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Growth performance of shrimp *Litopenaeus vannamei* under different carbon: Nitrogen (C/N) ratios of Bioflocs system

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Abstract
A 60 day feeding experimental trails were conducted to evaluate the effect of selected carbohydrate sources on growth performance in white shrimp *L. vannamei* under Biofloc system. The present set of Feeding trails consists of 8 Experimental groups with different Carbon sources in combinations. 1st group with Control diet, 2nd group with Control feed with Probiotics added, 3rd group Molasses & Tapioca, 4th group Molasses & Maize flour, 5th group Molasses & Sucrose, 6th group Molasses, Tapioca & Maize flour, 7th group Molasses, Maize flour & Sucrose and 8th group Molasses, Maize flour, Tapioca & Sucrose as Carbon sources in combinations. In the present study both Probiotics and combinations of Carbon sources used for the production of Bioflocs in the culture medium. The microbial community produced serve as additional food in addition to the regular feed supply for shrimp, produces relatively higher Growth rates and finally Productivity rates. The combined use of both Probiotics and BFT, generally acts as efficient alternative against pathogens and maintenance of ideal Water quality parameters, which in turn substantially enhances the growth potentials of shrimp *L. vannamei*. So the Probiotics selected in the present study *Bacillus licheniformis* and *Lactobacillus rhamnosus* and Bioflocs produced by combinations of molasses, tapioca, maize flour and sucrose yielded the best and highest Growth potentials in *L. vannamei*. Among the Experimental diets, Experimental diet (ED-7) formulated with both Probiotics and Bioflocs developed by the addition of Molasses, Tapioca, Maize flour and Sucrose yielded highest growth rates in *L.vannamei*.

Keywords: *L. vannamei*, probiotics, Bioflocs, carbon sources

1. Introduction
Shrimp farming is one of the essential fundamental sectors in Aquaculture and accounting fifty five % of the area's Crustacean production. Because of higher wishes of shrimp inside the global market has delivered about an enlargement of shrimp tradition and production, which become supported by the usage of numerous modern scientific technologies for increasing productivity rates. Extensive culture practices of shrimp entails an indiscriminate use of different types of chemical compounds and antibiotics to overcome higher mortality rates of Candidate species [1]. Probiotics are an alternative to antibiotics due to the fact antibiotics have caused resistance of bacterial pathogens [2]. Applications of Probiotic organisms in to the culture environment have shown several useful results inside the host organism, together with advanced increase, survival and health status [3, 4].
Biofloc technology (BFT) is based on the development of macroaggregates comprising heterotrophic bacteria, phytoplankton, and organic matter etc and capable of maintaining ideal Water quality for aquaculture operation [5, 6]. BFT have the right C: N ratio, they oxidize the overall Ammonia Nitrogen, excreted by candidate species of culture [7]. The nitrification phenomenon generally lead to substantial increase in microbial mass [8], which is rich in protein and is consumed as a food supplement by the species cultivated, thereby improving growth [9, 10]. BFT will have a Probiotic impact on culture operation of each fish and shrimp. It's been observed that, due to addition of Probiotics, the beneficial results of BFT became substantially more advantageous [11].

The BFT system severely relies upon on the powerful manipulation of C: N ratio thru the supply of Carbon deliver and Feed addition. Carbon supply addition in to the lifestyle surroundings considerably complements the conversion of heterotrophic micro-organism and facilitates in balancing the C: N ratio [7, 12]. The heterotrophs successfully take within the inorganic nitrogen and facilitate faster discount of TAN is a major project in shrimp farming and consists of ionized (NH4+) and unionized ammonia (NH_3) with later being are notably toxic to the cell membranes of shrimps and different aquatic organisms [13]. To decrease the TAN levels, it's far essential to apply Carbon sources with lower dissolution costs to favour the Carbon: Nitrogen ratio. However, first rate natural carbon resources can probably have an effect on the composition of the Biofloc [7, 11, 14].

Carbon resources in keeping a balanced C: N ratio have divergent roles such as the formation of Biofloc, lowering TAN levels and therefore improving the water satisfactory inside the tradition working media. Using Carbon sources like Molasses, Tapioca, Maize flour and Sucrose personally in Biofloc based shrimp farming have proven to sell heterotrophic bacterial increase, which correctly controlled the TAN ranges in the tradition system [5, 8, 15]. But there is no information about using the combinations of above mentioned Carbon sources with effective C: N ratio for *L.vannamei* rearing in Biofloc system. The application of Biofloc in the culture operation of *L.vannamei* could create natural food which is ideal for consumption in the present Feeding trail experiments. Thus, the main objective of the present study was to evaluate the Growth and Feed performance in *L.vannamei* under different C: N ratios with combinations of selected Carbon sources.

Carbon sources in maintaining a balanced C: N ratio have divergent roles which include the formation of Biofloc, decreasing TAN degrees and therefore improving the water quality inside the culture operating media. Using Carbon sources like Molasses, Tapioca, Maize flour and Sucrose in my view in Biofloc based totally shrimp farming have demonstrated to promote heterotrophic bacterial growth, which successfully controlled the TAN levels inside the culture system [5, 8, 15]. But there's no information approximately the use of the combinations of above stated Carbon resources with effective C: N ratio for *L.vannamei* rearing in Biofloc system. The application of Biofloc system in the way of life operation of *L.vannamei* could create natural food which is good for intake inside the present Feeding trail experiments. Thus, the principle objective of the present observe turned into to assess the Growth and Feed performance in *L. vannamei* beneath exceptional C: N ratios with combinations of selected on Carbon sources.

2. Materials and Methods

2.1 Experimental Design

A 60 days feeding Experimental trails were conducted using Penaeid shrimp *Litopenaeus vannamei* juveniles of weight 1.52±0.07 g, obtained from local Aquaculture farms transported through oxygenated bags at shrimp culture facility located in Ramayapatnam (Latitude 15º02'55''N; Longitude 80º02'50''E) Praksham District of Andhra Pradesh. The present study was conducted in cement tanks of 3000 litre capacity with a size of 5x10 mts located in an external area with natural illumination and covered by screen to prevent escape of shrimp. The Shrimp were acclimatized to Laboratory conditions for 7-8 days and fed twice a day both in the morning and evening with formulated Feed (35% Crude Protein Table.1). All the experimental feeding trail tanks were submerged in earth and earthern bottom was provided and a water depth of 1mt was maintained constantly throughout the Feeding trail experiments. All of the treatments had 3 replicates and allocation for each treatment turned into completely randomized. All the experimental tanks have been aerated with pieces of air stone suspended inside the water column in every tank linked thru a well described electrical blowers and compressors. The Experimental tanks were aerated continuously to maintain the Dissolved Oxygen level higher than 6 mg/lit and suspend of Biofloc without damage its form of Biofloc.

The volume of water all the Experimental feeding trail tanks were maintained constantly by adding desired amounts of sea water of 10±1 ppt weekly in order to replace discarded water and compensate evaporation. Water temperatures and salinity of the experimental setup over 60 days of Feeding trail experiments ranged 22-27°C and 10±1 ppt, respectively.

2.2 Probiotic Feed Preparation

Probiotic supplemented feeds were prepared by incorporating *Bacillus licheniformis* and *Lactobacillus rhamnosus* @ 10 billion CFU/Kg by following the procedures described by Naresh [16] and Aparna *et al.* [6].

2.3 Preparation of Bioflocs

A completely randomized experimental design was adopted for the conduction of feeding trails with three Carbon (C) to Nitrogen (N) ratios (10:1, 15:1 and 20:1) with selected four carbohydrate sources i.e. Molasses, tapioca, Maize Flour, Sucrose in combinations. The procedure for the preparation of Bioflocs and calculation formulae were followed as described earlier [17-21].

Carbon content [22], Total Ammonia Nitrogen (TAN) concentration and water quality parameters were measured by adopting the appropriate International methodologies [23].

2.4 Growth & Feed Performance Parameters

All the Growth related indices represented by average body weights, average body growth rates, specific growth rates, feed conversion ratio, protein efficiency ratio, feed efficiency ratio and productivity rates were monitored and tabulated. All of the growth related indices represented through common body weights, feed conversion ratio, protein efficiency ratio, feed performance ratio and productivity rates were monitored by adopting the standard formulae [6] and tabulated.

2.5 Feed Management

The daily feeding began at 10% body weight and declined step by step to 4% on the cease of the experiment. The daily ration become divided into two parts 50% feed twice each day at 06.00 AM and 18.00 hrs. evening.

2.6 Experimental Diets

Eight experimental diets one Control and seven Diet formulated with the addition of Probiotics and combinations of selected carbon sources with 35% crude protein level representing the C: N ratios of 10:1, 15:1, and 20:1, respectively were prepared. The details are as follows:

Experimental Diet 1 (ED1): Control diet

Experimental Diet 2 (ED2): Control diet + Probiotics added.

Experimental Diet 3 (ED3): Control diet + Probiotics + Molasses & Tapioca were added as carbon source for Biofloc formation.

Experimental Diet 4 (ED4): Control diet + Probiotics + Molasses & Maize flour were added as carbon source for Biofloc formation.

Experimental Diet 5 (ED5): Control diet + Probiotics + Molasses & Sucrose were added as carbon source for Biofloc formation.

Experimental Diet 6 (ED6): Control diet + Probiotics + Molasses, Tapioca & Maize flour were added as carbon source for Biofloc formation.

Experimental Diet 7 (ED7): Control diet + Probiotics + Molasses, Maize flour & Sucrose were added as carbon source for Biofloc formation.

Experimental Diet 8 (ED8): Control diet + Probiotics + Molasses, Maize flour, Tapioca & Sucrose were added as carbon source for Biofloc formation.

The tested diets have been analysed in line with the standard techniques of AOAC [25] for moisture, protein, lipid and ash. Moisture content material of the samples became estimated with the aid of drying in oven at 135°C for 2 hrs. to consistent weight. Crude protein and crude lipid contents were estimated by the Kjeldahl's method (N X 6.25) after acid digestion, Ether extraction technique by Soxhlet system, respectively. The Ash was determined by combusting dry samples in a Muffle furnace at 550°C for 6 hrs.

2.7 Statistical Analysis

One way analysis of variance (ANOVA; SPSS, 13.0) was adopted to determine relative significance between the treatments existed. By using DMRT Test, the difference between means were determined. All the tests used a significance level of $p<0.05$. Data are reported as Means ± Standard deviations.

3. Results and Discussion

In the present study, an attempt was made to assess the growth performance of *L.vannamei* after feeding with different types of Experimental diets through feeding trails, wherein both Probiotics and Bioflocs were added into the culture operation. In the present study one Control and seven Experimental diets were formulated with C:N of 10:1, 15:1 and 20:1 and proximate composition was analysed and presented in Tables 2-4. The organic matter was found to be in the range of 82-83%, Ash (17%), Crude Protein (33-35%), Crude Lipid (7%), Crude Fiber (4%), Nitrogen Free Extract (28-29%), Moisture Content (8%), in all the Experimental diets formulated in the present feeding trails study. All the Experimental diets formulated were considered to be iso-energetic i.e. contains 395 Kcal/100 g diet. Growth and Feed Performance details of *L. vannamei* under different Biofloc feeding trails i.e. C: N (10:1), (15:1), and (20:1) for a period of 60 days of Experimental Feeding Trails and presented in Table. 5 (C/N 10:1), Table. 6 (C/N 15:1) and Table 7 (C/N 20:1). The results obtained in the present study envisages that maximum growth rates and feed performance were recorded with Experimental Diet-7 (ED-7) fed with Control diet + Probiotics + Molasses + Maize flour, Tapioca and Sucrose were added as External carbon sources for the formation of Bioflocs in all the Carbon/Nitrogen ratios of 10:1, 15:1 and 20:1. Comparative performance of both Growth potentials and Feed performance under ED-7 were presented in Table. 8. The results obtained in the present study clearly demonstrates that C: N treated Shrimp groups showed better growth and feed performance compared to control group. The percent survival rates recorded highest of 98% with C: N (15:1) feed group compared to 95% with C:N (20:1) and 93% with C:N (10:1) group against 87% recorded with Control group (Table. 8). The Average Final Body Weights (ABW) recorded significantly ($p<0.05$) higher in all the Biofloc added groups compared to Control group. ABW recorded highest of 33.97 g with ED-7 of C/N 15:1 treated Shrimp group followed by 28.73 g with ED-7 (C/N 10:1) Biofloc group, 27.42 g with ED-7 (C/N 20:1) Biofloc group compared to 16.43 g with Control group (Figure.1). ABW recorded highest 107% increment with Biofloc (C/N 15:1) treated group and is significant ($p<0.05$) followed by 75% growth with Biofloc (C/N 10:1) treated group and 67% growth with Biofloc (C/N 20:1) treated group compared to control group. The Average Weight Gain (AGW) values were found to be significantly ($p<0.05$) higher +118% with Biofloc (C/N 15:1) treated group followed +83% with Biofloc (C/N 10:1) and +74% with Biofloc (C/N 20:1) treated group compared to control group. Daily Growth Rates (DGR) recorded significantly higher ($p<0.05$) +118% with Biofloc (C/N 15:1) treated group followed by +82% with Biofloc (C/N 10:1) treated group and + 73% with Biofloc (C/N 20:1) treated group compared to Control group. Specific Growth Rates (SGR) values recorded also showed significant ($p<0.05$) elevation of +36% with Biofloc (C/N 15:1) treated group, followed by +29% with Biofloc (C/N 10:1) treated group and +26% with Biofloc (C/N 20:1) treated group compared to control group (Figure.2). Overall, all the Growth associated Parameters recorded significantly ($p<0.05$) higher with Biofloc maintained group (C/N 15:1) followed by (C/N 10:1) treated group and finally C/N 20:1 treated group compared to Control group. Similarly Feed performance parameters including Protein Efficiency Ratio (PER) values recorded significantly ($p<0.05$) highest +63% with Biofloc (C/N 15:1) treated group, followed by +52% with Biofloc (C/N 10:1) treated group, +51% with Biofloc (C/N 20:1) treated group compared to control group. Feed Conversion Ratio (FCR) values obtained in the present study recorded 1.22, the best and the lowest with Biofloc group (C/N 15:1), followed by 1.41 with Biofloc group (C/N 10:1) and 1.57 with Biofloc group (C/N 20:1) compared to 2.56 recorded with Control group. The FCR values obtained were significantly ($p<0.05$) lower -53% with Biofloc group (C/N 15:1), followed by -45% with Biofloc group (C/N 10:1), -39% with Biofloc group (C/N 20:1) compared to Control group (Figure.2). Gross Feed Conversion Efficiency (GFCE) values were found to be significantly ($p<0.05$) higher 82% recorded with Biofloc Shrimp (C/N 15:1) followed by 71% with Biofloc group (C/N 10:1), 64% with Biofloc group (C/N 20:1) compared to Control group (39%). The highest percentage increment of GFCE +110% and is highly significant ($p<0.05$) with Biofloc group (C/N 15:1), followed by +82% with Biofloc group (C/N 10:1), +63% with Biofloc group (C/N 20:1), compared to Control group (Figure.3). The Feed Efficiency Ratio (FER), values obtained recorded higher with Biofloc groups compared to control group. The FER percent increase values showed significant (p<0.05) elevation of +110% with Biofloc group (C/N 15:1) followed by +82% with Biofloc group (C/N 20:1) and +63% with Biofloc group (C/N 10:1) compared to Control group. The Harvest Size (HS) recorded in the present set of feeding trails obtained were found to be significantly (p<0.05) higher in all the Biofloc groups i.e. +106% with Biofloc group (C/N 15:1) followed by +66% with Biofloc group (C/N 20:1), and +66% with Biofloc group (C/N 10:1) compared to Control group. The Highest HS obtained to be 33.45g with Biofloc group (C/N 15:1), followed by 27.13g with Biofloc group (C/N 20:1), and 27.03g with Biofloc group (C/N 10:1), compared to 16.31g with Control group. The Productivity rates obtained were found to highest 16.39 Kgs recorded with Biofloc group (C/N 15:1), followed by 12.89 Kgs with Biofloc group (C/N 20:1), 12.84 Kgs with Biofloc group (C/N 10:1) compared to 7.18 Kgs recorded with Control group (Figure.4).

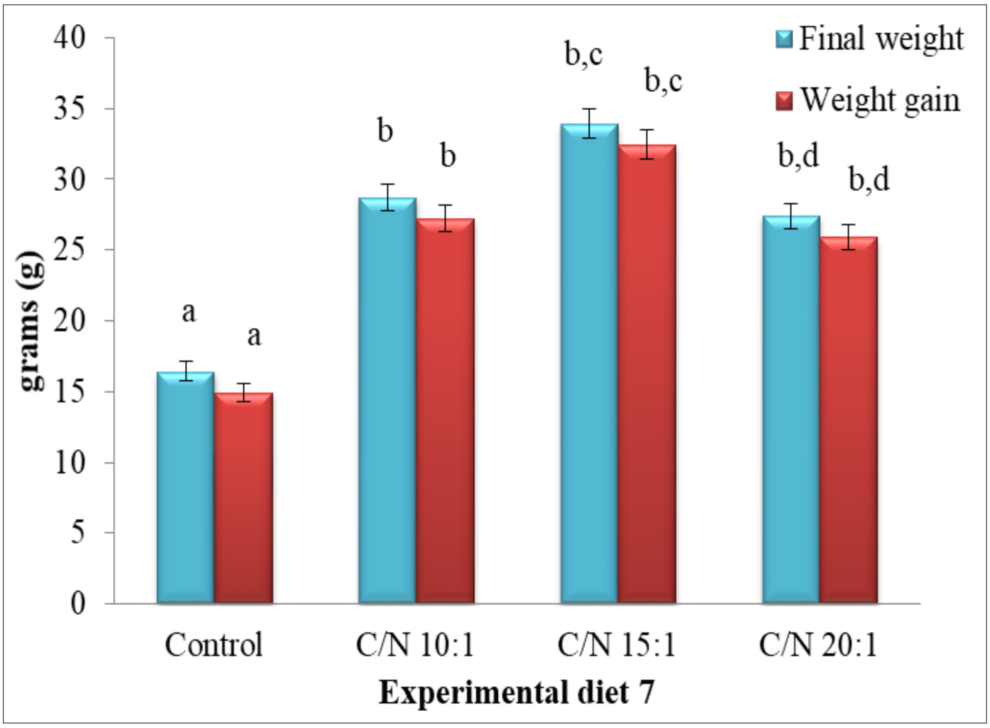

Fig 1: Growth Performance details – final weight and weight gain recorded in *L.vannamei* under different C/N ratios

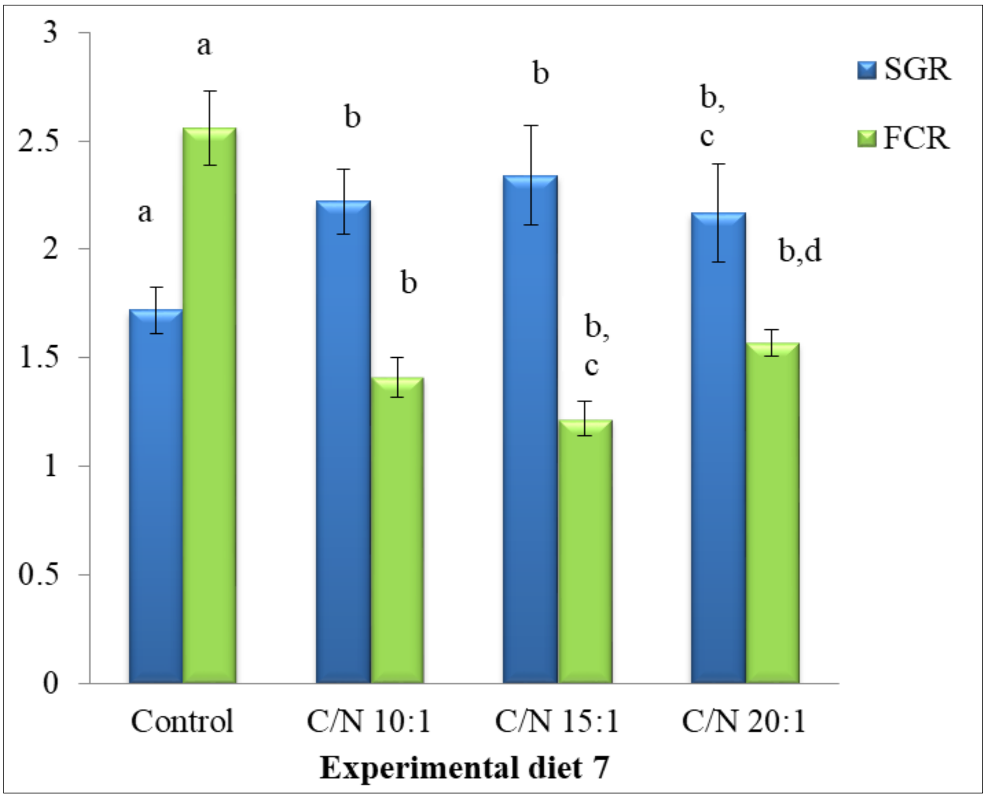

Fig 2: Growth Performance details – Specific Growth Rates (SGR) and Feed Conversion Ratio (FCR) recorded in *L.vannamei* under different C/N ratios

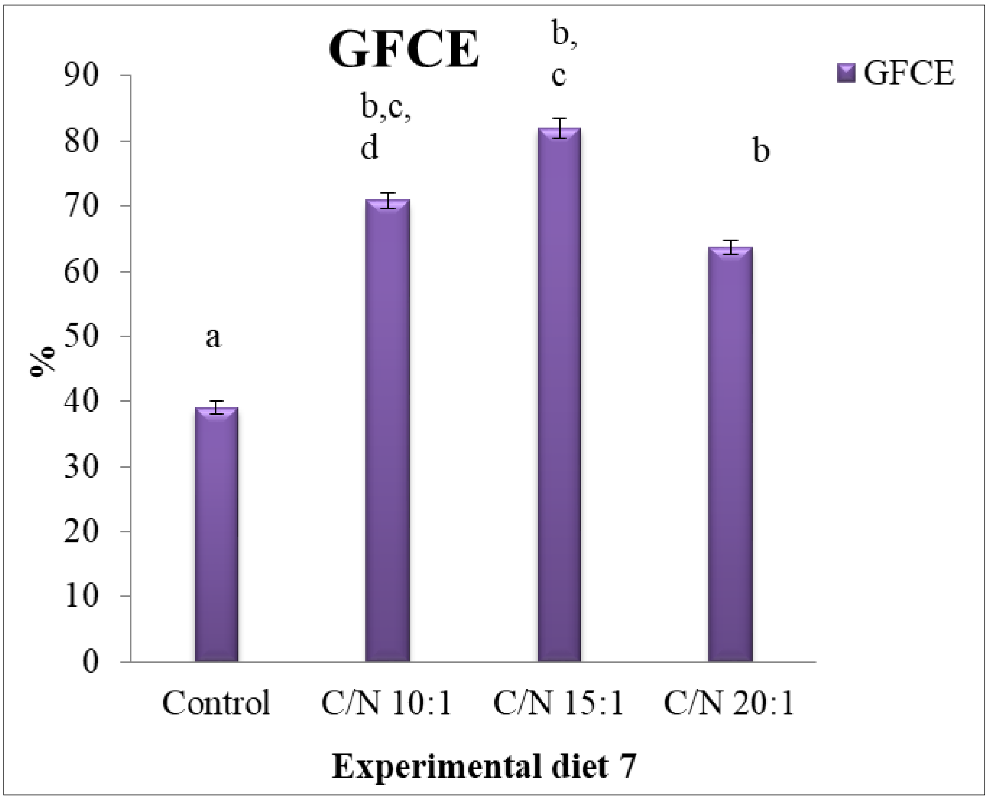

Fig 3: Growth Performance details – Gross Feed Conversion Efficiency (GFCE) recorded in *L.vannamei* under different C/N ratios

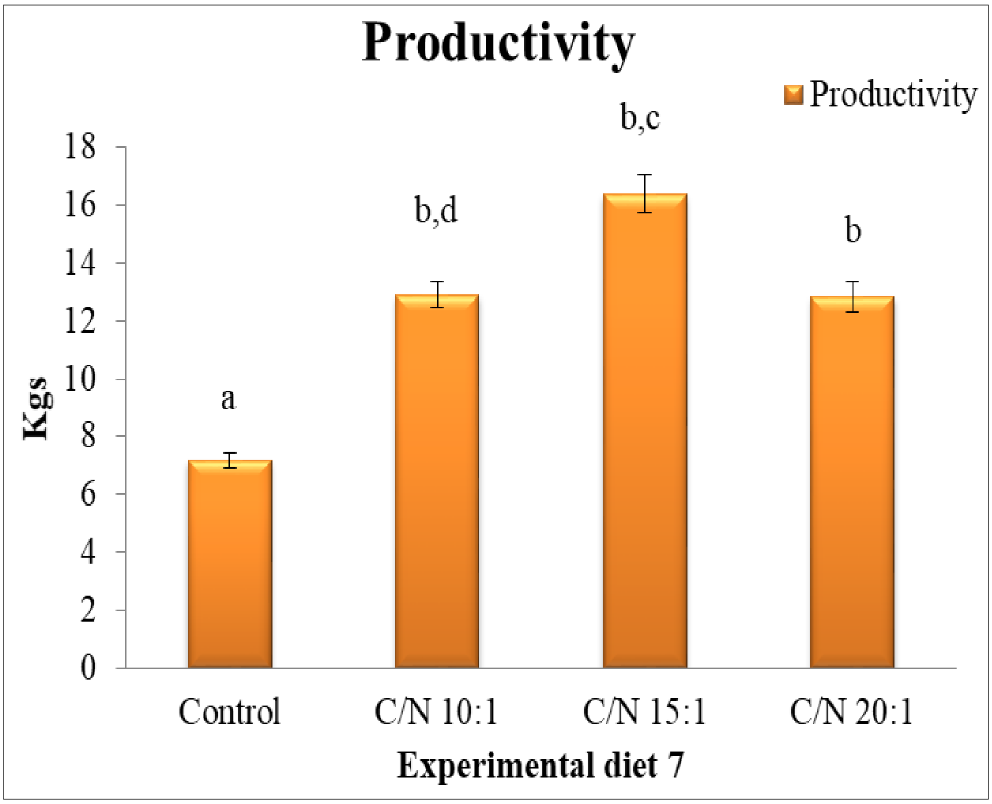

Fig 4: Growth Performance details – productivity rate recorded in *L.vannamei* under different C/N ratios

Performance studies associated with growth of shrimp *L. vannamei* conducted through feeding trails by the addition of both feed Probiotics and Bioflocs into the culture medium for a period of Sixty days. Results obtained for Growth studies were presented in Tables 5-8, clearly indicates that due to the addition of both feed Probiotics and Bioflocs used in the present study induced more growth rates in all the Experimental Feeding Trails compared to Control Feed, feeding trail experiment. All the water quality parameters were found to be ideally maintained during the 60 day Feeding trail experiments, which plays an important role in the induction of growth rates substantially in Shrimp *L. vannamei* [6] with an increasing demand for aquatic products like fish and shrimp, and for eco-friendly aquaculture, Probiotics are increasingly thought to be a viable alternative to pesticides and antibiotics in aquaculture, with the potential to significantly increase the productivity and quality of the produced fish. Numerous research conducted since probiotics were first used in aquaculture have shown that they have the power to suppress possible infections and speed up the growth of aquatic animals that contain Candidate species [26].

In the present study two bacterial species namely *Bacillus licheniformis* and *Lactobacillus rhamnosus* were added as the feed Probiotics, which induced maximum growth potentials compared to Control group, several species of Bacteria have been identified and used in shrimp aquaculture operation to control causative agents against *Vibriosis* including *bacillus*, *lactobacillus*, *vibrio*, *streptococcus*, *aeromonas*, *nitrosomonas* etc. [26, 27]. In the present study, the Probiotic-supplemented diets resulted in an increase of growth rates, indicates that Probiotics increased the growth performance in *L.vannamei*. This is a very inspiring result for the aqua culturists with use of Probiotics, the Production rates will be substantially improved and thereby earning of foreign exchange also improve. *Bacillus*, *Vibrio* and *Pseudomonas* were found frequently in aquatic environs and therefore naturally ingested by shrimp and other aquatic organisms, therefore periodically recommended as potential Probiotic bacteria for cultivating crustaceans and fish. Thru the usage of Probiotics, amendment of bacterial groups in the Aquaculture environment improves the cultivation of crustaceans which includes shrimp. It is very clear that the prevailing observe demonstrating that Probiotics preserve a balanced and bacterial community that not handiest improves water quality and additionally improves productivity rates in shrimp *L.vannamei* culture operation, that's contemplated via the addition of both *Bacillus* and *Lactobacillus spp* in the present feeding trail experiments. Several authors reported that due to incorporation of Probiotics, through the selection of *Bacillus* spp the growth rates were substantially increased not only in Post larvae and also Juveniles in *L.vannamei*, *P. monodon* and *M. rosenbergii* [26-28]. Probiotic bacteria were shown to be a strong contender for enhancing the growth and nutrient digestion of aquatic species, a finding that was corroborated by previous research. Therefore, probiotic bacteria have been demonstrated to promote digestive activity by producing relevant digestive enzymes, which in turn improves digestion and increases food absorption. This eventually improves feed utilisation efficiently and accounts for the low FCR in the current study. So the selected bacteria *Bacillus* and *Lactobacillus* are good candidates for Probiotic application and were found to be potential Probiotic strains.

The ability of *L. vannamei* to utilize natural Productivity and its effect on enhancing Shrimp growth is well studied and reported [7, 29, 30]. In order to boost the carbon to nitrogen ratio, Biofloc Technology (BFT), a microbial-based culture system, adds organic carbon sources to water or raises the carbon content of feed. Because BFT systems encourage nutrient cycling, they are better for the environment. BFT is basically a water quality management method that reduces water exchange by using microorganisms to transform hazardous nitrogen-containing materials like ammonia and nitrite into less dangerous nitrates [11, 31]. Because of its probiotic properties, BFT can suppress harmful microorganisms [32]. Some of the active ingredients in it, including phytosterol, carotenoids, chlorophyll, and poly-β-hydroxy butyrate (PHB), have antibacterial qualities [33, 34]. In addition to providing high-quality proteins, Bioflocs contents are abundant in growth promoters and bioactive substances, which enhance the candidate species' overall health. Shrimp aquaculture practices have recently made extensive use of Biofloc Technology, which raises the C/N ratio of the aquaculture water. Through the formation of microbial floc, it has been reported that controlling the C/N ratio can enhance water quality and support the growth and health of prawns [7, 29, 33, 35]. The idea that the prawns developed healthily in Biofloc-based tanks with the addition of external carbohydrate sources can also be supported by the shrimp's excellent survival rate and better development in the various Bioflocs treatments. Additionally, there was a significant (p<0.05) difference in the shrimp's survival and growth between the Control and Biofloc added groups, but not a significant (p<0.01) difference between the Biofloc groups, which included C/N 10:1, 15:1, and 20:1.

Undoubtedly, the microbial communities of Bioflocs may be significantly impacted by the first injection of beneficial bacteria or natural biota [36]. A practical and efficient way to quicken the growth of Bioflocs in zero-exchange high density prawn culture systems is to inoculate culture water with Biofloc-enriched water and add specific sources of carbohydrates [8, 37]. The end result suggests that it's far viable to control a correctly excessive C/N ratio of the feed formulated with the addition of decided on Carbohydrate assets to acquire a properly- appearing Biofloc system, and has a positive utility prospect in big-scale shrimp aquaculture. The higher survival rates, better performance and improved growth rates of *L. vannamei* in the present study clearly demonstrates that, all the Biofloc systems developed by the addition of different carbon sources helps the Shrimp to grow faster in a healthy condition. The Biofloc possess not only good amount of protein and also provides better essential amino acid composition through natural microbial food would have resulted enhanced growth performance in the present study. The usage of microbial protein depends at the capacity of the goal organism to harvest the bacteria and its capacity to digest and utilize the microbial protein [7, 33, 35]. All the external Carbon sources used in the present study, effectively produced Bioflocs showing promising results in terms of Shrimp net production rates. A comparison of all the Carbon sources added for Biofloc production showed a significant effects not only in maintaining water quality but also inducing the highest growth rates, through usage in combinations, but reached maximum with ED-7 diet in which Molasses, Tapioca, Maize flour and Sucrose were added as carbon sources for the production of Bioflocs. In terms of weight gain and FCR values recorded also the best with ED-7 diet. The better yield in the mixture of carbohydrates brought in the present study showed that *L. vannamei* can nicely make use of the extra protein derived from the accelerated bacterial biomass. Numerous authors mentioned that flocculated debris rich in micro-organism and phytoplankton could make contributions substantially to the nutrients of *L.vannamei.*

Due to the addition of organic carbon, in the culture medium promotes the dominance of heterotrophic bacterial community, which subsequently taken by the Candidate species of culture. By the addition of Probiotics and Bioflocs, the total Pathogenic *vibrio* content were significantly reduced, indicates that the combination have beneficial microorganisms that competes and reduces the pathogens especially *vibrios*. The Heterotrophic micro-organism quick decompose the easy carbohydrates, which are added constantly to preserve C: N levels within the tank.

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 (C: N 10:1) (% DM basis)

Parameter	Experimental diets							
	Control	ED-1	ED-2	ED-3	ED-4	ED-5	ED-6	ED-7
Organic matter (%)	82.79±2.02	82.52±1.99	82.53±2.00	82.59±2.02	82.36±1.99	82.40±1.98	82.32±1.99	82.31±1.97
Ash (%)	17.21±0.74	17.48±0.75	17.47±0.78	17.41±0.75	17.64±0.77	17.60±0.76	17.68±0.72	17.69±0.72
Crude Protein (%)	34.18±1.15	34.22±1.16	34.28±1.14	34.27±1.16	34.68±1.16	34.71±1.15	34.74±1.15	35.20±1.19
Crude Lipid (%)	7.14±0.47	7.18±0.47	7.21±0.48	7.19±0.41	7.18±0.48	7.18±0.49	7.23±0.49	7.21±0.47
Crude Fiber (%)	4.18±0.29	4.17±0.30	4.15±0.31	4.17±0.33	4.19±0.35	4.17±0.29	4.16±0.37	4.15±0.31
Nitrogen Free Extract (NFE) (%)	29.14±0.94	28.71±0.83	28.71±0.91	28.77±0.91	28.17±0.90	28.16±0.89	28.02±0.93	27.58±0.92
Moisture (%)	8.15±0.52	8.24±0.50	8.18±0.52	8.19±0.50	8.16±0.52	8.18±0.55	8.17±0.50	8.18±0.56
Gross Energy (Kcal/100g)	395	394	395	395	394	395	394	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

Table 3: Proximate composition of experimental diets (C: N 15:1) (% DM basis)

Parameter	Experimental diets							
	Control	ED-1	ED-2	ED-3	ED-4	ED-5	ED-6	ED-7
Organic matter (%)	82.84±2.03	82.47±2.01	82.55±2.02	82.63±2.02	82.45±2.01	82.45±2.01	82.31±1.98	83.12±2.14
Ash (%)	17.16±0.79	17.53±0.84	17.45±0.82	17.38±0.81	17.55±0.84	17.55±0.85	17.69±0.98	16.88±0.71
Crude Protein (%)	34.12±1.06	34.34±1.11	34.38±1.13	35.48±1.18	36.17±1.19	36.41±1.26	36.77±1.29	36.97±1.30
Crude Lipid (%)	7.11±0.560	7.14±0.573	7.18±0.610	7.19±0.614	7.23±0.638	7.19±0.610	7.24±0.646	7.23±0.633
Crude Fiber (%)	4.31±0.34	4.33±0.36	4.38±0.40	4.41±0.411	4.43±0.422	4.45±0.434	4.41±0.423	4.53±0.456
Nitrogen Free Extract (NFE) (%)	28.73±0.996	28.08±0.969	28.07±0.959	26.93±0.768	25.94±0.706	25.81±0.67	25.31±0.672	25.78±0.685
Moisture (%)	8.57±0.427	8.58±0.435	8.54±0.509	8.61±0.544	8.68±0.588	8.59±0.597	8.58±0.613	8.61±0.634
Gross Energy (Kcal/100g)	395	395	394	394	394	395	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

Table 4: Proximate Composition of Experimental diets (C: N 20:1) (% DM basis).

Parameter	Experimental diets							
	Control	ED-1	ED-2	ED-3	ED-4	ED-5	ED-6	ED-7
Organic matter (%)	82.68±2.08	82.58±2.04	82.61±2.05	82.64±2.07	82.48±2.01	82.44±1.98	82.44±1.96	82.49±2.01
Ash (%)	17.32±0.80	17.42±0.83	17.39±0.82	17.36±0.81	17.52±0.85	17.56±0.86	17.56±0.86	17.51±0.84
Crude Protein (%)	34.31±1.12	34.38±1.14	34.37±1.13	34.42±1.18	34.41±1.17	34.42±1.18	34.59±1.25	34.79±1.56
Crude Lipid (%)	7.18±0.60	7.19±0.61	7.22±0.63	7.19±0.61	7.21±0.62	7.19±0.61	7.24±0.64	7.22±0.63
Crude Fiber (%)	4.17±0.56	4.16±0.55	4.17±0.56	4.18±0.57	4.17±0.56	4.16±0.54	4.19±0.58	4.18±0.57
Nitrogen Free Extract (NFE) (%)	28.83±1.08	28.64±1.03	28.67±1.01	28.66±1.00	28.48±0.94	28.46±0.93	28.22±0.87	28.09±0.84
Moisture (%)	8.19±0.59	8.21±0.62	8.18±0.58	8.19±0.59	8.21±0.63	8.21±0.62	8.20±0.61	8.21±0.63
Gross Energy (Kcal/100g)	394	395	395	395	395	395	394	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

Table 5: Performance details of *L.vannamei* under different Biofloc Feeding trails (C/N 10:1)

Parameter	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 (%)	87	89	92	92	94	94	94	93
Final weight (g)	16.43±0.74^{a}	18.42±0.82^{a}	20.34±0.83^{b}	22.13±0.87^{b}	23.42±0.89^{b,c}	25.04±0.93^{b,c}	26.34±0.94^{b,c,d}	28.73±0.96^{b,c,d}
	PDC	+12.11	+23.79	+34.69	+42.54	+52.40	+60.31	+74.86
Relative Growth Rate (g)	14.91±0.66^{a}	16.90±0.74^{a}	18.82±0.80^{b}	20.61±0.82^{b}	21.90±0.85^{b,c}	23.52±0.90^{b,c}	24.82±0.92^{b,c,d}	27.21±0.95^{b,c,d}
	PDC	+13.34	+26.22	+38.22	+46.88	+57.74	+66.46	+82.49
Daily Growth Rates (DGR) (g)	0.249±0.008^{a}	0.282±0.02^{a}	0.314±0.02^{b}	0.334±0.022^{b}	0.365±0.021^{b,c}	0.392±0.022^{b,c}	0.414±0.022^{b,c,d}	0.454±0.023^{b,c,d}
	PDC	+13.25	+26.10	+38.15	+46.58	+57.42	+66.26	+82.32
Specific Growth Rates (SGR)	1.72±0.10^{a}	1.79±0.10^{a}	1.84±0.11^{a}	1.90±0.13^{a}	1.96±0.13^{a}	2.11±0.15^{b}	2.15±0.14^{b}	2.22±0.15^{b,c}
	PDC	+4.06	+6.97	+10.46	+13.95	+22.67	+25.0	+29.06
Protein Efficiency Ratio (PER)	5.34±0.22^{a}	6.39±0.25^{b}	6.79±0.27^{b}	7.14±0.29^{b}	7.64±0.31^{b,c}	7.68±0.32^{b,c}	7.83±0.33^{b,c}	8.13±0.34^{b,c}
	PDC	+19.66	+27.15	+33.70	+43.07	+43.82	+46.62	+52.24
Feed Conversion Ratio (FCR)	2.56±0.17^{a}	2.42±0.16^{a}	2.38±0.15^{a}	2.14±0.13^{a}	2.03±0.11^{b}	1.82±0.10^{b}	1.78±0.11^{b,c}	1.41±0.09^{b,c}
	PDC	-5.46	-7.03	-16.40	-20.70	-26.17	-30.46	-44.92
Gross Feed Conversion Efficiency (GFCE) (%)	39.06±0.96^{a}	41.32±0.98^{a}	42.02±1.01^{a}	46.73±1.05^{b}	49.26±1.08^{b}	52.91±1.09^{b,c}	56.18±1.15^{b,c}	70.92±1.21^{b,c,d}
	PDC	+5.79	+7.57	+19.64	+26.11	+35.46	+43.83	+81.57
Feed Efficiency Ratio (FER)	0.391±0.018^{a}	0.413±0.020^{a}	0.421±0.01^{a}	0.467±0.021^{a}	0.493±0.024^{b}	0.529±0.025^{b,c}	0.562±0.026^{b,c}	0.715±0.312^{b,c,d}
	PDC	+5.62	+7.67	+19.43	+26.08	+35.29	+43.73	+81.58
Harvest Size (g)	16.32±0.68^{a}	17.38±0.71^{a}	18.92±0.72^{a}	21.13±0.76^{a}	23.14±0.77^{b,c}	24.19±0.78^{b,c}	25.38±0.81^{b,c}	27.13±0.84^{b,c,d}
	PDC	+6.49	+16.29	+29.47	+41.78	+48.22	+55.51	+66.23
Productivity (kgs)	7.26±0.29^{a}	7.73±0.33^{a}	8.73±0.35^{b}	9.93±0.44^{b,c,d}	10.88±0.49^{b,c,d}	11.37±0.52^{b,c,d}	11.93±0.53^{b,c,d}	12.89±0.58^{b,c,d}
	PDC	+6.47	+20.25	+36.78	+49.86	+56.61	+64.33	+79.53

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 @ $p<0.05$.

Table 6: Performance details of *L.vannamei* under different Biofloc Feeding trails (C/N 15:1)

Parameter	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	92	94	94	93	94	96	98
Final weight (g)	16.48±0.72^{a}	19.31±0.82^{a}	20.18±0.83^{b}	21.83±0.86^{b}	23.14±0.87^{b,c}	25.17±0.88^{b,c}	29.79±0.95^{b,c,d}	33.97±0.98^{b,c,d}
	PDC	+17.17	+22.45	+32.46	+40.41	+52.73	+80.76	+106.12
Relative Growth Rate (g)	14.96±0.64^{a}	17.79±0.72^{a}	18.66±0.78^{b}	20.31±0.84^{b}	21.62±0.87^{b,c}	23.65±0.92^{b,c}	28.27±0.95^{b,c,d}	32.45±1.02^{b,c,d}
	PDC	+18.91	+24.73	+35.76	+44.51	+58.20	+89.17	+116.91
Daily Growth Rates (DGR) (g)	0.249±0.008^{a}	0.297±0.012^{a}	0.311±0.014^{b}	0.339±0.016^{b}	0.361±0.022^{b,c}	0.394±0.036^{b,c}	0.472±0.042^{b,c,d}	0.541±0.047^{b,c,d}
	PDC	+19.27	+24.89	+36.14	+44.97	+58.23	+89.55	+117.26
Specific Growth Rates (SGR)	1.73±0.13^{a}	1.81±0.10^{a}	1.83±0.12^{a}	1.91±0.15^{a}	1.95±0.16^{a}	2.10±0.19^{b}	2.17±0.21^{b}	2.34±0.23^{b,c}
	PDC	+4.62	+5.78	+10.40	+12.71	+21.38	+25.43	+35.26
Protein Efficiency Ratio (PER)	5.38±0.20^{a}	6.77±0.23^{b}	7.24±0.27^{a}	7.58±0.31^{b,c}	7.74±0.34^{b,c}	7.89±0.41^{b,c}	8.34±0.43^{b,c}	8.72±0.51^{b,c}
	PDC	+25.83	+34.57	+40.89	+43.86	+46.65	+55.01	+62.08
Feed Conversion Ratio (FCR)	2.54±0.161^{a}	2.21±0.134^{a}	2.14±0.116^{a}	1.79±0.0966^{b}	1.67±0.0782^{b}	1.58±0.076^{b,c}	1.42±0.056^{b,c}	1.22±0.081^{b,c,d}
	PDC	-12.99	-15.74	-29.52	-34.25	-37.79	-44.09	-51.96
Gross Feed Conversion Efficiency (GFCE) (%)	39.37±0.97^{a}	45.25±1.09^{a}	46.73±1.11^{a}	55.87±1.17^{b}	59.88±1.19^{b}	63.29±1.29^{b,c}	70.43±1.36^{b,c,d}	81.97±1.62^{b,c,d}
	PDC	+14.94	+18.70	+41.91	+52.10	+60.76	+78.90	+108.20
Feed Efficiency Ratio (FER)	0.393±0.018^{a}	0.453±0.024^{a}	0.467±0.032^{a}	0.559±0.041^{b}	0.599±0.047^{b}	0.633±0.054^{b}	0.704±0.067^{b,c}	0.820±0.065^{b,c,d}
	PDC	+15.27	+18.83	+42.24	+52.42	+61.07	+79.14	+108.65
Harvest Size (g)	16.37±0.62^{a}	19.42±0.77^{a}	20.39±0.79^{b}	22.14±0.91^{b}	23.44±0.93^{b,c}	25.72±1.03^{b,c}	29.74±1.12^{b,c,d}	33.45±1.19^{b,c,d}
	PDC	+18.63	+24.55	+35.24	+43.18	+57.11	+81.67	+104.33
Productivity (kgs)	7.28±0.29^{a}	8.93±0.34^{b}	9.58±0.39^{b}	10.41±0.44^{b,c}	10.90±0.46^{b,c}	12.59±0.55^{b,c,d}	14.28±0.58^{b,c,d}	16.39±0.67^{b,c,d}
	PDC	+22.67	+31.59	+42.99	+49.73	+72.93	+96.15	+125.13

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 @ $p<0.05$.

Table 7: Performance details of *L.vannamei* under different Biofloc Feeding trails (C/N 20:1)

Parameter	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 (%)	88	89	93	94	94	95	94	95
Final weight (g)	16.59±0.66^{a}	18.36±0.70^{a}	20.14±0.73^{b}	21.49±0.78^{b}	22.48±0.84^{b,c}	23.12±0.86^{b,c}	25.74±0.88^{b,c,d}	27.42±0.90^{b,c,d}
	PDC	+10.66	+21.39	+29.53	+35.50	+39.36	+55.15	+65.28
Relative Growth Rate (g)	15.07±0.62^{a}	16.84±0.67^{a}	18.62±0.73^{b}	19.97±0.74^{b}	20.96±0.79^{b,c}	21.60±0.81^{b,c}	24.22±0.84^{b,c,d}	25.90±0.87^{b,c,d}
	PDC	+18.91	+24.73	+35.76	+44.51	+58.20	+60.71	+71.86
Daily Growth Rates (DGR) (g)	0.252±0.013^{a}	0.281±0.014^{a}	0.311±0.015^{b}	0.333±0.01^{b}	0.349±0.018^{b}	0.360±0.020^{b,c}	0.404±0.021^{b,c,d}	0.432±0.025^{b,c,d}
	PDC	+11.50	+23.41	+32.14	+38.49	+42.85	+60.31	+71.42
Specific Growth Rates (SGR)	1.72±0.14^{a}	1.78±0.16	1.86±0.17^{a}	1.92±0.20^{a}	1.97±0.21^{a}	2.04±0.22^{a}	2.11±0.23^{a}	2.17±0.22^{b}
	PDC	+4.62	+5.78	+10.40	+12.71	+21.38	+25.43	+35.26

Protein Efficiency Ratio (PER)	5.39±0.27^{a}	6.58±0.32^{a}	7.12±0.34^{b}	7.44±0.33^{b}	7.62±0.37^{b}	7.74±0.40^{b,c}	7.82±0.41^{b,c}	8.04±0.46^{b,c}
	PDC	+22.07	+32.09	+38.03	+41.37	+43.59	+45.08	+49.16
Feed Conversion Ratio (FCR)	2.56±0.152^{a}	2.38±0.137^{a}	2.27±0.124^{a}	2.18±0.114^{a}	2.13±0.092^{b}	2.04±0.069^{b}	1.78±0.080^{b,c}	1.57±0.060^{b,c,d}
	PDC	-7.03	-11.32	-14.84	-16.79	-20.31	-30.46	-38.67
Gross Feed Conversion Efficiency (GFCE) (%)	39.06±0.904^{a}	42.01±0.949^{a}	44.06±0.955^{a}	45.88±0.98^{b}	46.95±1.019^{b}	49.02±1.046^{b,c}	55.87±1.088^{b,c,d}	63.69±1.126^{b,c,d}
	PDC	+7.55	+12.80	+17.46	+20.20	+27.88	+43.04	+63.06
Feed Efficiency Ratio (FER)	0.391±0.020^{a}	0.420±0.023^{a}	0.441±0.025^{a}	0.459±0.02^{b}	0.469±0.036^{b}	0.491±0.037^{b}	0.562±0.038^{b,c}	0.637±0.041^{b,c,d}
	PDC	+7.41	+12.78	+17.39	+19.94	+25.57	+43.73	+62.91
Harvest Size (g)	16.31±0.59^{a}	17.78±0.61^{a}	19.83±0.73^{b}	20.14±0.77^{b}	21.39±0.78^{b}	23.14±0.80^{b,c}	25.12±0.82^{b,c}	27.03±0.85^{b,c,d}
	PDC	+9.07	+21.58	+23.48	+31.14	+41.87	+54.01	+65.72
Productivity (kgs)	7.18±0.29^{a}	7.91±0.31^{a}	9.22±0.33^{b}	9.47±0.36^{b}	10.04±0.42^{b}	10.99±0.47^{b,c}	11.81±0.52^{b,c,d}	12.84±0.54^{b,c,d}
	PDC	+10.17	+28.41	+31.89	+39.83	+53.06	+64.49	+78.84

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 @ $p<0.05$.

Table 8: Comparative performance details of *L.vannamei* under different C: N ratios

	Type of Diet used			
Parameter	**Control**	**ED-7**	**ED-7**	**ED-7**
		C/N 10:1	**C/N 15:1**	**C/N 20:1**
Number of shrimp stocked (nos)	500	500	500	500
Percent survival (%)	87	93	98	95
Final weight (g)	16.43±0.74^{a}	28.73±0.96^{b}	33.97±0.98^{b,c}	27.42±0.90^{b,d}
	PDC	+74.86	+106.12	+66.89
Relative Growth Rate (g)	14.91±0.66^{a}	27.21±0.95^{b}	32.45±1.02^{b,c}	25.90±0.87^{b,d}
	PDC	+82.49	+117.64	+73.71
Daily Growth Rates (DGR) (g)	0.249±0.008^{a}	0.454±0.023^{b}	0.541±0.04^{b,c}	0.432±0.025^{b,d}
	PDC	+82.33	+117.69	+73.44
Specific Growth Rates (SGR)	1.72±0.106^{a}	2.22±0.15^{b}	2.34±0.23^{b}	2.17±0.226^{b,c}
	PDC	+29.07	+36.05	+26.16
Protein Efficiency Ratio (PER)	5.34±0.22^{a}	8.13±0.34^{b}	8.72±0.51^{b}	8.04±0.468^{b,c}
	PDC	+52.25	+63.30	+50.56
Feed Conversion Ratio (FCR)	2.56±0.17^{a}	1.41±0.09^{b}	1.22±0.081^{b,c}	1.57±0.060^{b,d}
	PDC	-44.92	-52.34	-38.67
Gross Feed Conversion Efficiency (GFCE) (%)	39.06±0.96^{a}	70.92±1.21^{b,c,d}	81.97±1.62^{b,c}	63.69±1.12^{b}
	PDC	+81.57	+109.86	+63.06
Feed Efficiency Ratio (FER)	0.391±0.018^{a}	0.715±0.31^{b,c,d}	0.820±0.065^{b,c}	0.637±0.041^{b}
	PDC	+81.59	+109.72	+62.92
Harvest Size (g)	16.31±0.68^{a}	27.13±0.84^{b}	33.45±1.198^{b,c}	27.03±0.85^{b}
	PDC	+66.34	+105.09	+65.73
Productivity (kgs)	7.18±0.28^{a}	12.89±0.45^{b,d}	16.39±0.67^{b,c}	12.84±0.54^{b}
	PDC	+79.53	+128.27	+78.83

4. Conclusion

The results obtained in the present feeding trail study reveals that the incorporation of Probiotics and manipulation of C/N ratios by the addition of selected external Carbohydrate sources such as Molasses, Tapioca, Maize flour and Sucrose in combinations can significantly promote growth and feed associated parameters of shrimp and increase the Biofloc volume in the culture tanks. The higher ideal growth performance of *L.vannamei* was recorded with C/N ratio of 15:1, compared to C/N 10:1 or 20:1, indicating the adequacy of C/N ratio of 15:1 to achieve maximum productivity. The current take a look at highlights the significance of various Carbon sources in improvising the Biofloc system and Shrimp overall performance with an advanced survival rates with *L.vannamei*. The C/N ratio of 15:1 increased the relative abundance of a few capacity beneficial bacteria and could sell the accumulation of various bioactive metabolites such as flavonoids and many others that may similarly inhibit the growth of destructive bacteria.

5. References

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