

**RESEARCH ARTICLE :**

# Growth performance and survival of *L. vannamei* in biofloc treatments grown with different carbon sources

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**ARTICLE CHRONICLE :**

**Received :**  
20.07.2017;

**Accepted :**  
16.08.2017

**KEY WORDS :**

*L. vannamei*, Carbon, Source

**SUMMARY :** Impact of *L. vannamei* rearing with biofloc by using different carbohydrate materials (wheat flour, tapioca flour and molasses) as a carbon source to boost the production by improving the conversion of nutrients into harvestable products while maintaining good water quality. The carbohydrate sources for this study were selected based on easy availability and economic viability. In the present study it has been evaluated to identify the efficient carbon source to develop the quality biofloc which play significant role in growth and survival of *L. vannamei*. Enhanced shrimp growth was noticed in biofloc treatment tanks. There was a significant difference in the final average body weight of (15.92±0.07g) in the wheat flour treatment than those treatments and control group of shrimp. The FCR differs significantly between biofloc treatment group and control (P<0.05). FCR lowest (0.5±0.07) was recorded in wheat flour as carbohydrate source biofloc treatment. Highest SGR (4.59) was observed in the wheat flour treatment than those treatments and control. Wheat flour utilization as carbohydrate source to biofloc development for rearing of *L. vannamei* was proved to be the best option among all treatments. The addition of carbohydrate for biofloc development affected the survival of *L. vannamei*. The highest survival of (73.36%) was recorded for wheat flour used as carbohydrate source in biofloc treatments. All the carbohydrate sources (wheat flour, tapioca flour and molasses) utilized for biofloc treatments indicated highest growth and survival than control treatment.

**How to cite this article :** Pamanna, K. Veeranjaneyulu, K. and Gouthamchowdary M (2017). Growth performance and survival of *L. vannamei* in biofloc treatments grown with different carbon sources, *Agric. Update*, **12** (TECHSEAR-8) : 2150-2159.

## **BACKGROUND AND OBJECTIVES**

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; Matos *et al.*, 2006 and Subasinghe, 2005).

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). In biofloc culture systems, the “natural food” consists of diatoms, macroalgae, food and faecal

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remnants, exoskeletons, bacteria and invertebrates.

The application of biofloc technology (BFT) in shrimp aquaculture has gained great attention in recent years because it provides a practical solution for effective control of water quality with negligible water exchange and improves shrimp growth performance, thus achieving efficient and healthy culture of shrimp (Avnimelech, 2012; Crab *et al.*, 2012; De Schryver *et al.*, 2008; Stokstad, 2010; Xu and Pan, 2013).

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 (Avnimelech, 2009; Crab *et al.*, 2010a) or can be harvested for use as a feed ingredient (Kuhn *et al.*, 2009, 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; Avnimelech, 2006). In order to evaluate the use of biofloc as a food source, general criteria of aquaculture feed can therefore, be applied, *i.e.*, the size of particles, attractiveness, palatability, digestibility and nutritional content (Tacon, 1987b).

Pacific white shrimp, *L. vannamei*, is one of the most important farmed species in the world. However, farming activities of this species have been largely affected by diseases, mostly diseases such as the White Spot Syndrome Virus (WSSV) and Early Mortality Syndrome (EMS). Producers and researchers are constantly looking for methods to reduce massive shrimp losses due to disease outbreaks. Growing shrimp using biofloc technology (BFT) was proposed as a tool to reduce water exchange and minimize the introduction of viral pathogen through incoming water. In addition, observations on the effects of BFT on reducing viral disease outbreaks were reported (Avnimelech, 2012).

The objectives of present study are:

- To collect the selective carbon sources like wheat flour, tapioca flour and molasses which will be utilized for growing biofloc.
- To study the effect of biofloc on growth and survival of *L. vannamei*.
- To find out the effect on growth and survival of *L. vannamei* fed on biofloc grown with different carbon sources.

## RESOURCES AND METHODS

### Site of the experiment :

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.

### Experimental animals and their acclimatization :

*Litopenaeus vannamei* (1000 numbers) were obtained from BMR Hatchery, Nellore, who has been authorized by Coastal Aquaculture Authority (CAA), Chennai to produce the seed. Shrimp seed were packed in double plastic bags filled with oxygen and water in the ratio of 3:1 in each bag and the density of shrimp was 300/bag. Post larvae (PL 20) transported by road in plastic bags containing 5 ppt saline water. PL transferred to the same salinity water in the wet lab. Acclimatization was carried out over 2 weeks. During this time salinity was lowered from 5 ppt to 3ppt. During this period the seed were fed with crumble, sinking starter feed having a crude protein percentage of 35 (Manamei shrimp feeds AVANTI Company).

### Tank allocation :

Indoor experiments were conducted in FRP tanks with 1000 ltr capacity and with an effective bottom area of 1.03 m<sup>2</sup>, three triplicate treatments were maintained in the Wet Laboratory (Plate A). Tanks were filled with bore water with a depth of 60 cm. All tanks were facilitated with 2 air stone-hoses type of diffuser system which is fitted to 2 HP blower. Aeration was provided 24 hours throughout the experiment for better biofloculation. Tanks were kept one week for dechlorination. Urea and super phosphate were added as fertilizers at a dosage of 4 and 1 g/m<sup>2</sup> during the first three weeks (Varghese, 2007). After two week all tanks were stocked with shrimps at a rate of 15/m<sup>2</sup> (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 (*Manihotesculaneta*), wheat flour (*Triticumaestivum*), and molasses



**Plate A: Experimental set up in the wet - laboratory**

(*Saccharum officinarum*) were selected as carbohydrate sources for bioflocation (Plate B). Wheat flour were purchased from the local market in powdered form which was meant for the culinary purpose. Molasses were purchased from the local sugar factory. While tapioca were purchased from vegetable 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 35 µm 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 daily feeding ration for each treatment was calculated and adjusted by estimating the weekly sampled mean biomass. The ration was divided and distributed twice daily with similar



**Plate B: Experimental carbon sources molasses, wheat flour and tapioca flour used as biofloc agent**

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) and Hari *et al.* (2004, 2006). 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 T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub>, respectively (Plate B). 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.

#### Statistical analysis :

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

#### OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads :

#### Water quality parameters :

In the present study important water quality parameters such as Dissolved oxygen, Temperature, pH, Total alkalinity, TAN were observed for every seven days of sampling in all FRP tanks and presented in tables from 2-6 and in figures from 1-5. The water quality parameters were similar during the experimental period and maintained in acceptable level.

The average values recorded for the various physiochemical parameters like dissolved oxygen, temperature, pH and total alkalinity are presented in Tables (2-6). These parameters were well within the optimum range and were not found to be affected by the addition of different sources of carbohydrate (New and Singholka, 1985). Water quality parameters, such as dissolved oxygen, temperature, pH and total alkalinity, were in the range of 7.12±0.05 - 7.98±0.02 mg/l, 25.8±0.01 - 30.7±0.04 °C, 7.71±0.03-8.03±0.01 and 252±1.41 - 290±1.41 mg/l, respectively. Among the

various treatments, the water TAN is lower in control ( $T_1$ ) ( $0.04\pm 0.01$  mg/l) and maximum ( $0.27\pm 0.02$  mg/l) was in where Molasses ( $T_4$ ) was used as carbohydrate source. Concentrations of TAN recorded from water showed fluctuating trends. When comparing week-wise values, the TAN concentration significantly lower in water was in initial days of the culture period, especially in the 1st week where higher values were recorded in

Molasses ( $T_4$ )  $0.09\pm 0.01$  mg/l and lower value recorded in control ( $T_1$ )  $0.04\pm 0.01$  mg/l. Similarly second week (14th day) higher values were recorded in Molasses ( $T_4$ )  $0.09\pm 0.02$  mg/l and lower TAN values was in Wheat flour ( $T_2$ )  $0.05\pm 0.02$  mg/l. Similar trend continued on the 21st day also. The highest and lowest water TAN observed were  $0.13\pm 0.02$  and  $0.09\pm 0.02$  mg/l for Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. During the 28th

**Table 1: Weekly variation of Dissolved Oxygen (mg/l) in the tanks treated with various carbohydrate sources as biofloculating agents**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	$T_1$	$T_2$	$T_3$	$T_4$
0	$7.98\pm 0.02$	$7.62\pm 0.02$	$7.63\pm 0.04$	$7.19\pm 0.01$
7	$7.24\pm 0.03$	$7.34\pm 0.01$	$7.30\pm 0.05$	$7.25\pm 0.04$
14	$7.21\pm 0.01$	$7.30\pm 0.03$	$7.39\pm 0.01$	$7.41\pm 0.03$
21	$7.16\pm 0.02$	$7.12\pm 0.05$	$7.16\pm 0.03$	$7.14\pm 0.02$
28	$7.27\pm 0.01$	$7.23\pm 0.02$	$7.27\pm 0.02$	$7.26\pm 0.03$
35	$7.24\pm 0.04$	$7.40\pm 0.04$	$7.43\pm 0.05$	$7.44\pm 0.05$
42	$7.31\pm 0.03$	$7.46\pm 0.01$	$7.34\pm 0.04$	$7.39\pm 0.01$
49	$7.28\pm 0.02$	$7.38\pm 0.01$	$7.41\pm 0.03$	$7.37\pm 0.02$
60	$7.33\pm 0.05$	$7.34\pm 0.03$	$7.38\pm 0.01$	$7.35\pm 0.04$

**Table 2: Weekly variation of temperature (°C) in the tanks treated with various carbohydrate sources as biofloculating agents :**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	$T_1$	$T_2$	$T_3$	$T_4$
0	$27.6\pm 0.02$	$27.6\pm 0.03$	$27.6\pm 0.03$	$27.6\pm 0.01$
7	$25.8\pm 0.01$	$25.8\pm 0.01$	$25.8\pm 0.05$	$25.8\pm 0.05$
14	$27.4\pm 0.04$	$27.4\pm 0.03$	$27.4\pm 0.01$	$27.4\pm 0.02$
21	$28.4\pm 0.02$	$28.4\pm 0.01$	$28.4\pm 0.02$	$28.4\pm 0.01$
28	$28.0\pm 0.03$	$28.0\pm 0.03$	$28.0\pm 0.03$	$28.0\pm 0.03$
35	$28.1\pm 0.05$	$28.1\pm 0.02$	$28.1\pm 0.01$	$28.1\pm 0.01$
42	$30.6\pm 0.02$	$30.6\pm 0.01$	$30.6\pm 0.04$	$30.6\pm 0.02$
49	$30.7\pm 0.03$	$30.7\pm 0.03$	$30.7\pm 0.02$	$30.7\pm 0.04$
60	$30.5\pm 0.02$	$30.5\pm 0.02$	$30.5\pm 0.03$	$30.5\pm 0.03$

**Table 3: Weekly variation of pH in the tanks treated with various carbohydrate sources as biofloculating agents:**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	$T_1$	$T_2$	$T_3$	$T_4$
0	$8.01\pm 0.01$	$7.97\pm 0.04$	$8.00\pm 0.02$	$7.81\pm 0.03$
7	$7.92\pm 0.03$	$7.93\pm 0.02$	$7.89\pm 0.03$	$7.93\pm 0.01$
14	$7.90\pm 0.02$	$7.86\pm 0.01$	$7.87\pm 0.01$	$7.95\pm 0.05$
21	$7.94\pm 0.02$	$7.88\pm 0.05$	$7.86\pm 0.04$	$7.89\pm 0.03$
28	$7.92\pm 0.04$	$7.88\pm 0.02$	$7.92\pm 0.02$	$7.94\pm 0.01$
35	$8.03\pm 0.01$	$8.01\pm 0.01$	$8.02\pm 0.02$	$8.01\pm 0.04$
42	$7.95\pm 0.03$	$7.91\pm 0.04$	$7.90\pm 0.05$	$7.84\pm 0.02$
49	$7.78\pm 0.01$	$7.74\pm 0.01$	$7.79\pm 0.01$	$7.82\pm 0.01$
60	$7.71\pm 0.05$	$7.71\pm 0.03$	$7.79\pm 0.05$	$7.77\pm 0.05$

day, the highest and lowest water TAN observed were  $0.16\pm 0.0$  mg/l and  $0.05\pm 0.01$  mg/l in Molasses ( $T_4$ ) and Control ( $T_1$ ), respectively. Wheat flour ( $T_2$ ) and Tapioca flour ( $T_3$ ) stood in second and third positions with water TAN of  $0.11\pm 0.02$  mg/l and  $0.09\pm 0.02$  mg/l, respectively. On the 35th day of the experiment the highest and lowest water TAN value observed were  $0.18\pm 0.02$  mg/l and  $0.08\pm 0.02$  mg/l in Wheat flour ( $T_2$ ) and in Control ( $T_1$ ), respectively. Molasses ( $T_4$ ) and Tapioca flour ( $T_3$ ) stood in second and third positions with TAN value of  $0.15\pm 0.02$  mg/l and  $0.13\pm 0.02$  mg/l, respectively. On the 42nd day highest TAN value of  $0.27\pm 0.02$  mg/l and lowest value of  $0.09\pm 0.02$  mg/l were recorded in Molasses ( $T_4$ ) and Control ( $T_1$ ), respectively. On the 49th day highest TAN value of  $0.23\pm 0.03$  mg/l and lowest value of  $0.08\pm 0.01$  mg/l were recorded in Molasses ( $T_4$ ) and Control ( $T_1$ ), respectively. On the 60th day highest increment of  $0.22\pm 0.03$  mg/l and lowest value of  $0.09\pm 0.02$  mg/l were recorded for the Molasses ( $T_4$ ) and Control ( $T_1$ ), respectively. Wheat flour ( $T_2$ ) and Tapioca flour ( $T_3$ ) stood in second and third positions with TAN value of

$0.21\pm 0.02$  mg/l and  $0.10\pm 0.02$  mg/l, respectively. An overall study indicated that the Molasses ( $T_4$ ) recorded highest TAN value of  $0.27\pm 0.04$  mg/l in the 60 days experimental period.

#### Growth parameters :

*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 were presented in Tables 7, 9 and Fig. 6 and 7. Observations on the growth during the first week (7th day) revealed that the weight increment varied between  $2.09\pm 0.11$  and  $2.70\pm 0.08$ g in Control ( $T_1$ ) and Wheat flour ( $T_2$ ). On the 14th day highest weight increment of  $4.44\pm 0.11$ g and lowest weight increment of  $3.21\pm 0.08$ g were recorded for the Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. Similar trend continued during the 21st day also. The highest and lowest weight increment were observed were  $4.43\pm 0.05$  and  $5.75\pm 0.11$ g in Control ( $T_1$ ) and Molasses ( $T_4$ ), respectively. During the 28th day, the highest and

**Table 4: Weekly variation of Total Alkalinity (mg/l) in the tanks treated with various carbohydrate sources as biofloculating agents :**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
0	252±1.41	258±2.82	254±4.24	256±5.65
7	266±2.82	278±1.41	270±1.41	268±1.41
14	268±1.41	280±1.41	268±2.82	272±2.82
21	270±1.41	276±2.82	272±1.41	278±1.41
28	258±4.24	282±1.41	270±2.82	280±1.41
35	268±2.82	262±4.24	284±2.82	268±4.24
42	276±1.41	274±1.41	280±1.41	278±2.82
49	280±2.82	286±1.41	278±2.82	274±1.41
60	288±1.41	280±2.82	290±1.41	286±1.41

**Table 5: Weekly variation of TAN (mg/l) in the tanks treated with various carbohydrate sources as biofloculating agents :**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
7	0.04±0.01	0.07±0.01	0.06±0.01	0.09±0.01
14	0.06±0.02	0.05±0.02	0.08±0.01	0.09±0.02
21	0.09±0.02	0.13±0.02	0.11±0.02	0.12±0.02
28	0.05±0.01	0.11±0.02	0.09±0.02	0.16±0.03
35	0.08±0.02	0.18±0.02	0.13±0.02	0.15±0.02
42	0.09±0.02	0.23±0.02	0.12±0.02	0.27±0.02
49	0.08±0.01	0.19±0.01	0.11±0.03	0.23±0.03
60	0.09±0.02	0.17±0.02	0.10±0.02	0.21±0.03

lowest weight increments observed were  $7.97\pm 0.09\text{g}$  and  $5.72\pm 0.07\text{g}$  in Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. In treatments of Tapioca flour ( $T_3$ ) and Molasses ( $T_4$ ) stood in second and third positions in weight increment of  $7.42\pm 0.08$  and  $7.40\pm 0.05\text{g}$ , respectively. Similar trend continued during the 35th day of the experiment also. Highest and lowest weight growth increments observed were  $9.78\pm 0.11$  and  $7.08\pm 0.02\text{g}$  in Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. Tapioca flour ( $T_3$ ) and Molasses ( $T_4$ ) stood second and third positions with growth weight gain of  $9.24\pm 0.11$  and  $9.11\pm 0.01\text{g}$ , respectively. On the 42nd day the highest weight increment of  $11.71\pm 0.04\text{g}$  and lowest increment of  $8.59\pm 0.11\text{g}$  were recorded in Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. On the 49th day the highest increment of  $13.65\pm 0.12\text{g}$  and lowest increment of  $10.26\pm 0.12\text{g}$  were recorded in Wheat flour ( $T_2$ ) and Control ( $T_1$ ), respectively. On the 60<sup>th</sup> day the highest increment of  $15.92\pm 0.07\text{g}$  and lowest increment of  $12.27\pm 0.09\text{g}$  were recorded in Wheat flour ( $T_2$ ) and in Control ( $T_1$ ), respectively. Treatments of Tapioca flour ( $T_3$ ) and Molasses ( $T_4$ ) stood in second and third

positions with growth weight gain of  $15.17\pm 0.07$  and  $14.82\pm 0.04\text{g}$ , respectively. An overall study indicated that the Wheat flour ( $T_2$ ) recorded total weight increment of  $15.92\pm 0.07\text{g}$  in 60 days experimental period. This was followed by the Tapioca flour ( $T_3$ )  $15.17\pm 0.07\text{g}$  and Molasses ( $T_4$ )  $14.82\pm 0.04\text{g}$ , they stood in second and third positions, respectively.

The growth data was subjected to analysis of variance (ANOVA) at 5% level of significance and the observations are given in Table 8. The statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classification. The Treatment Wheat flour ( $T_2$ ) is found to be significantly superior when compare to other Treatments. Treatment Wheat flour ( $T_2$ ) has shown significantly different from all other Treatments. The second and third positions were occupied by Tapioca flour ( $T_3$ ) and Molasses ( $T_4$ ), respectively. There was a significant difference between the culture periods also.

**Table 6: Growth performance (g) of *L. vannamei* fed on biofloc grown with different carbon sources:**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
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

**Table 7 : Weight gain (g) in *L. vannamei* fed on biofloc grown with different carbon sources:**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
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

**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 bioflocs were calculated and presented in Table 8.

Control (T<sub>1</sub>) group has the lowest Specific Growth Rate of 4.13%. The highest value was in Wheat flour (T<sub>2</sub>) with 4.59%. The treatments that stood second and third positions were Tapioca flour (T<sub>3</sub>) 4.49% and Molasses (T<sub>4</sub>) 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 9.

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

During the first sampling (7th day), the Feed Conversion Ratio ranged between 0.50±0.07 and 0.80±0.08. The highest value during this period was recorded in Control (T<sub>1</sub>) and the lowest was in Wheat flour (T<sub>2</sub>). On the 14th day of sampling the highest value

of (FCR) 1.39±0.02 was in Control (T<sub>1</sub>) and the lowest value 1.08±0.05 was in Wheat flour (T<sub>2</sub>). The highest value of 1.58±0.05 was observed in Control (T<sub>1</sub>) on 21th day while the lower value of 1.42±0.04 was recorded in Tapioca flour (T<sub>3</sub>). On 28th day recorded Molasses (T<sub>4</sub>) with highest Feed Conversion Ratio value of 1.58±0.05 and lowest value was in Wheat flour (T<sub>2</sub>) the value of 1.31±0.04. The highest value of 1.28±0.04 was observed in Control (T<sub>1</sub>) on 35th day while the lowest value of 1.21±0.02 was recorded in Molasses (T<sub>4</sub>). Sampling on 42nd day recorded highest value of 1.24±0.04 in Molasses (T<sub>4</sub>) and lowest value of 1.03±0.08 was in Tapioca flour (T<sub>3</sub>). Sampling on 49th day recorded a highest Feed Conversion Ratio value of 1.12±0.05 in Molasses (T<sub>4</sub>) and lowest value of 1.00±0.02 in Control (T<sub>1</sub>). In the last sampling on 60th day recorded Control (T<sub>1</sub>) with highest Feed Conversion Ratio value of 1.80±0.05 and lowest value of 1.59±0.07 in Molasses (T<sub>4</sub>).

The feed conversion ratio was subjected to analysis of variance (ANOVA) and presented in Table 15. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classification. The Control (T<sub>1</sub>) was found to be significantly superior when compared to the other Treatments. The Molasses (T<sub>4</sub>), Tapioca (T<sub>3</sub>) and Wheat flour (T<sub>2</sub>) occupied second, third and fourth positions.

**Table 8: Specific Growth Rates (%) of *L. vannamei* fed on biofloc grown with different carbon sources.**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
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 9: Feed conversion Ratio of *L. vannamei* fed on biofloc grown with different carbon sources.**

Treatment	Control	Wheat flour	Tapioca flour	Molasses
Period(Days)	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
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



There was a significant difference between the experimental periods also.

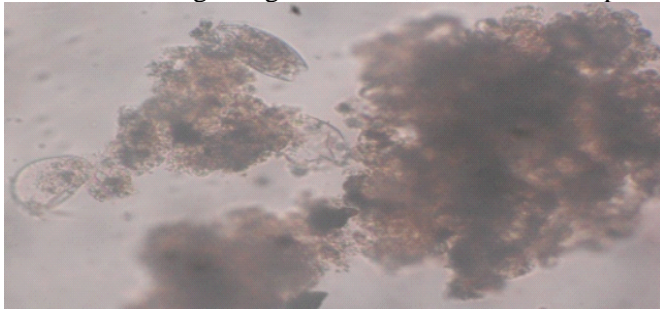
**Composition of biofloc :**

Composition of biofloc in the biofloc treatment group, floc was seen in anomalous flocculation with bacteria and zooplankton especially rotifers (Plate 8). The compositions of different floc associated planktonic organisms are given in Plate 8-12. The group of organisms was identified as Rotifer-*Brachionusplacatilis* (Plate 8), Ciliophoraprotzoaon - *Stenterroeseli* (Plate 9), *Globiginasps* (Plate 10), *Verticellasps* (Plate 11), *Parameciumsps*(Plate 12). Rotifers were the most dominant group in all the biofloc treatment tanks. The total number of organisms was significantly high in the Wheat flour (T<sub>2</sub>) followed by Tapioca flour (T<sub>3</sub>), Molasses (T<sub>4</sub>) and Control (T<sub>1</sub>). These microorganisms were found to be grazing on the flocs, when fresh samples

were observed under micro scope. In all the biofloc treatment tanks, mostly the zooplanktons were dominant and very limited number of phytoplankton could be visualized under the microscope.

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

In the present experiment tapioca flour used as carbohydrate source showed higher growth performance than molasses and control but lower than wheat flour. This may be due to ready availability of carbohydrate to microbes in tapioca flour are in more than in molasses and lower than in wheat flour. Varghese, (2007) carried out similar studies in extensive culture system of *P. monodon* with the same carbohydrates sources and the results were similar. Increase in growth performance of *M. rosenbergii* was noticed in tapioca flour as



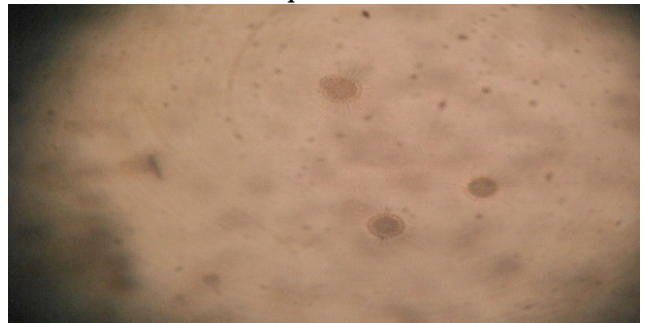
**Biofloc composition**



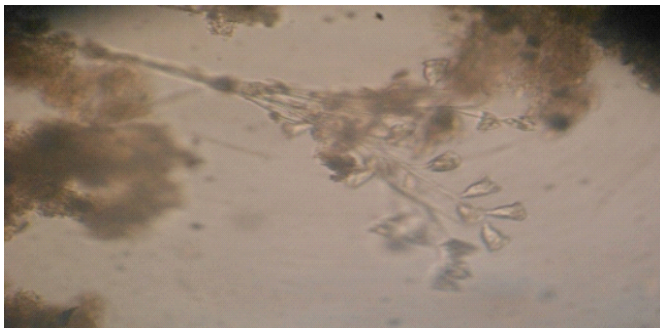
**Rotifer-*Brachionusplacatilis* observed in biofloc**



**Ciliophoraprotzoaon -*Stenterroeseli* observed in biofloc**



***Globiginasps* observed in biofloc**



***Verticellasps* observed in biofloc**



***Parameciumsps* observed in biofloc.**



carbohydrate source treatment tanks compare to control tanks (Hari *et al.*, 2004; Crab *et al.*, 2010a and Prajith, 2011).

The different organic sources, however appeared to have an effect on the assimilation of protein and lipid by the shrimp in the biofloc treatment was higher than that of the control shrimp (Ekasari *et al.*, 2014). Molasses as a carbohydrates source in the present study showed higher growth performance than that of control shrimp. A similar observation was obtained for the lipid and protein assimilation resulted in increased growth that was significantly higher for the molasses and tapioca treatments (Ekasari *et al.*, 2014).

Wheat flour as a carbohydrate source biofloc grown shrimp documented higher weight gain ( $2.27 \pm 0.01$ ) 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 (Raj kumar *et al.*, 2015).

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 (Avnimelech, 2007; Burford *et al.*, 2004; Kuhn *et al.*, 2009; Li *et al.*, 2013). Similar results were reported by Anand *et al.* (2014) in *P. monodon* at 4% and 8% supplementation of biofloc treatments.

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*.

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