81[b]	
	PDC		+130.57		+122.53	
Chlorophyll-a	42.79	65.17	54.78	96.38	51.94	98.47
Average	53.98±1.49[a]		75.58 ±1.75[b]		75.21±1.73[b]	
	PDC		+40.01		+39.33	
PhytoPlankton Density ($x10^5$ Cells/Lit)	2.03	3.94	6.18	10.13	6.79	11.19
Average	2.99±0.32[a]		8.16 ±0.46[b]		8.99±0.48[b,c]	
	PDC		+172.91		+200.67	

Values are Mean ± SD of six individual observations.
Means with different superscripts for each in a column differ significantly at $p<0.05$.

Table 4. Culture details of *L.vannamei* and *P.monodon* in Monoculture and Polyculture during Biofloc feeding trails.

	Control		**Probiotics + Biofloc Adde**		
	L.vannamei	***P.monodon***	***L.vannamei***	***P.monodon***	***L.van***
Culture Pond Area (ha)	0.5	0.5	0.5	0.5	
Shrimp Stocked (Nos)	10,000	10000	10,000	10,000	50
Days of Culture (in days)	120	120	120	120	
Percent Survival (%)	82	83	95	96	9
Final Weight (g)	16.87±0.58[a]	22.12±0.71[a]	34.38±0.78[b]	42.47±0.81[b]	35.42
	PDC	PDC	+103.79	+92.01	+1
Weight gain (g)	16.20±0.53[a]	21.45±0.68[a]	33.71±0.73[b]	41.80±0.78[b]	34.75
	PDC	PDC	+108	+95	+1
Daily Growth Rates (DGR) (g)	0.135±0.008[a]	0.179±0.012[a]	0.281±0.013[b]	0.348±0.018[b]	0.290±
	PDC	PDC	+108	+94	+1
Specific Growth Ratios (SGR)	1.74±0.090[a]	1.85±0.095[a]	2.27±0.143[b]	2.32±0.157[b]	
	PDC	PDC	+30.46	+25.41	
Feed Conversion Ratio (FCR)	2.41±0.15[a]	2.14±0.11[a]	1.32±0.09[b]	1.21±0.08[b]	
	PDC	PDC	-45.22	-43.46	
Feed Efficiency Ratio (FER)	0.415±0.024[a]	0.467±0.026[a]	0.758±0.049[b]	0.826±0.051[b]	
	PDC	PDC	+82.65	+76.87	

	Control		Probiotics + Biofloc Adde		
	L.vannamei	***P.monodon***	***L.vannamei***	***P.monodon***	***L.van***
Protein Efficiency Ratio (PER)	5.39±0.17[a]	5.58±0.24[a]	8.34±0.32[b]	8.58±0.36[b]	
	PDC	PDC	+54.73	+53.76	
Feed Conversion Efficiency (%)	41.5±0.79[a]	46.73±0.88[a]	75.75±1.31[b]	82.65±1.44[b]	
	PDC	PDC	+82.49	+76.87	
Harvest Size (g)	16.28±0.48[a]	21.75±0.60[a]	33.41±0.72[b]	41.89±0.83[b]	35.12
	PDC	PDC	+105.22	+92.60	+11
Productivity (in kgs)	1335±149[a]	1805±187[a]	3174±221[b]	4021±321[b]	16
	PDC	PDC	+137.75	+122.77	

Initial Weight: 0.67±0.05

Values are Mean ± SD of six individual observations.

Values are Statistically Significant at a $p<0.05$ from its respective Controls.

Means with different superscripts for each in a column differ significantly at $p<0.05$.