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Effect of Distillery Spent Wash at Different C/N Ratio on Biofloc based Nursery Culture of *Penaeus vannamei*

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ABSTRACT

Background: Biofloc technology is one of the methods to overcome the problems associated with high ammonia-nitrogen and nitrate-nitrogen concentration in the water. The addition of carbon source is one of the practical ways to increase the C/N ratio and to promote the floc development in the culture system. The present research was carried out to assess the effect of distillery spent wash (DSW) at different C/N ratios on floc formation and growth of the shrimp under an intensive culture system.

Methods: A 35-day experiment was conducted to study the effect of DSW at different C/N ratios (10, 15, 20 and 25) on the development of biofloc in the nursery culture of *Penaeus vannamei*. Floc development, water quality and growth performance of *P. vannamei* were also studied.

Result: It was observed that significantly ($p<0.05$) higher specific growth rate (12.03±0.15), survival, final weight gain and better feed conversion ratio (1.25±0.02) of the shrimp at C/N 15 treatment. The study also recorded an increase in C/N ratio decreases the growth performance of the shrimp. The present study reported that, in a DSW based biofloc system, C/N ratio of 15 showed a positive effect on water quality parameters, survival and enhanced growth of cultured shrimp.

Key words: Bio-floc, Carbon-Nitrogen ratio, Distillery spent wash, Growth performance, *Penaeus vannamei*.

INTRODUCTION

The pacific white leg shrimp, *Penaeus vannamei* is a commercially important shrimp species cultured in many countries. It contributes 53% of the total crustacean production (FAO, 2018). The rapid development of intensive shrimp culture resulted in environmental degradation and disease outbreak in the culture system due to the frequent water exchange and effluent discharge (Ekasari *et al*. 2014; Thitamadee *et al*. 2016). An intrinsic feature of such a system is a rapid accumulation of uneaten feed, organic matters and toxic inorganic nitrogen. Several new methods have been proposed to overcome the problems associated with a high ammonia-nitrogen concentration and nitrate-nitrogen in the water. One such method is biofloc technology (BFT); floc generated in this system has numerous advantages such as it does not only control the water quality it also helps to increase the feed utilization, remove the toxic nitrogen, reduces the potential spread of the pathogen, minimizes the feed conversion ratio and improves the growth of the animal (da Silva *et al*. 2013; Wang *et al*. 2015; Xu *et al*. 2018). The C/N ratio of commercial shrimp feed used in the intensive aquaculture system is around 10:1. The addition of the carbon source is one of the practical ways to increase the C/N ratio and to promote the floc development in the culture system (Xu *et al*. 2018; Hargreaves, 2006; Avnimelech, 1999). Carbon source and C/N ratio are the two major factors that influence the character and development of the floc and it also affects the growth of the cultured shrimp. Several types of carbon sources are used to promote the growth of floc, such as wheat flour, acetate, tapioca, corn, wheat, glycerol, molasses starch, *etc*., (Avnimelech, 1999; Azim and Little, 2008; Crab, 2010; Ekasari *et al*. 2014).

Distillery spent wash (DSW) is the residual liquid generated during alcohol production. It is classified as organic liquid fertilizer with high potassium content (Samuels, 1980). Cane molasses is one of the most common raw materials used in alcohol production. More than 40 billion liters of DSW have generated annually in India (AIDA, 2007). Chidankumar *et al*. (2010) reported that the DSW contains a higher concentration of essential nutrients and it also increased the irrigation yield by 33% (Jain and Srivastava, 2012). The previous studies suggest DSW could be used as a substrate for BFT production (Liu *et al*. 2017). Hence, the present research was carried out to assess the effect of DSW at different C/N ratios on floc formation, water quality and growth of the shrimp under an intensive culture system.

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MATERIALS AND METHODS

The experiment was carried out for 35 days in 500-liter tanks at Brackish Water Research Farm Facility, TNJFU-OMR Campus, an outstation facility of Dr. M.G.R Fisheries College and Research Institute, Ponneri, TNJFU. DSW (24% carbon source) was collected from M/s. Rajshree Biosolutions Pvt. Ltd., situated at Coimbatore, DSW was used as a carbon source to promote floc development under the zero-water exchange system. In BFT tanks, four different C/N ratios were maintained, such as C/N 10:1, C/N 15:1, C/N 20:1 and C/N 25:1 in triplicate. Carbon addition was calculated based on the ammonia level in the tank concerning treatments (Avnimelech, 1999). There was no water exchange done in the BFT tanks. Dechlorinated water was added to all the tanks to compensate for the evaporation loss.

PL15 shrimps (*P. vannamei*) were used in the experiment and were procured from Aqua Nova shrimp hatchery, Kanathur, OMR Road, Chennai. OIE listed diseases were tested in shrimp before three days of the stocking. The healthy shrimp were stocked in the raceway tanks with proper acclimatization at the rate of 1500 PL/m^3. Shrimp fed with 35% crude protein, feeding was done four times a day (6.00, 10.00, 14.00 and 18.00) at 20% of the body weight.

Water samples were collected from the experimental tanks during morning hours. Visual analysis of water quality parameters such as color and flocculation was observed daily. Temperature, salinity, floc volume and pH were tested daily in the experimental unit. Other parameters such as total alkalinity, dissolved oxygen (DO), total hardness, nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen, total suspended solids and total solid were estimated in the lab following standard protocols (Eaton *et al*. 2005) once in three days. Floc volume was obtained using Imhoff cones once in three days (Avnimelech and Kochaba, 2009). Floc volume index was determined by the ratio of TSS and floc volume.

At the end of the experiment, growth parameters were calculated using the following growth indices,

Percentage weight gain =

$$\frac{[\text{Final weight (g)} - \text{Initial weight (g)} \times 100]}{\text{Initial weight (g)}}$$

Specific growth rate {SGR (%/day)} =

$$\frac{[(\ln \text{ final weight} - \ln \text{ initial weight}) \times 100]}{\text{Culture period (day)}}$$

(Tanuja *et al*., 2017).

$$\text{Feed conversion ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

$$\text{Feed efficiency ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Body weight gain (wet weight)}}{\text{Crude protein fed}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of shrimp harvested}}{\text{Total number stocked}} \times 100$$

(Paripuranam *et al*., 2011).

Statistical analysis

All the data were statistically analyzed using SPSS 22.0 for Windows. One-way analysis of variance was conducted to examine the significant difference among the treatments in growth parameters, water quality parameters and AMF volume. The mean was compared in Duncan's multiple range test at a significance level of P<0.05.

RESULTS AND DISCUSSION

The present study confirmed that the DSW could promote floc development and positively affect shrimp production and water quality maintenance in a zero water exchange system. It is clearly explained that the key to BFT is the increasing conversion of microbial protein by carbon addition (Zhao *et al*. 2012). Ammonia, nitrite and nitrate have significantly differed among the treatments (Fig 1). In all treatments, ammonia level was maintained at <0.4 ppm. In C/N 15 and 20, the ammonia level kept <0.1 ppm; at the end, the culture

Table 1: Water quality parameters of experimental groups for the 35 days of culture trial.

Parameter	C/N 10	C/N 15	C/N 20	C/N 25
DO (ppm)	5.53±1.48[a]	6.33±3.61[a]	6.58±3.32[a]	6.5±3.1
	(5.2-8.6)	(5.2-8.6)	(5.2-7)	(5-7)
Temperature (°C)	27.84±1.34[a]	27.03±1.05[a]	27.05±1.14[a]	27.03±1.05
	(25-30)	(25-30)	(25-30)	(25-30)
Salinity (ppt)	19.41±1.56[a]	19.63±0.48[a]	19.96±0.29[a]	19.96±0.45[a]
	(19.5–21.5)	(19.5-21.5)	(19.5-21.5)	(19.5-21.5)
pH	7.63±0.30[a]	7.67±0.25[a]	7.69±0.23[a]	7.69±0.53[a]
	(7-8.5)	(7-8.5)	(7-8.5)	(7-8.5)
Alkalinity (mg/L^{-1})	115.71±33.79[a]	162.55±19.45[b]	163.13±23.18[b]	125.71±33.79[a]
	(46-180)	(124-200)	(139-230)	(46-180)

Values (Mean ± SE) in the same row with different superscript differ significantly (Duncans multiple range test (p<0.05)). All the values in percentage were transformed for ANOVA.

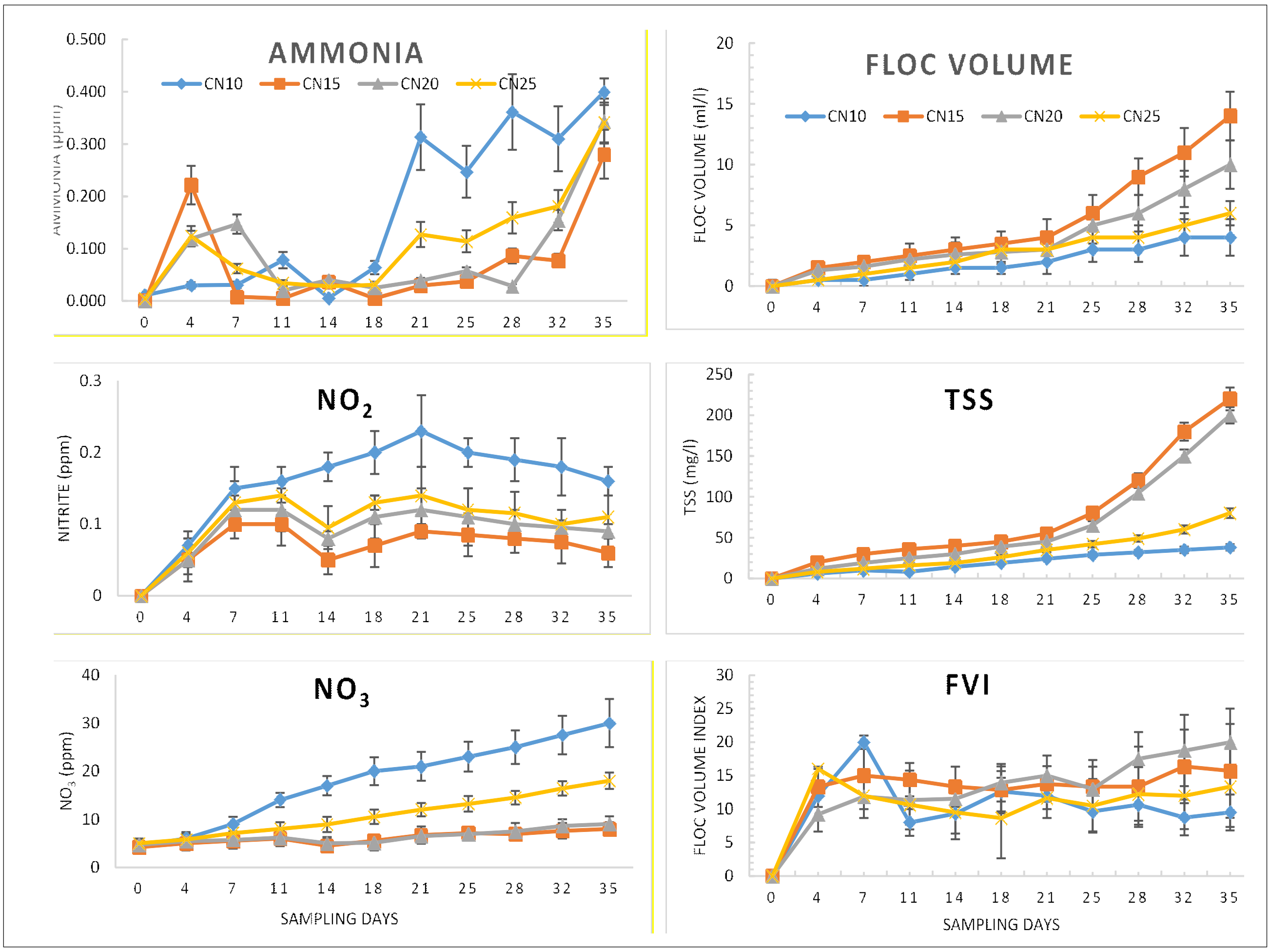

Fig 1: Water quality fluctuation in experimental trail throughout the culture period.

ammonia level increased. In C/N 10 treatment, NO_2 and NO_3 levels were higher than the other treatments. When ammonia-nitrogen and nitrite-nitrogen concentrations are higher than the 0.1 mg/l and 5 mg/l, respectively, it will harm the aquatic animals (Qiao *et al*. 2006). Nitrate-nitrogen can also harm the shrimp when the animal is exposed for a longer period at a concentration of >60 mg/l (Wang *et al*. 2015). When sugar cane, molasses and dextrose were used as the carbon source (Samocha *et al*. 2007 and Hari *et al*. 2004) in the culture system, ammonia was rapidly removed. Similar results were observed in this study. Alkalinity maintained above 110 mg/l and C/N 15 and C/N 20 having the alkalinity of >160 mg/l. This reduction of alkalinity occurs due to the consumption of inorganic carbon by the heterotrophic and nitrifying bacteria that form the bioflocs (Ebeling *et al*. 2006). No significant difference was observed in dissolved oxygen, temperature, salinity, pH and alkalinity (Table 1). All water quality parameters remained within the limit for shrimp culture throughout the culture period (Ponce-Palafox *et al*. 1997; Zhang *et al*. 2006).

Floc volume, TSS and FVI were often used as indicators for the quantitative determination of the microbial floc (De Schryver *et al*. 2008) as their changes over time can be reflected in the development of the floc in water. In the present study, floc density, in terms of floc volume, TSS and FVI was gradually increased over the culture period in all treatments. Total suspended solids, floc volume index and floc volume differed between treatments throughout the study (Fig 1). The average floc volume and TSS range from 8-16 ml L^{-1} and 150 - 220 mg L^{-1}, respectively (Fig 1). Initially, floc volume was less in all treatments and after two weeks, floc volume increased based on the addition of C/N ratio and activity of heterotrophic bacteria and proliferation (Liu *et al*. 2017). Floc volume steadily increased from C/N 10 to C/N 15. Total suspended solids significantly improved, based on the floc volume in the system and higher TSS was recorded in C/N 15 and C/N 20 at the range of 150-250 mg/ The lowest TSS was recorded in the C/N 10 treatment.

Furthermore, the microbial biomass yield per unit substrate of heterotrophic bacteria is 40 times greater than the nitrifying bacteria (Ebeling *et al*. 2006; Hargreaves, 2006). Avnimelech (1999) determined that using carbon sources to raise the C/N ratio within the culture system was a practical and inexpensive way to reduce inorganic nitrogen

Table 2: Zoo technical performance of *P. vannamei* at the end of experimental period.

Growth parameters	Treatments			
	C/N10	C/N15	C/N20	C/N25
Initial body weight (g)	0.02±0.003[a]	0.02±0.001[a]	0.02±0.002[a]	0.02±0.002[a]
Final body weight (g)	0.68[b]±0.052	0.86[a]±0.01	0.75[b]±0.025	0.57[b]±0.052
Daily growth rate (g)	0.022[b] ±0.001	0.028[a]±0.004	0.024[a]±0.003	0.018[c]±0.001
Survival rate (%)	70.99[c]±0.5	90.88[a]±1.1	81.44[b]±1.23	65.99[d]±0.5
SGR (% day^{-1})	11.75[b]±0.2	12.53[a]±0.15	12.08[a]±0.12	11.16[a]±0.12
FCR	1.52[b]±0.03	1.25[a]±0.02	1.44[a]±0.02	1.89[b]±0.03
PER	0.01813[b]±0.01	0.024[c] ±0.01	0.020[a]±0.01	0.015[b]±0.01

Values (Mean±SE) in the same row with different superscript differ significantly (Duncans multiple range test ($p<0.05$). All the values in percentage were transformed for ANOVA. *SGR: Specific growth rate; FCR: Feed conversion ratio; FER: Feed efficiency ratio; PER: Protein efficiency ratio.

accumulation in the culture system. In the present study, use of distillery spent wash to raise the C/N ratio in treatments C/N10 to C/N25 has efficiently maintained the low level of nitrogen species without affecting the shrimp growth. This may result from high-density protozoans, bacteria, ciliates rotifer, zooplankton and organic matter present in the floc. The reduction in ammonia and development of floc volume shows a similar pattern when floc volume increases the ammonia concentration maintained below the average level and this strongly proves the utilization of ammonia-nitrogen by heterotrophic bacteria.

In the present study, biofloc was effectively utilized by the P. *vannamei* nursery at high stocking density with a zero water exchange system. Shrimp survival was higher in the treatment C/N 15, suggesting that BFT resulting from the addition of a carbon source had a beneficial effect on the shrimp survival. A higher level of shrimp mortality was recorded in C/N 25 caused by the hypoxic condition of the culture system due to the over addition of carbon source in the initial phase of the culture system. These indicate that attention should be paid to a hypoxic condition in treatments with a high C/N ratio culture system and biochemical composition of the carbon source.

Growth performance was appraised through final weight, weight gain, specific growth rate and survival of the shrimp (Table 2) at the end of the culture period. The specific growth rate and PER, survival and net production of the shrimp in treatment C/N 25 significantly ($P<0.05$) lower than other treatments. The FCR of the shrimp was better in C/N 15 than in other treatments. This indicates that the appropriate quantity of carbon source addition was helpful in the good growth and survival of the shrimp. These might be due to the synergistic effect of the improved water quality, higher bacterial and zooplankton density. Studies have indicated that carbon addition can result in the production and accumulation of biofloc (Avnimelech, 2007; Emerenciano *et al*. 2011; Gao *et al*. 2012), which could serve as an essential food source for the zooplankton and thus could increase the growth of the shrimp. It has been demonstrated that zooplankton serves as an *in-situ* food source for *P.vannamei* (Chen and Chen, 1992).

CONCLUSION

The application of BFT allows minimal or zero water exchange practice during the culture period and thereby can improve the sustainable, bio secured and production in shrimp aquaculture. The driving force of BFT is the development of floc, which is responsible for water quality, waste assimilation and nutrient cycling, improved growth performance of the shrimp under high stocking density. The present study suggests that modifying the C/N ratio significantly influenced the growth and feed utilization of the shrimp and floc development under the zero water exchange system. Two significant conclusions can be drawn from the present study as follows (1) distillery spent wash can be used as a carbon source to develop the BFT (2) Once a mature floc developed, the heterotrophic bacteria can effectively control ammonia and NO_2- N concentration under intense aeration that helps in the maintaining water quality to an acceptable range for shrimp culture under high stocking density.

Conflict of interest: None.

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