EFFECT OF THREE DIFFERENT CARBOHYDRATE SOURCES USED IN BIOFLOC TECHNOLOGY IN REDUCING TAN LEVELS DURING THE NURSERY PHASE OF *LITOPENAEUS VANNAMEI*.

Raghu Ram.M[1], Gnana Mani.G[2], Shameem.U[2], Padma Dorathy.K[3] and Padmini Devi.K[1]
[1]Research Scholar, [2] Professor, Department of Zoology, Andhra University, Visakhapatnam.
[3] Assistant Professor, Department of Biotechnology, GITAM University, Visakhapatnam, Andhra Pradesh, India.

ABSTRACT

The objective of this study was to evaluate the effect of different carbohydrate sources in Bio-floc technology in reducing the (Total Ammonia Nitrogen) TAN levels during the nursery phase of *L.vannamei*. In recent days, Bio-floc technology is gaining the attention of shrimp farmers and scientists from all over the world. Nutrient recycling and water quality management are the main advantages in Bio-floc technology over the conventional farming practices. Many countries have successfully implemented this technology in *Litopenaeus vannamei* farming. However, in India, application of this technology is still at a nascent stage. Even though some studies are available on this technology, most of these are mainly focused on vannamei farming, not on nursery phase. It may be noted that the three phase culture system which includes nursery phase is much recommended by the experts.

During August, 2016 to December, 2017, an attempt has therefore been made to study the effect of three carbohydrate sources for controlling the TAN levels in nursery phase of *L.vannamei* using Biofloc technology. Maintenance of Carbon: Nitrogen (C:N) ratio is the key factor for the success of the biofloc system and for the control of toxic TAN levels. Three different sets of experimental tanks and one set of control tanks in triplicate were established. The experiment was carried out over a period of 21 days in closed system. Biological and water quality parameters were recorded. The results were analyzed with one way ANOVA and pertinent statistical tests were conducted. The study revealed that the Biofloc technology was effective in reducing the toxic TAN values in nursery phase of *L.vannamei*. It was also evident that all the three carbohydrate sources were found to be effective in regulating the TAN values.

Key Words: Aquaculture, *L.vannamei*, Bio-floc technology, carbohydrate source, protein recycling, Total Ammonical Nitrogen (TAN), C:N ratio, Bio-flocculating agents.

INTRODUCTION:

Application of Bio-floc technology has been receiving attention of scientists as well as aqua farmers in India in recent times, for its efficient and eco-friendly nature for rearing shrimp/fish. The sustainable approach of such system is based on development of beneficial heterotrophic micro-organism in the culture environment. Furthermore, protein utilization is twice as high in this system, compared to the existing conventional culture practices. Maintaining Carbon to Nitrogen ratio is very vital in this system, in which toxic ammonia is immobilized by dominant heterotrophic bacteria, converting the available inorganic nitrogen into bacterial protein.

The shrimp need a high concentration of protein in their feed because their energy production pathways depend on oxidation and catabolism of proteins. As earlier studies indicated, nearly 3/4th quantity of the nutrients (Phosphorous and Nitrogen) used in shrimp feeds, are excreted as organic waste, because of the poorly developed digestive system in the shrimp. The unutilized feed and excreta lead to the accumulation of TAN (Total Ammonical Nitrogen), which becomes one of the limiting factors to intensive aquaculture. This TAN can be utilized as a source of nitrogen, for thriving of heterotrophic bacteria when carbohydrate is added externally to maintain the carbon, nitrogen (C:N) ratio more than 10.

In the present investigation, three different substances namely sugar, jaggery and rice flour have been chosen as carbohydrate source to examine their effectiveness in regulating the TAN values during the nursery phase of *Litopenaeus vannamei.*

MATERIAL AND METHODS

Three different carbohydrates namely, sugar, jaggery and rice flour were used to examine their effectiveness in controlling TAN values in vannamei nursery system in the application of Bio-floc technology. The study was carried out in HDPE circular tanks, each with 60 liters capacity. Altogether 12 nursery tanks were employed for this study, which were grouped into 3 experimental and control tanks, maintained in triplicates.

Litopenaeus vannamei (SPF) shrimp seed at PL 10 stage were procured from a CAA approved hatchery (Rajkamal Hatchery, Visakhapatnam). Necessary tests have been conducted to evaluate the health condition of shrimp and to ensure their disease free status. The seed was transported to the laboratory in oxygen filled polythene bags. Following acclimatization of the seed to the laboratory conditions, the experimental (9 Nos.) and control (3 Nos.) tanks were stocked randomly with 200 post larvae (10 PL/L). The initial length and weight of the PL were measured randomly for at least 20 specimens from each tank.

Each experimental tank was filled with 19L of treated sea water (28ppt salinity) and 1L of Bio-floc water. Bio-floc was developed separately in a tank following Avnimelech (2012) protocol. Each control tank contained 20L of treated sea water without Biofloc.

Sugar (S), Jaggery (J) and Rice flour (R) were used as carbohydrate source for each of the experimental tanks. The PL's were fed with 36% protein containing commercial feed (J.D. Kalyan shrimp feed) for the first 10 days of the experiment. The feed was proportional to 30% of average body weight of the shrimp. For the remaining period of the experiment, feed quantity was reduced to 14%, based on the body weight of the shrimp recorded on 10th day. The study was carried out for 21 days.

Along with the feed, necessary quantity of the carbohydrate was added to each one of the culture tanks for the maintenance of the Carbon to Nitrogen (C:N) ratio. Carbohydrate quantity was calculated taking into consideration both the TAN and Nitrite values, which were recorded the previous day. Data was also recorded on other necessary parameters such as D.O, Temperature, pH, Salinity, Nitrate (No_3), Biological Oxygen Demand (BOD), chlorophyll-a, Total Heterotrophic Bacteria (THB) at specified intervals. Additionally, other biological parameters namely, Average Daily Gain (ADG), Final Weight Gain (FWG), Specific Growth Rate (SGR), Survival rate and Feed Conversion Ratio (FCR) were recorded.

RESULTS AND DISCUSSIONS

The Biological parameters of *L.vannamei*, such as mean length, mean weight, survival rate, ADG, FWG%, total quantity of the feed used and FCR observed in this study were shown in Table-1. Initially the post larvae, ranging in size from 7.11-7.22mm and weight 0.00239-0.00241g, were stocked randomly in both experimental and control tanks. On 11th day, the length of *L.vannamei* ranged between 22.84 - 23.52mm and weight 0.23-0.24g. Survival rate was better in the experimental tanks (92-94%) compared to the control (89.33%). Mean length gain in experimental tanks was between 15.9-16.4mm and in control tanks 15.6mm. Mean weight gain in experimental tank ranged from 0.229-0.234g and in control 0.225g.

After 21 days, the mean length of the juveniles varied between 33.64 - 34.49mm in the experimental tanks, while their counterparts in the control tanks were measured 31.5mm. The mean weight in all the experimental tanks was 0.34g and in the control it was 0.33g. Survival rate was 87.67-89.34% in experimental tanks, while it was considerably lower in control (84.5%). Mean length gain and mean weight gain in experimental and control tanks was 26-27mm, 0.23g and 24mm, 0.225g respectively. Average daily Gain was almost equal in all the experimental tanks (0.016±0.00g) and it was 0.0155±0.00g control tank. Specific Growth Rate was in the range of 10.23-10.24g in experimental tanks and it was 10.16±0.09g in control tank. Feed Conversion Ratio (FCR) ranged from 1.13-1.14 in experimental tanks and 1.20 in control tank. Total feed used and total yielded mass was in the range of 67.37-68.54g, 59.34-60.80g respectively in experimental tanks and 65.99g, 55.23g in control tank.

The water quality parameters, D.O, temperature, pH, TAN, Nitrite, Salinity, Nitrate, Cholorophyll-a, BOD and THB recorded during the study are furnished in Table-2. In experimental as well as control tanks, Dissolved oxygen ranged from 5.95 - 5.97 irrespective of the time of sample collection. Likewise water temperature and pH did not show much variation. These parameters were found to be conducive for growth of *L.vannamei*. BOD levels were high in control tank (3.14±0.54) and low in experimental tank fed with sugar (3.04±0.56). TAN values were almost equal (0.54±0.21mg/L) in all experimental tanks and it was high (0.72±0.34mg/L) in control tank. Nitrite and Nitrate value were high (0.26, 0.15) in the experimental tank fed with Jaggery, low in the control tank (0.17, 0.14). Chlorophyll-a and THB values were found lowest in the control tank (12.4±1.6, 146.47±156.61) and highest in the culture tank fed with rice flour (13.9±2.4, 169.74±167.25) which is found significantly differ among experimental and control tanks.

The study demonstrated that the growth of *L.vannamei* was significantly higher in the experimental tanks compared to the control. Similar findings were made by earlier workers (Wyban *et al.*, 1988; Samocha *et al.*, 1993). Similarly, survival of *L.vannamei* in experimental tanks was more relative to the control. Further it is evident that the heterotrophic bacteria utilized the accumulated TAN as a source of nitrogen. The carbohydrate sources supplemented in the form of sugar, jaggery and rice flour facilitated the uptake of TAN by the biofloc and conversion into microbial protein, also maintained the water quality in the experimental tanks.

Feed conversion ratio (FCR) value was also significantly higher in the control compared to the BFT treatment tanks. The consumption of biofloc by shrimp has demonstrated innumerous benefits such as improvement of growth rate, decrease of Feed Conversion Ratio (FCR) and associated costs in feed (Burford et al. 2004). Hence, BFT is being successfully expanded in shrimp farming units in almost all parts of world.

Temperature has pervasive controlling effect on growth. Wang *et al.*, (2004) reported that the favorable pH range of 7.6-8.6 for *L.vannamei.* Several works have reported good growth and survival of *L.vannamei* in brackish water range from 10-35ppt was ideal for shrimp culture.

TAN value in the control was recorded high and found significant in ANOVA, which indicates that the biofloc technology have a significant impact in reducing the TAN values during the nursery phase of *L.vannamei* culture. Average TAN value was recorded almost similar for all the three sets of experimental tanks, which show that the three carbohydrate sources were equally effective in reduction of TAN values in Biofloc system during the nursery phase of *L.vannamei*. Hence, it is proven that the addition of carbohydrates successfully decrease the TAN levels in shrimp ponds.

The increase in ammonia concentrations is followed by an increase in nitrite concentrations, which is in turn, followed by an increase in nitrate concentrations. This series of events occurs because there is a lag time between the development of populations of ammonia-oxidizing and nitrite oxidizing bacteria (Avnimelech, Y. 2012). Ammonia will act as a substrate for the Nitrate production. It was evident from the graphs that the nitrate levels were going to further increase as the Ammonia levels were coming down in the treatments. In control tanks ammonia levels were high and Nitrate levels were comparatively low, which indicates that the toxic ammonia accumulation took place in the control tank.

But there was found no significant difference among the treatments and control as per the ANOVA. Toxic ammonia converts into Nitrate and then finally into less toxic Nitrate by the process of nitrification. In this study, even conversion of Nitrite is not fully happened and hence, Nitrate levels were lowest.

Table-1: Biological parameters of *L.vannamei* in Biofloc system with three carbohydrate source

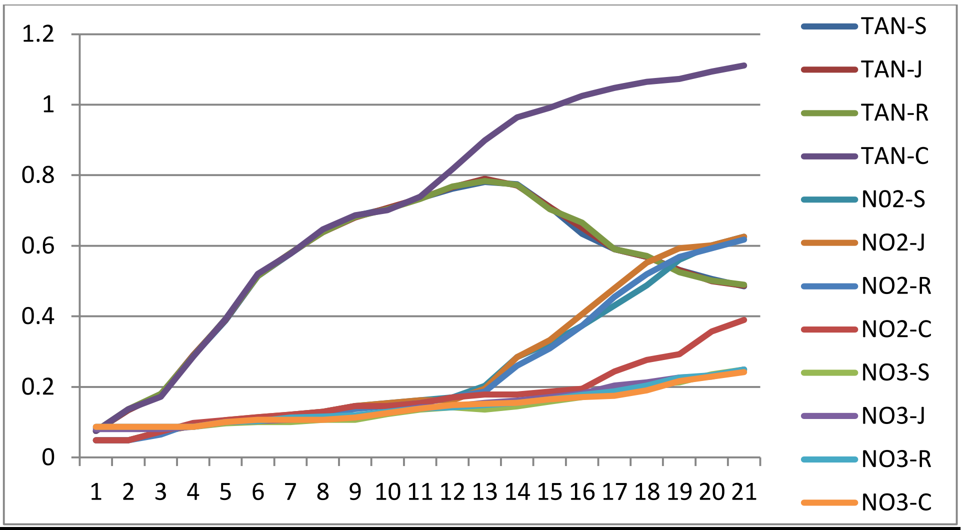
Parameter	Duration	Sugar	Jaggery	Rice Flour	Control
Mean Length (mm)	1st day	7.16±0.36	7.17±0.36	7.11±0.27	7.22±0.19
	11th day	23.52±1.20	23.06±1.21	23.40±1.09	22.84±0.99
	21st day	34.49±2.75*	33.64±2.04*	34.15±1.95*	31.49±3.78*
Mean Weight (g)	1st day	0.00241±0.00E	0.00239±0.00E	0.00240±0.00E	0.00240±0.00E
	11th day	0.23±0.02	0.23±0.01	0.24±0.01	0.23±0.01
	21st day	0.34±0.015*	0.34±0.017*	0.34±0.015*	0.33±0.019*
Survival Rate %	11th day	92.6*	94*	92.33*	89.33*
	0-21 days	87.67*	89.34*	87.67*	84.5*
Mean Length Gain		27.32±2.61*0	26.46±1.78*	27.034±1.77*	24.266±3.81*
Mean Weight Gain		0.3373±0.015*	0.3379±0.017*	0.336±0.0151*	0.3244±0.0188*
ADG		0.016±0.000717*	0.16±0.000814*	0.16±0.000723*	0.155±0.0009*
SGR%		10.233±0.097*	10.246±0.084*	10.2305±0.049*	10.167±0.096*
FCR		1.14*	1.13*	1.14*	1.20*
Total feed used (g)		67.97*	68.54*	67.37*	65.99*
Total yielded mass (g)		59.56*	60.80*	59.34*	55.23*

(* Indicates the significance at p=0.05 level).

Table-2: Water quality parameters recorded during the study

Parameter	Time/ Duration	Sugar	Jaggery	Rice Flour	Control
DO (mg/L) 9hr	Twice a day	5.96±0.085	5.97±0.085	5.95±0.080	5.96±0.078
DO (mg/L) 16hr		5.97±0.07	5.97±0.076	5.96±0.076	5.96±0.068
Temperature (^{0}C) 7hr		28.14±1.44	28.08±1.41	28.15±1.43	28.14±1.41
Temperature (^{0}C) 13hr		28.50±1.48	28.48±1.46	28.50±1.47	28.49±1.46
pH (9hr)		7.77±0.05	7.74±0.05	7.77±0.06	7.76±0.06
pH (16hr)		7.77±0.05	7.75±0.05	7.77±0.06	7.77±0.06
TAN – (mg/l)	Daily	0.54±0.21*	0.54±0.21*	0.54±0.21*	0.72±0.34*
Nitrite (mg/l)		0.25±0.19*	0.26±0.20*	0.25±0.19*	0.17±0.09*
Salinity (mg/l)	Once in 5 days	27.47±0.65	27.27±0.44	27.27±0.37	27.27±0.43
Nitrate (mg/l)		0.14±0.05*	0.15±0.05*	0.15±0.05*	0.14±0.05*
BOD (mg/l)		3.04±0.56*	3.12±0.64*	3.12±0.63*	3.14±0.54*
Chlorophyll-a (mg/l)		13.5±2.2*	13.8±2.2*	13.9±2.4*	12.4±1.6*
THB (10^3cfu)	Once in 10 days	168.12±157.4*	164.53±142.81*	169.74±167.25*	146.47±156.61*

(* Indicates the significance at p=0.05 level).



Graph:-1: TAN, Nitrite and Nitrate Patterns in the nursery phase of *L.vannamei* tested with three different carbohydrate sources

CONCLUSIONS

The three carbohydrate sources i.e., sugar, jaggery and rice flour examined in this study were found to be equally effective in reducing the TAN levels during the nursery phase of *L.vannamei*. It was evident that, Carbon to Nitrogen ratio adjustment promoted the formation of bio-flocs by the up-take of TAN from the water column. Biofloc formation facilitated in maintaining the water quality and there by minimizes the need for water exchange, which are of the major constraints in aquaculture. Nutrient recycling takes place in this system, contributes to reduction of FCR considerably. Carbohydrates source employed in this study are cost effective and also do not cause any negative effect on shrimp or environment. The farmers may use any of the above carbohydrate sources (Sugar, Jaggery or Rice flour), in order to control the Toxic TAN levels in Biofloc system. More research is required to determine the efficiency of utilizing other cost-effective carbohydrate sources and also different types of carbohydrates like monosaccharide, disaccharides, polysaccharides and oligosaccharides as bio- floculating agents. There is also a significant need for further studies on composition and nutritional value of the bio- floc developed in the culture system.

REFERENCES

1. A.P.H.A., (American Public Health Association) 1989. Standard methods for the examination of water and waste water, 17th edition. Washington, D.C., U.S,A.
2. Avnimelech,Y. 2012. *Biofloc Technology- A practical Guide Bok, 3rd Edition.* The World Aquaculture Society, Baton Rouge, Louisiana, United States.
3. Boyd, C.E., Tucker, C.S., 2009. *Pond aquaculture water quality management*, Springer international editor.
4. Burford, M.A., Thompson, P.J., Mc Intosh, P.R., Bauman, R.H., Pearson, D.C.,, 2004. The contribution of flocculated material to shrimp, Litopenaeus vannamei nutrition in a high-intensity, zero-exchange system. *Aquaculture*. 232, 525-537.
5. Fast. A.W. and J.E. Lannan. 1992. Pond Dynamic Processes. In: Fast A.W. and J. Lester (eds). Marine Shrimp Culture; Principles and Practices, *Elsevier Science Publication*, Amsterdam. pp.431-455.
6. Samocha,T.M., et.al., 1993. Production of the pacific white shrimp Litopenaeus vannamei in high density greenhouse enclosed raceways using low salinity groundwater.
7. Wyban. J.A., Lee., C.S., Sato, V.T., Sweeney, J.N., Richards Jr., W.K. 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. *Aquaculture* 61, 23-32.