EFFECT OF BIOFLOC SYSTEM ON GROWTH PERFORMANCE IN SHRIMP LITOPENAEUS VANNAMEI UNDER DIFFERENT C:N RATIOS WITH SUGARCANE MOLASSES

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ABSTRACT

An attempt has been made to study the effect of biofloc System on water quality and performance in shrimp *Litopenaeus vannamei* under different Carbon : Nitrogen (C:N) ratios, using sugarcane molasses as carbon source for the development of Bioflocs in the present investigation. In the present investigation three C:N ratios i.e. 10:1, 15:1, and 20:1 were maintained with sugarcane molasses as carbon source for period of 6 weeks with *L. vannamei*. Certain water quality parameters like Temperature, Dissolved oxygen, pH were found to be almost static, without any change during biofloc treatments. TAN, Nitrate, Nitrite, Alkalinity, TSS, Biofloc volume, BOD and DO reduction rates were found to be significantly changed during biofloc treatments compared to control group. Similarly the growth performance studies monitored also clearly evinces that, bioflocs addition efficiently induces the growth rates which were manifested through ABW, WG, DGR, SGR, PER & FER and finally productivity rates. In the present investigation among the three C:N ratios maintained 10:1, 15:1 & 20:1 with sugarcane molasses, the 15:1 C:N ratio appears to be reasonably very good, because it is efficiently inducing highest and best growth potentials in *L. vannamei*.

Keywords : *L. vannamei*, C:N ratios, Bioflcos, growth

1.0 Introduction

Aquaculture is considered to be one of the fastest growing food production sectors and representing around 45% of the world fish production (FAO, 2016). Among the various aquaculture activities, commercial shrimp farming is highly preferred due to its high protein content and economic returns over investment as, these shrimps were considered as valued seafoods. The shrimp culture activity played a major role in the economic growth of several developing countries including India. But after meeting a tremendous success, the penaeid shrimp culture activity was plagued with several diseases by pathogens including Bacteria, Virus and Protozoans. Several of the diseases caused by pathogens in many Asian countries induce catastrophic mortality with severe economic losses (Karunakar *et al*, 2004; Lightner *et al*, 2012). Due to this particular region, the researchers and producers are actively engaged in exploring the alternative, innovative therapeutic and preventive measures to reduce the mortality rates and increasing the productivity by way of using certain technologies. i.e. use of Probiotics, Immune-stimulants and Biofloc technologies etc. Maintenance of Ecological sustainability of shrimp farming activity is a major challenge, because several of the inputs especially the feed and its ingredients were known to be accumulating in the culture environment and finally contributing towards the deterioration of the water quality, results in the imposing of stress finally lead to disease outbreaks (Kautsky *et al*, 2000; Funge-Smith and Briggs, 1998). In recent years, the introduction of usage of certain substances including Probiotics, Immuno-stimulants and adopting of newer technologies like Biofloc technology paved way for the establishment of sustainability to a certain extent and also reduced the rate of disease outbreaks to a large extent. Biofloc technology is based on microbial manipulation with in the aquaculture system i.e. Carbon : Nitrogen (C:N) ratio in feed has been shown promising results (Avnimelech, 1999; 2007; Crab *et al*, 2012). This Biofloc Technology was generated by supplementation of external Carbon sources in the feed and contributes for increased productivity rates in the candidate species of culture (Kumar *et al*, 2014; 2017; Hari *et al*, 2006; Xu and Pan, 2012; 2014)

By keeping the above literature, the present investigation is aimed to probe in to the aspects concerning the use of sugarcane molasses as carbohydrate source for the development of biofloc for the Penaeid shrimp *L. vannamei* and to monitor the water quality parameters, growth potentials and to arrive the exact ratio of C:N source for the production of approximate feeding technologies towards increasing the productivity rates.

2.0 Materials and Methods

2.1 Experimental Design

The present set of Experiments were carried out in shrimp culture units located in Ramayapatnam (Latitude 15^{0} 02^{1} 55^{11} N; Longitude 80^{0} 02^{1} 50^{11} E) Prakasam Dist of Andhra Pradesh, India. The Experiments were randomly designed which includes Four feeding trail Experiments i.e.

Group – I : Control group without Biofloc addition

Group – II : Biofloc maintenance @ C:N 10 : 1

Group – III : Biofloc maintenance @ C:N 15 : 1

Group – IV : Biofloc maintenance @ C:N 20 : 1

Sugarcane molasses was selected as carbon source to produce bioflocs. Penaeid shrimp *Litopenaeus vannamei* of 2.52±0.12 g of uniform size were obtained from local Aquafarms and were tested for disease occurrence and finally pathogen specific free shrimp were selected for experimentation. The selected shrimp were acclimatized in experimental tanks with a specific salinity of 10±0.5 ppt. Feeding was done with a commercial feed obtained from local market and the shrimp were fed *ad libitum* twice a day both in the morning and evening at 6.00 AM and 6.00 PM @ 30% of the total biomass. The experimental trails were conducted for a period of 6 weeks i.e. for 42 days.

Sugarcane molasses was selected as a source for Carbon, and known to contain 36% Carbon, 53% Carbohydrate, 24% moisture content was incubated for 2 days in warm water at 40^{0}C and the same was added to use culture medium in the ratio 1:3 Molasses : Water. To stimulate Nitrogen loading in an aquaculture system, NH_4Cl, KH_2PO_4 and Na_2HPO_4 were added to each tank. 96, 31 and 64 mg/lit, respectively (Ekasari, 2010). The ratio between sugarcane molasses and the feed to reach desired Carbon : Nitrogen (C:N) ratio was calculated based on assuming 50% nitrogen from feed eaten by the shrimp excreting in to water environment (Avnimelech, 1999). On the above basis, the formula of the ratio on weight between Carbon source and feed can be given as follows :

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

Where :

ΔCH : Weight of Carbon Source

ΔF : Weight of the Feed

CN : C:N ratio need 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

Carbon content was determined by adopting the method of Walkley and Black (1934). Total Ammonia Nitrogen (TAN) concentration and other water quality parameters were measured with the procedures according to APHA (2005). Growth parameters including Average body weights (ABW), Average daily growth rates (ADGR), Specific growth rates (SGR), Feed conversion ratio (FCR), Protein efficiency ratio (PER), Feed efficiency ratio (FER) and Productivity were monitored and tabulated. All the above parameters were calculated by adopting the following Formulae

Weight gain :

Weight of the Shrimp (G) At the end of the Experiment - Weight of the Shrimp (G) at the start of the Experiment

Survival rate (%)

$$\frac{\text{Total number of Live shrimp}}{\text{Total number of shrimp stocked}} \times 100$$

Weight Gain (g)

Weight of the shrimp (g) At the End of the Expt - Weight of the shrimp (g) at the Start of the Expt

Feed Conversion Ratio (FCR)

$$\frac{\text{Total amount of Feed Consumed (Kgs)}}{\text{Total Biomass of shrimp (Kgs)}}$$

Average daily growth rates (ADGR)

Weight of the shrimp (g) At the End of the Expt - Weight of the shrimp (g) at the Start of the Expt

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Total number of days of Experiment

Specific growth rates (SGR)

$$\frac{\text{Log Weight of the shrimp (g) At the End of the Expt} - \text{Log Weight of the shrimp (g) at the Start of the Expt}}{\text{Total number of days of Experiment}} \times 100$$

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

Where

W_1 : Weight of the shrimp at start of the Experiment

W_2 : Weight of the shrimp at the end of the Experiment

T : Total number of days of Experiment

Vibrio Bacteria rate (%)

$$\frac{V}{H} \times 100$$

Where

V : Average density of Vibrio bacteria

H : Average density of Total Bacteria in each Tank

Biofloc volume (FV)

$$V_{Floc} / V_{collection}$$

Where

V_{Floc} : Biofloc Volume (ml)

$V_{collection}$: Collected Sample Volume (ml)

The data obtained was analyzed statistically through Microsoft excel. The difference in variants between treatments was determined according to One-Way ANOVA using SPSS.

3.0 Results and Discussion

3.1 Water quality analysis

The Water quality parameters were monitored in all the Experimental groups i.e. control and three Biofloc groups and presented in Table.2. The water parameters like salinity, temperature and pH were found to be almost constant without and significant change throughout the experimental period of 6 weeks. The salinity was found to be 10 ± 0.4 ppt. The Temperature of water medium also found to ranges between 25-27^{0}C. The Dissolved Oxygen (DO) content of the water samples range from 6.2-6.8. pH of the medium also in the range of 7.5-7.7. No significant changes were recorded with salinity, temperature, pH and DO during the present Experimental Period. The Total Ammonia Nitrogen (TAN) content, Nitrite and Nitrate contents were found to be significantly decreased in all the Experimental groups i.e. Bioflocs maintained at C:N (10:1), 15:1 & 20:1 ratios, when compared to control group maintained without any addition of bioflocs. The Alkalinity, Total Suspended Solids (TSS), Biofloc volume, Biological Oxygen Demand (BOD) and DO reduction rate were found to be significantly increase in all the Experimental groups maintained with different ratios of C:N ratios compared to control group (Table.2). The Total Hetero Bacterial (THB) count showed a significant increases gradually from Ist to VIth week of experimental period in all the experimental group added with Bioflocs compared to control group (Figures. 1 & 2), when as the Total Vibrio Bacterial (TVB) content was significantly decreased with an increase in the experimental period up to 6th week, but in the control group the TVB content showed a increment from 1st week onwards and reaching maximum in the 6th week (Figures. 3 & 4). The performance details of Penaeid shrimp *Litopenaeus vannamei* were monitored for a period of 6 weeks and presented in Table.3. The percent survival rates recorded to be 95, 96, 98 & 96 for control, C:N (10:1), 15:1 & 20:1 Biofloc maintained groups, respectively.

At the start of the experiment specific pathogen free shrimp of average body weight of 2.52 ± 0.12 g were selected and stoked in experimental tanks. For each Experimental group 100 nos were stocked and for each set six groups were maintained. Performance parameters were monitored regularly and at the end were tabulated. The find weights of *L. vannamei* were recorded to be 13.14 g for control group, with a weight gain (%) of +322, which as highly significant (P<0.001). Among the Experimental groups, maximum weight of 18.49 g was recorded with Biofloc with C:N (15:1) ratio compared to 17.38 g with Biofloc C:N (10:1) ratio followed by 17.14 g with Biofloc C:N (20:1) ratio, and the present changes were observed to be +40.72, +32.27 and +30.44 for Biofloc C:N (15:1), C:N (10:1) & C:N (20:1), respectively and were found to be statistically significant over control group. Similarly the weight percentages obtained were +322, +490. +534 & +480 for control, Biofloc C:N (10:1), 15:1, 20:1 maintained groups, respectively. The feed conversion ratios (FCR) was recorded to be 2.58 for control group, where as minimum of 2.03, followed by 2.33 and 2.34 were obtained for Biofloc C:N (15:1), 20:1 & 10:1 maintained groups. The daily growth rates (DGR) recorded to be maximum 0.388 with Biofloc C:N (15:1) maintained group compared to 0.354, 0.349 and 0.253 for Biofloc C:N (10:1), 20:1 & control group, respectively. The percent change was observed to be maximum +53.36 with Biofloc C:N (15:1) maintained group followed by +39.92, +37.94 with Biofloc C:N (10:1) and 20:1 maintained group and all the values are found to be statistically significant over the control group. The Specific Growth Rates (SGR) were found to maximum of 2.06 with a percent increment of +20.47 with Biofloc C:N (15:1) maintained group compared with 1.97 (+15.21), 1.98 (+15.79), with Biofloc C:N (10:1), & 20:1 maintained groups, respectively. The Protein Efficiency Ratios (PER) were found to be recorded maximum 7.94 (+49.25), followed by 7.53 (+41.54), 7.31 (+37.41) for Biofloc C:N (15:1), 10:1, 20:1 maintained groups, respectively and all the values are formed to be statistically significant over its control group. The Feed Efficiency Ratio (FER) were also found to be recorded maximum of 0.624 (+37.44); 0.605 (+33.26), 0.595 (+31.06) for Biofloc C:N (15:1), 20:1, 10:1 maintained groups, respectively and the values are statistically significant at $P<0.001$ over its control group. Soon after the termination of the experiment the harvested size of the shrimp was recorded and maximum sized shrimp of 18.44 (+40.72), followed by 17.38 (+32.27) and 17.14 (+30.44) with Biofloc C:N (15:1), 10:1, 20:1 maintained groups and the values are statistically significant over its control group. The productivity or the Total Biomass was increased after 6 weeks of Experimental period and maximum of 10.87 kgs (+45.13), followed by 10.01 kgs (+33.64), 9.87 kgs (+31.78) recorded with Biofloc C:N (15:1), 10:1, 20:1 maintained group compared to 7.49 kgs with control group. All the values obtained for Experimental groups pertaining to productivity were found to be significant over its control group.

The proximate composition of different Bioflocs used along with control diet were analyzed and presented in Table.1. The organic matter values ranged in and around 82-83% in all the experimental diets selected. The Ash values were also ranged from 16-18%. The crude protein content also ranges from 35-37% in all the practical diets preferred in the present investigation. The crude lipid content was approximately around 6-6.8%. The crude fiber values falls in and around 3-4%. The NFE values were in the range of 25-29%. The moisture content was around 8-9%. The gross energy values calculated for all the practical feeds and found to be in the range around 390-396 kcal/100 g.

In the present experiment, the Biofloc development was recorded in terms of Floc volume (FV) and Total suspended Solids (TSS), both were found to be significantly increased in all the Biofloc added groups compared to control group. The color obtained on the Biofloc added experimental groups was brown and supposed to contain suspended organic particles in the form of flocculated aggregates, with a heterotrophic bacteria colonies along with protozoans & microalgae. Control of the concentration of TSS is very important and plays a vital role in biofloc systems. It is unclear that how particle concentration management leads to improved shrimp production, although some possibilities include reduced gill clogging, promotion of a younger and potentially healthier microbial community, possible removal of nuisance organisms or reduced biochemical oxygen demand which may lead to increased oxygen availability for culture organisms. By adopting mechanisms like reducing the overall abundance of bacteria, cyanobacteria, nematods, rotifers, adopting radiation in the water column will lead to the substantial changes in microbial communities lead to tncrease in productivity. In the present investigation, all the water quality parameters recorded were found to be with in a suitable ranges for *L. vannamei* survival and growth for a period of 6 weeks and are with in the acceptable range for brackish water shrimp culture (Xu *et al*, 2016). In the present study, Biofloc C:N ratios were manipulated and maintained at 10:1, 15:1 and 20:1 by addition of external carbohydrate helps in Biofloc production (Azhar *et al*, 2016; Hari *et al*, 2004; 2006). In the present study sugarcane molasses was used as carbon source for successful augmentation of microbial growth. Due to sufficient aeration of all the experimental tanks in the present study provided adequate quantities of DO levels. The alkalinity levels obtained in the present investigation were found to be relatively higher as it was recommended to be 130 mg/lit for shrimp culture (Boyd *et al*, 2002). In the present investigation, due to addition of sugarcane molasses as carbohydrate source to induce Biofloc production, significantly reduced the TAN levels in the water column of experimental media. The concentrations of TAN, Nitrite-N, Nitrate-N, obtained in the present investigation were found to fall in the optimum range of levels as recommended for juveniles of Pacific white shrimp *L. vannamei* (Lin & Chen, 2003; Samocha *et al*, 2007). Similar kind of observations were reported, where in due to the addition of additional carbohydrate sources significantly reduced, TAN concentrations in the Tilapia (Azim & Little, 2008), *P.monodon* (Hari *et al*, 2004; Kumar *et al*, 2014) and *L. vannamei* (Wasielesky *et al*, 2006). There was reports regarding the successful utilization of TAN and carbon by the Bacteria for the production of microbial floc with in the culture system (Avnimelech, 1999; Azim & Little, 2008). Moreover significantly reduced levels of TAN also helps to maintain better water quality in the Biofloc added culture systems. In the present investigation, relatively very low levels of Nitrite-N were recorded during the experimental period, suggests the successful oxidation of ammonia to nitrate (Cohen *et al*, 2005). It has been observed that ammonia nitrite and nitrate were found to be removed from culture systems by microbial communities towards the production of higher quantities of protein, i.e. due to assimilation of nitrogen during Biofloc treated culture organisms (Ebeling *et al,* 2006; Burford *et al* , 2004; Ray *et al,* 2010; Vinatea *et al,* 2010). Avnimelech (2007), in his series of experiments, he reported that the addition of carbohydrate as carbon source, reduces the need of dietary protein concentration and also decrease the TAN concentrations in the culture systems. In the present study, sugarcane molasses used as carbohydrate source significantly reduced the TAN concentrations, when compared to control group, which was maintained without the addition of sugarcane molasses. In the present investigation due to ammonia, an excretory product generated by shrimp under go oxidation and produces Nitrate via the formation of Nitrite, by adopting the nitrification phenomenon in the culture operation. The concentrations obtained for Nitrate and Nitrite in the present investigation also suggests that due to nitrification phenomenon the nitrate concentrations arte relatively high compared to nitrite. But during Biofloc treatments, the Nitrate concentration was relatively decreased in all the Ratios of C:N maintained, clearly suggests the replace of nitrate by microbes for the production of Bioflocs and also due to low availability of limited availability of TAN also facilitates to absorb Nitrate by microbes (Hargreaves, 1998). Generally ammonia present in the culture operation, be taken by Heterotrophic bacteria, while Nitrate available will be taken for higher rate production of Phytoplankton (Middelburg and Nieuwenbuize, 2000). The higher level of nitrogen compounds i.e. Ammonia, Nitrate and Nitrite generally affect physiological processes that are important to aquaculture activities (Sabrina et al, 2015). Among these processes, affected osmoregulation and respiration results in loss of appetite maximum subsequently food consumption was reduced significantly in turn effects growth rates and some times even lead to mortality of shrimp (Kuhn *et al*, 2010). The generation of Ammonia and its transformed products such as TAN, Nitrate and Nitrite seems to be significantly reduced during Biofloc treatment groups clearly reveals that the nitrogen was successfully transformed into the protein component under Biofloc presence in the culture operation. The TSS contents recorded in the present investigation were falls in the recommended level for penaeid shrimp including *P.monodon* and *L. vannamei* in the establishment of system stability (Samocha *et al,* 2007 ; Baloi *et al,* 2013) The Biofloc volume levels were shown to be increased gradually with an increase in C:N ratios. The Floc volume plays a vital role in the production and density of phytoplankton, i.e. the presence of carbon source and light. The results obtained in the present study clearly showed that the addition of carbon source enhanced the microbial growth rather than microalgae. DO reduction can be taken as an index to assess the microbial load and occurrence of plankton amount, the higher amounts results in efficient reduction of DO reduction rates. In the present investigation DO reduction rates were significantly increased with the addition of carbon source, which enhances the microbial load and phytoplankton quantity substantially, there by reduces the DO levels. Similar kind of observations were also reported by Kumar *et al* (2017) in P. monodon during usage of different carbon sources in the form of Bioflocs. The Total Heterotrophic Bacteria (THB) amounts were found to be significantly increased with increase in C:N ratios from 10:1 to 15:1 & 20:1 by using sugarcane molasses as the carbon source compared to its control group maintained without addition of molasses. The increase in the THB counts are found to be highly significant in all the Experimental groups ($P<0.001$) compared to control group. In the general the THB absorb the toxic substances such as NH_4^+ and NO_2^- and subsequently converted them in to biomass there by leading to increase in the Bacterial density and also Floc volume. Several authors reported the presence of THB in a biofloc system ranges around 10×10^6cfu/ml and more than that will be found to be harmful (Anderson, 1993; Avnimelech, 2015). But interestingly the Total Vibrio Bacteria (TVB) count seems to be reduced significantly with the addition of sugarcane molasses added as carbohydrate source in the culture media. Several authors reported the reduction of TVB counts and increased Survival rates after the addition of carbon sources like molasses in the culture operation of shrimp including *P. monodon, Farfantepenaeus brasiliensis*, (Tao *et al*, 2017; Souza *et al*, 2014; Ngan *et al*, 2008; Ngan and Heip, 2010). The results obtained clearly demonstrate that C:N ratios maintained the present investigation facilitated the production of beneficial bacteria and subsequently inhibit the development of vibrio bacteria in culture operation. The growth performance parameters recorded for *L. vannamei* during different biofloc treatments with the addition of sugarcane molasses, parameters like Final weights, Daily Growth Rates (DGR), Specific Growth Rates (SGR), Protein Efficiency Ratio (PER), and related parameters were found to be significantly increased during different Biofloc treatments, prepared with the addition of sugarcane molasses as carbon sources. The results obtained in the present investigation clearly indicates that addition of appropriate quantities of carbohydrates was helpful in promoting adequate growth rates and also survival rates of *L. vannamei*. Several authors also reported that, due to addition of carbohydrates results in the production and accumulation of Bioflocs, which will serve as an important food source for plankton increase and thus its plankton will be taken as food by shrimp and finally contributes for the increased productivity (Gao *et al*, 2012; Avnimelech, 2007). Phuoc (2017) demonstrated that Zooplankton available in culture operation serve as supplemental food source for *L. vannamei* and subsequently increase the productivity rates. Similar kind of situation may be prevailing which lead to the increased ABR and finally contributing towards increased productivity. Avnimelech *et al* (2015) demonstrated that carbohydrate addition to the culture medium lead to increase in protein utilization and supply of essential lipids and vitamins for the growth of shrimp.

The proximate composition of the bioflocs prepared and used in the present study clearly demonstrates that all the Bioflocs supplied in the Experimental study was known to contain sufficient and adequate amounts of protein, lipid, fiber, ash and energy which will be highly useful for maintains or supplying nutrients for the shrimp species). So the bioflocs produced in the present study providing all the nutrient materials for shrimp. Similar kind of proximate composition of bioflocs were reported earlier by Cuzon *et al* (2004) and was able to provide sufficient nutrient content and able to induce maximum growth in the case of shrimp from the results obtained in the present investigation, it is very clear that among the three Carbon : Nitrogen (C:N) ratios i.e. 10:1, 15:1 & 20:1 with sugarcane molasses as carbon source during culture operation, the shrimp showed highest and best growth potentials in the (15:1) C:N ratio.

4.0 Conclusion

The present investigation may be concluded that the Biofloc showed the effect on the growth potentials of *L. vannamei*, water quality of the culture medium. The Biofloc with C:N (15:1) ratio effectively reduced the TAN, there by maintain good water quality for shrimp culture operation. More over the sugarcane molasses used in the present investigation as a carbon source seems to be easily digestible and promotes best growth potentials and also growth of the microorganisms. The higher nutritional values of the Bioflocs developed by the molasses also play an important role in the promotion of growth rates in *L. vannamei*. Due to the additional supplementation of nutritional requirements supplied through the Biofloc are being absorbed efficiently and converted in to the study of the body and hence productivity were significantly increased in Biofloc treated shrimp. From the results it can be concluded that, the addition of Bioflocs provided sufficient nutritional requirements, which subsequently lead to the growth of the shrimp. Biofloc shrimp culture systems are viable alternative to traditional aquaculture, when the microbial communities in these systems are managed appropriately, according to the goals of the shrimp producers. Though the use of bioflocs brings greater risk of mechanical or biological complications such as dissolved oxygen depletion, substantially larger shrimp crops can be produced using very little land or water, but it requires some what unique set of engineering and management criteria. So by way of use of Biofloc addition; not only water quality and supplementation of nutritional requirements to the shrimp and also promotes the growth potentials in a successful way leading to sustainability of shrimp aquaculture operations.

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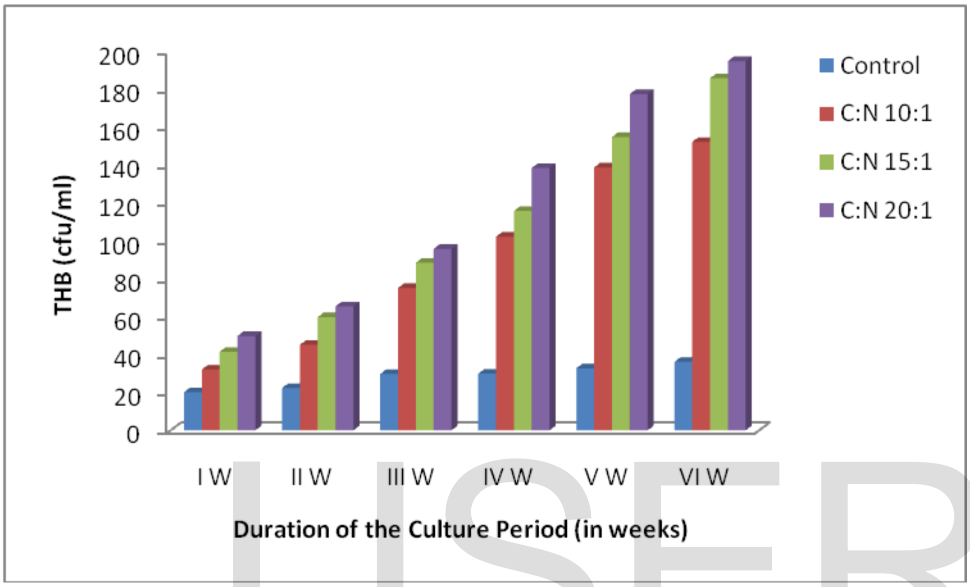

Figure.1 : Total Heterotrophic Bacteria (THB) content in *L. vannamei* culture tanks

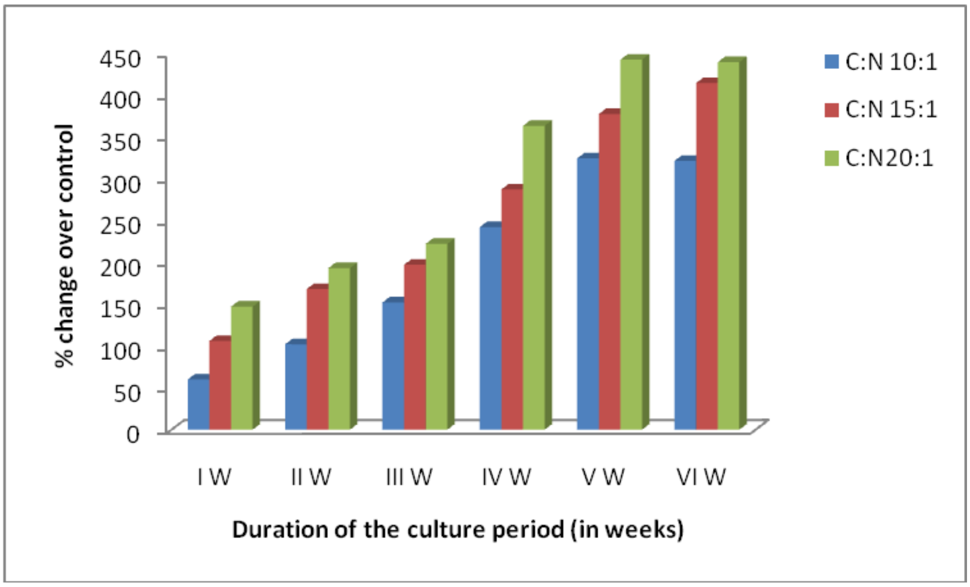

Figure.2 : % Change of Total Heterotrophic Bacteria (THB) content in *L. vannamei* culture tanks at different periods

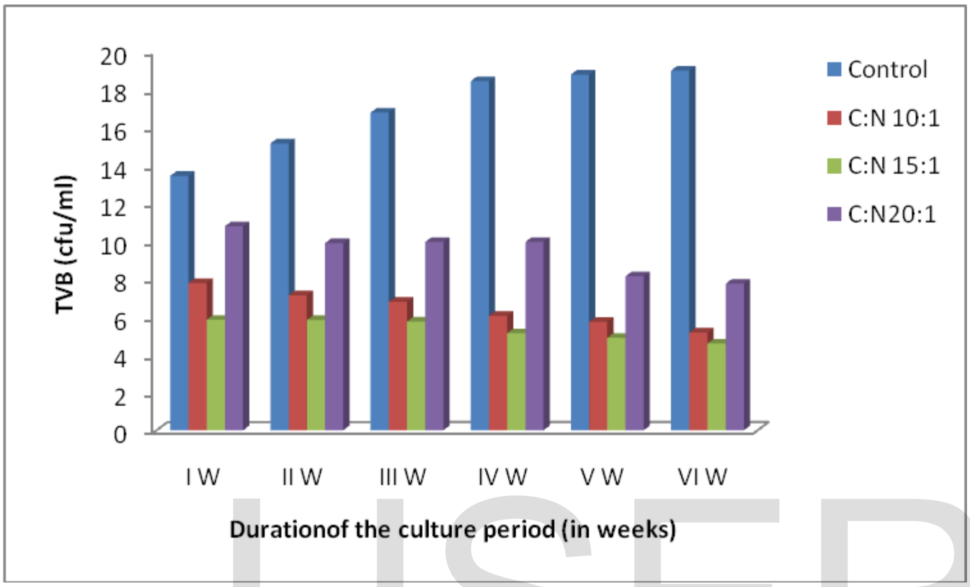

Figure.3 : Total Vibrio Bacteria (TVB) content in *L. vannamei* culture tanks

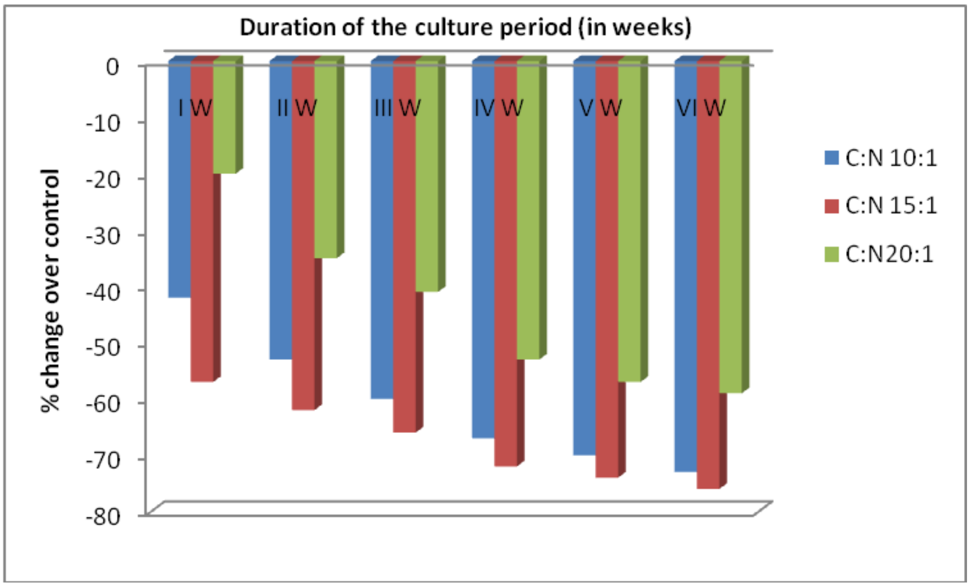

Figure.4 : % Change of Total Vibrio Bacteria (TVB) content in *L. vannamei* culture tanks at different periods

Table.1: Proximate composition of different Bioflocs

Parameter	Control	C:N (10:1)	C:N (15:1)	C:N (20:1)
Organic Matter	83.22 ± 2.89	82.79± 2.54	82.44±3.02	81.79± 2.48
Ash	16.78 ± 0.78	17.21 ± 0.84	17.56± 0.84	18.24± 0.79
Crude Protein	35.89 ± 1.77	36.74± 1.75	36.88± 1.84	36.72± 1.65
Crude Lipid	6.32 ± 0.11	6.48± 0.12	6.39± 0.14	6.79± 0.14
Crude Fiber	3.77 ± 0.11	4.12± 0.12	4.10± 0.12	4.08± 0.13
Nitrogen Free Extract	29.30 ± 1.12	26.68 ± 1.14	26.11± 1.34	25.11± 1.12
Moisture	7.94 ± 0.65	8.77± 0.74	8.96± 0.85	8.79± 0.74
Gross Energy (kcal/100 g)	396	393	390	390

All Values are Mean ± SD of six individual observations
Organic Matter : 100 – Ash
NPE : 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.2: Water quality parameters during*L. Vannamei* culture operation under different biofloc feeding trails

Parameter	**Control**	**C:N (10:1)**	**C:N (15:1)**	**C:N (20:1)**
Temperature (^{0}C)	27 $\pm$ 1	25$\pm$ 1^b	25$\pm$ 1^b	26 $\pm$ 1^b
pH	7.5 $\pm$ 0.5	7.6 $\pm$ 0.6^b	7.6 $\pm$ 0.5^b	7.7 + 0.6^b
Salinity (ppt)	10 $\pm$ 0.4	10 $\pm$ 0.5^b	10 $\pm$ 0.5^b	10 + 0.5^b
Dissolved Oxygen (DO) (mg/lit)	6.24 ± 0.21	6.35 ± 0.22^b	6.49 ± 0.23^b	6.78± 0.22^b
Total Ammonia Nitrogen (TAN) (mg/lit)	2.13 ± 0.11 PDC	0.32 ± 0.03^a (-85)	0.38 ± 0.03^a (-82)	0.36± 0.04^a (-83)
Nitrite (mg/lit)	1.37 ± 0.12 PDC	0.73 ± 0.07^a (-47)	0.68 ± 0.06^a (-50)	0.64± 0.06^a (-53)
Nitrate (mg/lit)	4.22 ± 0.24 PDC	2.23 ± 0.14^a (-47)	2.98± 0.15^a (-29)	2.77± 0.14^a (-34)
Alkalinity (mg/lit)	163.35 ± 28.74 PDC	490.13 ± 27.13^a (+200)	513.44±27.38^a (+214)	528.44±28.38^a (+224)
Total Suspended Solids (TSS) (mg/lit)	263.45 ± 25.14 PDC	374.34± 29.73^a (+42)	548.44 $\pm$ 27.14^a (+108)	448.33 + 28.13^a (+70)
Biological Oxygen Demand (BOD) (mg/lit)	17.37 ± 1.39 PDC	75.18± 2.39^a (+333)	79.77± 2.12^a (+359)	94.72±3.24^a (+445)
DO Reduction Rate (mg/lit/hr)	0.44 ± 0.05 PDC	1.22± 0.12^a (+177)	1.34± 0.14^a (+205)	1.39± 0.13^a (+216)
Biofloc volume (mg/lit)	12.34 ± 0.58 PDC	31.38± 1.72^a (+154)	37.78± 1.94^a (+207)	42.73±2.04^a (+246)

All Values are Mean ± SD of six individual observations
Values presented in parenthesis are Percent Change over their respective Control
All values are Statistically Significant at [a]P<0.001; [b]NS

Table.3 : Performance details of *L. vannamei* under different Biofloc feeding trails

Parameter	Control	C:N (10:1)	C:N (15:1)	C:N (20:1)
Shrimp stocked (nos)	600	600	600	600
Percent survival (%)	95	96	98	96
Final Weight (g)	13.14± 0.21 PDC	17.38± 0.32[a] (+32)	18.49± 0.34[a] (+41)	17.14 ± 0.34[a] (+30)
Weight gain (g)	10.62± 0.28 PDC	14.86± 0.312[a] (+40)	15.97± 0.33[a] (+50)	14.62 ± 0.29a (+38)
Weight gain (%)	+322	+490[a]	+534[a]	+480[a]
Daily Growth Rate (DGR) (g)	0.253 PDC	0.354[a] (+40)	0.388[a] (+54)	0.349[a] (+38)
Specific Growth Rates (SGR)	1.71 PDC	1.97[c] (+15)	2.06[b] (+21)	1.98[c] (+16)
Protein Efficiency Ratio (PER)	5.32 PDC	7.53[a] (+42)	7.94[a] (+49)	7.31[a] (+37)
Feed Conversion Ratio (FCR)	2.58 PDC	2.34[b] (-20)	2.03[b] (-21)	2.33[c] (-10)
Feed Efficiency Ratio (FER)	0.454 PDC	0.595[b] (+16)	0.624[a] (+23)	0.605[a] (+33)
Harvest size (g)	13.14 PDC	17.38[a] (+32)	18.49[a] (+41)	17.14[a] (+31)
Productivity (kgs)	7.49 PDC	10.01[a] (+34)	10.87[a] (+45)	9.87[a] (+32)

Shrimp with an Average Weight of 2.52± 0.12 were stocked
Values presented in parenthesis are Percent Change over their respective Control
All values are Statistically Significant at [a]P<0.001; [b]P<0.05; [c]NS