

GROWTH PERFORMANCE OF *LITOPENAEUS VANNAMEI* IN BIOFLOC TREATMENTS GROWN WITH DIFFERENT CARBON SOURCES

D. Pamanna*, A. Chandrasekhara Rao, D. Ravindra Kumar Reddy, N. Madhavan, V. Venkatesan¹, E. Nehru and P. Ranjith Kumar

College of Fishery Sciences, Sri Venkateswara Veterinary University, Muthukur, Nellore - 524 344, India.

¹Central Marine Fisheries Research Institute, ICAR, P.O. Box 1603, Ernakulam North Post Office, Ernakulam, India.

*e-mail: pami.neeru@gmail.com

(Accepted 1 August 2017)

ABSTRACT : Experiments were conducted with three biofloc treatments and one control in triplicate in 1000 ltr capacity indoor tanks and water level filled up to 600 ltr. Enhanced shrimp growth was noticed in biofloc treatment tanks and a significant difference in the final average body weight of (15.92±0.07g) was found in the wheat flour treated shrimps than those of control group shrimp. The Feed Conversion Ratio differs significantly ($P<0.05$) between biofloc treatment group and control groups. Lowest FCR (0.5±0.07) was recorded in wheat flour as carbohydrate source biofloc treatment during the experiment. Highest Specific Growth Rate (4.59) was observed in the wheat flour treatment groups than that of other treatments and control groups. Wheat flour utilization as carbohydrate source to biofloc development for rearing of *L. vannamei* was proved to be the best option among all treatments. All the carbohydrate sources (wheat flour, tapioca flour and molasses) utilized for biofloc treatments indicated highest growth than control treatment.

Key words : Growth, biofloc, carbon source, *Litopenaeus vannamei*, specific growth rate.

INTRODUCTION

World Aquaculture is growing with an annual rate of 8.9–9.1% since the 1970s. This high growth rate is needed to solve the problem of shortage in protein food supplies, which is particularly situated in the developing countries (Gutierrez-Wing and Malone, 2006). The global shrimp market has expanded from less than \$1 billion to \$12 billion (US) from 2000 to 2013 (FAO, 2014). To meet this growing demand, the shrimp industry is shifting from extensive rearing systems to more intensive rearing systems.

In order for aquaculture to be completely successful, the industry need to develop technology that will increase the economic and environmental sustainability. In conventional and semi-intensive ponds, natural food can supply up to 70% of the nutritional requirements of shrimp, benthic organisms and zooplankton constituting the essential components of this food source (Martinez-Cordova *et al*, 2003). The prime goal of aquaculture expansion in to produce more aquaculture products without significantly increasing the usage of the basic natural resources of water and land (Avnimelech, 2009). Biofloc technology is not only an adequate approach in maintaining water quality in the aquaculture system, but it also generates biomass that can contribute as a protein

source for the cultured organisms *in situ*, Crab *et al* (2010a) or can be harvested for use as a feed ingredient (Kuhn *et al*, 2010). Hence, the use of biofloc as a food source implies a decrease in the requirement of formulated feed protein and also improve nitrogen utilization efficiency by the cultured animals (Xu *et al*, 2012).

As the microbial communities develop, bioflocs are formed from heterogeneous aggregates of microorganisms and organic particles (De Schryver *et al*, 2008). As a supplemental food source available for cultured shrimp, the biofloc can be consumed and provide a significant fraction of protein demand (Xu *et al*, 2012).

The availability of natural food will be influence by the production system, which may interfere with the dependence on and availability of nutrients, such as protein from inert diets. In heterotrophic biofloc-based shrimp culture systems, the driving force is dense populations of active heterotrophic bacteria which can be promoted by increasing the C/N ratio of feed input and assimilate the waste nitrogen from culture water resulting in the production of new microbial biomass (cellular proteins) (Avnimelech, 2006).

By adding a carbon source to the culture medium in limited-discharge systems (*i.e.*, by changing the C : N

ratio), it is possible to obtain a significant enhancement of bacterial growth and of the fixation of toxic nitrogen metabolite species (Crab *et al*, 2010a). In addition to the improvement of water quality, increase in bacterial biomass, providing supplemental feed, improved shrimp survival, growth and to reduce the releases of nutrient rich water into receiving streams (Krummenauer *et al*, 2011).

MATERIALS AND METHODS

The experiment was conducted in Wet Laboratory of the Department of Aquaculture, College of Fishery Science, Sri Venkateswara Veterinary University, Muthukur, for a period of 60 days.

Litopenaeus vannamei (1000 numbers) were obtained from BMR Hatchery, Nellore. Acclimatization was carried out over 2 weeks. During this time salinity was lowered from 5 ppt to 3ppt. The seeds were fed with crumble, sinking starter feed having a crude protein percentage of 35 (Manamei shrimp feeds AVANTI Company).

Indoor experiments were conducted in FRP tanks with 1000 ltr capacity and with an effective bottom area of 1.03 m², triplicate treatments were maintained in the Laboratory. Tanks were filled with bore water with a depth of 60 cm. Aeration was provided 24 hours throughout the experiment for better biofloculation. Urea and super phosphate were added as fertilizers at a dosage of 4 and 1 g/m² during the first three weeks (Varghese, 2007). After two week all tanks were stocked with shrimps at a rate of 15/m² (New, 2002). Before stocking initial weight of the organism (1.025±0.05g), initial water parameters were recorded. Commercial pelletized sinking shrimp feed with a dietary protein level 35% was selected as experimental feed in pellet form and for initial feeding, it was repelletized into smaller size.

Preparation of carbohydrate source and feeding

Three easily and locally available carbohydrate sources *viz*, tapioca flour (*Manihot esculaneta*), wheat flour (*Triticum aestivum*) and molasses (*Saccharum officinarum*) were selected as carbohydrate sources for biofloculation. Wheat flour, Molasses and Tapioca were purchased from the local market. Raw tubers were purchased, peeled and washed thoroughly, made into small pieces and soaked in water overnight. Next morning water drained and the pieces were kept in oven at 60°C till it dried completely. After that slices were powdered in a mixer grinder, sieved through 35µm sieves and powder stored in air-tight container (Saritha, 2009). By processing 1 kg of raw tuber, 500 g of corresponding powder was obtained.

Shrimps were fed with experimental feed at 12% of initial body weight and adjusted gradually to 2.5% at the end of the culture (1-60 days). The ration was divided and distributed twice daily with similar portions between 9:00 and 10:00 hours in the morning and between 17:00 and 18:00 hours in the evening. The C:N ratio of the treatments was calculated using the formula of Avnimelech (2000) and it was found to be 10:1 for all the treatments. The quantity of carbohydrate added was calculated following the theory of Avnimelech (1999). Pre-weighed carbohydrate source was mixed in a glass beaker with the water collected from the corresponding culture tanks. The culture tanks treated with wheat flour, tapioca flour, molasses were represented as T₂, T₃, and T₄, respectively. All the systems were maintained for 60 days without any water exchange. Water loss due to evaporation was compensated by the addition of dechlorinated water as per requirement. Proximate analysis of the feed was estimated by the method of AOAC (1995).

Table 1 : Proximate composition of experimental feed & different carbon sources.

Feed code	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture (%)
3S	35	5	4	4	11
Wheat flour	12.8	1.8	13.3	1.2	6
Tapioca flour	2.1	1.23	0.6	0.89	9.4
Molasses	5.9	0.06	9.4	0.1	29.6

Growth parameters

Yield study was done by using hand net in the culture tanks. Individual length and weight were recorded. Individual shrimp weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and average daily weight gain (ADG) were calculated. The growth parameters of all the shrimps of each tank were individually estimated by taking their total body length and weight at 7 days interval.

Statistical analysis

Statistical analyses were performed using web agristat package (WASP) version 2.0. The data obtained on Growth and Food Conversion Ratio was statistically analyzed by applying Randomized Block Design (RBD) of two-way classification.

RESULTS

Growth of *L. vannamei* fed on biofloc grown with different carbon sources

Weight of shrimp in grams and weight increment data observed weekly for different treatments is presented in

Table 2 : Growth performance (g) of *L. vannamei* fed on biofloc grown with different carbon sources.

Treat-ment Period (days)	Control T ₁	Wheat flour T ₂	Tapioca flour T ₃	Molasses T ₄
0	1.025±0.12	1.025±0.12	1.025±0.12	1.025±0.12
7	2.09±0.11	2.70±0.08	2.53±0.12	2.54±0.05
14	3.21±0.08	4.44±0.11	4.12±0.07	4.13±0.09
21	4.43±0.05	5.19±0.05	5.74±0.02	5.75±0.11
28	5.72±0.07	7.97±0.09	7.42±0.08	7.40±0.05
35	7.08±0.02	9.78±0.11	9.24±0.11	9.11±0.01
42	8.59±0.11	11.71±0.04	11.12±0.08	10.79±0.09
49	10.26±0.12	13.65±0.12	13.05±0.12	12.64±0.12
60	12.27±0.09	15.92±0.07	15.17±0.07	14.82±0.04

Tables 2-3. Observations on the growth during the overall study indicated that the Wheat flour (T₂) recorded total weight increment of 15.92±0.07g in 60 days experimental period. This was followed by the Tapioca flour (T₃) 15.17±0.07g and Molasses (T₄) 14.82±0.04g, they stood in second and third positions, respectively.

Specific Growth Rates

Specific Growth Rates (%) of *L. vannamei* fed on biofloc grown with different carbon sources

Specific growth rates by the end of experimental period (60 days) were calculated for all the treatments. Specific growth rates for *L. vannamei* treated with different carbon source biofloc were calculated and presented in Table 4.

Control (T₁) group has the lowest Specific Growth Rate of 4.13%. The highest value was in Wheat flour (T₂) with 4.59%. The treatments that stood second and third positions were Tapioca flour (T₃) 4.49% and Molasses (T₄) 4.45%, respectively.

Feed Conversion Ratio

Feed Conversion Ratio of *L. vannamei* fed on biofloc grown with different carbon sources

The Feed Conversion Ratio (FCR) in different experiments of *L. vannamei* groups were calculated and presented in Table 5.

The range for Feed Conversion Ratio varied during the experimental period was 0.50±0.07 (Wheat flour) – 1.80±0.05 (Control).

The Control (T₁) was found to be significantly superior when compared to the other treatments. The Molasses (T₄), Tapioca (T₃) and Wheat flour (T₂) occupied second, third and fourth positions. There was a significant difference between the experimental periods also.

Table 3 : Weight gain (g) in *L. vannamei* fed on biofloc grown with different carbon sources.

Treat-ment Period (days)	Control T ₁	Wheat flour T ₂	Tapioca flour T ₃	Molasses T ₄
7	1.07±0.08	1.68±0.05	1.54±0.11	1.52±0.09
14	1.12±0.02	1.74±0.08	1.59±0.09	1.59±0.05
21	1.22±0.05	1.75±0.02	1.62±0.05	1.62±0.02
28	1.29±0.11	1.78±0.11	1.68±0.08	1.65±0.11
35	1.36±0.09	1.81±0.09	1.82±0.02	1.71±0.01
42	1.51±0.02	1.93±0.07	1.88±0.12	1.68±0.12
49	1.67±0.07	1.94±0.12	1.93±0.09	1.85±0.08
60	2.01±0.12	2.27±0.01	2.12±0.11	2.18±0.09

DISCUSSION

Growth Performance of *L. vannamei* in biofloc treatments grown with different carbon sources

Biofloc consist of variety of bacteria, fungi, micro algae, detritus and other suspended organism (Hargreaves, 2006). The microbial community associated with biofloc improves feed utilization and animal growth (Kim *et al*, 2014). The growth performance of shrimp in biofloc reared with different carbon sources were significantly ($p < 0.05$) higher than those in control group in this study. Many of previous studies have shown that growing *L. vannamei* in biofloc system can improve shrimp growth as compared to clear water (Kim *et al*, 2014). Growth performance was significantly higher ($p < 0.05$) in wheat flour treated tank than to control and other carbohydrate source treatments. In the present

Table 4 : Specific Growth Rates (%) of *L. vannamei* fed on biofloc grown with different carbon sources.

Treat-ment Period (days)	Control T ₁	Wheat flour T ₂	Tapioca flour T ₃	Molasses T ₄
Initial	1.025	1.025	1.025	1.025
Final	12.27	15.92	15.17	14.82
SGR	4.13	4.59	4.49	4.45

Table 5 : Feed Conversion Ratio of *L. vannamei* fed on biofloc grown with different carbon sources.

Treat-ment Period (days)	Control T ₁	Wheat flour T ₂	Tapioca flour T ₃	Molasses T ₄
7	0.80±0.08	0.50±0.07	0.55±0.05	0.56±0.02
14	1.39±0.02	1.08±0.05	1.19±0.02	1.19±0.05
21	1.58±0.05	1.52±0.02	1.42±0.04	1.53±0.04
28	1.56±0.05	1.31±0.04	1.54±0.07	1.58±0.05
35	1.28±0.04	1.23±0.08	1.23±0.05	1.21±0.02
42	1.08±0.07	1.15±0.05	1.03±0.08	1.24±0.04
49	1.00±0.02	1.05±0.04	1.09±0.04	1.12±0.05
60	1.80±0.05	1.65±0.02	1.69±0.02	1.59±0.07

study increasing in growth performance in biofloc with wheat flour as carbohydrate source are in the agreement with findings of Megahed (2010) in *P. semisulcatus* and Anand *et al* (2014) in *P. monodon*.

The growth performance in biofloc group were significantly higher than those in control group in our study. One of the reason for improved performance is probably related to consuming biofloc by the shrimp.

Wheat flour as a carbohydrate source biofloc grown shrimp documented higher weight gain (2.27 ± 0.01) than to control and other carbohydrate source treatment grown shrimp. In the case of biofloc treatments in the shrimp culture with different carbohydrate sources wheat flour as carbohydrate source showed significantly higher growth performance and weight gain in *L. vannamei* than those of others (Rajkumar *et al*, 2015).

In the present study, the shrimp fed on biofloc grown with different carbon sources showed better SGR than the control shrimp. Wheat flour utilized as carbohydrate source for biofloc development to provide diet for *L. vannamei* was resulted higher SGR than the control. The results reported in the present study were correlated with the finding of Rajkumar *et al* (2015) in *L. vannamei* and Anand *et al* (2014) in *P. monodon*.

In the present experiment, biofloc grown shrimp with different carbohydrate sources showed significant ($p < 0.05$) reduction in FCR compared to control treatment. FCR in the present study has been demonstrated fluctuating trend, initially from 7th day to 21st day, it was increased and gradually followed declining trend. It may be due to fluctuating in the density of floc in different carbohydrate sources treatments. Biofloc supplementation of 12% did not resulted in proportionate increase in growth rate or improvement in FCR of *P. monodon* compared with control (Anand *et al*, 2014). On 28th day in regard to wheat flour, 42nd day for tapioca flour and 60th day for molasses lowest FCR was noticed in different carbohydrate sources utilization for biofloc treatment except on 49th day all the treatments of *L. vannamei* fed on biofloc grown with different carbohydrate sources performed with better FCR than control treatments. Biofloc is a proven food source for cultured species and results in a decreased requirement for supplemental feeding (Li *et al*, 2013). Similar results were reported by Anand *et al* (2014) in *P. monodon* at 4% and 8% supplementation of biofloc treatments.

CONCLUSION

Wheat flour biofloc treatment gave best result in the enhancement of growth, SGR and better FCR among all treatments.

ACKNOWLEDGEMENT

The authors sincerely thanks Dr. Manmohan Singh, Vice-Chancellor, SVVU, Tirupati for extending facilities to carry out this work. The SVVU, MFSc fellowship granted to the first author, is gratefully acknowledged.

REFERENCES

- Anand P S S, Kohli Sujeet, Kumar M P S, Sundaray J K, Dam Roy S, Venkateshwarlu, Archana Sinha G and Pailan G H (2014) Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture* **418-419**, 108-115.
- AOAC (1995) Official Methods of Analysis of the Association of Official Analytical Chemists. Washington, D. C. pp: 101.
- Avnimelech Y (1999) Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **176**, 227-235.
- Avnimelech Y (2000) Nitrogen control and protein recycle. Activated suspension pond. *The Aquac. Advo.* April: 23-24.
- Avnimelech Y (2006) Bio-filters: The need for a new comprehensive approach. *Aquac. Eng.* **34**, 172-178.
- Avnimelech Y (2009) *Biofloc Technology- A Practical Guide Book*. The World Aquac. Socie. Baton Rouge, Louisiana, United States. pp: 182.
- Crab R, Chielens B, Wille M, Bossier P and Verstraete W (2010a). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquac. Res.* **41**, 559-567.
- De Schryver P, Crab R, Defoirdt T, Boon N and Verstraete W (2008) The basics of bioflocs technology: the added value for aquaculture. *Aquaculture* **277**, 125-137.
- FAO (Food and Agriculture Organization) (2014) *The state of world fisheries and aquaculture*. Rome, Italy: Food and Agriculture Organization of the United Nation.
- Gutierrez-Wing M T and Malone R F (2006) Biological filters in aquaculture: trends and research directions for freshwater and marine applications. *Aquac. Eng.* **34**(3), 163-171.
- Hargreaves J A (2006) Photosynthetic suspended-growth systems in aquaculture. *Aquac. Eng.* **34**, 344-363.
- Kim K S, Pang Z, Seo H C, Cho Y R, Samocha T and Jang I K (2014) Effect of bioflocs on growth and immune activity of Pacific white shrimp, *Litopenaeus vannamei* post larvae. *Aquac. Res.* **45**, 362-371
- Krummenauer D, Peixoto S, Cavalli R O, Poersch L H and Wasielesky W (2011) Super intensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern Brazil at different stocking densities. *J. World. Aquac. Socie.* **42**, 726-733.
- Kuhn D D, Lawrence A L, Boardman G D, Patnaik S, Marsh L and Flick G J (2010) Evaluation of two types of biofloc derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* **303**, 28-33.
- Li Y, Liu L P, Zhao G X, Hu Z X and Su X M (2013) The effect of wheat starch addition on water quality and feed efficiency in tilapia pond (In Chinese with English abstract). *J. Dalian Ocean Univ.* **28**, 1-6.

- Martinez-Cordova L R, Torres A C and Porchas-Cornejo M A (2003) Dietary protein level and natural food management in the culture of blue shrimp (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. *Aquac. Nutr.* **9**, 155-160.
- Megahed M E (2010) The effect of Microbial Biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus Semisulcatus*) fed with different crude protein levels. *J. Arabian Aquac. Socie.* **5**, 119-142.
- New M B (2002) Farming Freshwater Prawns: A Manual for the Culture of the Giant River Prawn (*Macrobrachium rosenbergii*), vol: **428**. FAO, Rome.
- Rajkumar M, Pandey P K, Alagarsamy R A, Vennila Vivekanand, Bharti V and Purushothaman C S (2015) Effect of different biofloc system on waterquality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquac. Res.* 1-13.
- Saritha T (2009) Development of innovative low cost larviculture technologies of the Giant Freshwater Prawn, *Macrobrachium rosenbergii* (de Man). *Ph.D. Thesis*, Cochin University of Science and Technology.
- Varghese J T (2007) Carbon/nitrogen ratio optimization and periphyton development on the production and sustainability of *Penaeus monodon* (fabricius) in extensive culture system. *Ph.D. Thesis*. Cochin University of Science and Technology, Cochin, India.
- Xu W J, Pan L Q, Zhao D H and Huang J (2012) Preliminary investigation into the contribution of biofloc on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture* **350-353**, 147-153.