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Original research article

Semi Intensive and Semi Biofloc Methods for the Culture of Indian White Prawn, *Fenneropenaeus indicus* in High-density Polyethylene Liner Ponds

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ABSTRACT

An experiment (triplicated) was conducted to assess the growth and production of Indian white prawn, *Fenneropenaeus indicus* in semi intensive and semi biofloc culture technique for a period of 120 days in polythene liner ponds (300 m^2). Water exchange was done at 10% in semi intensive culture method (control) and zero water exchange was done in semi biofloc method (treatment). Soya hull and molasses were added as carbon sources to induce biofloc formation in treatment ponds. Post larvae (PL20) were stocked at the rate of $100/m^2$ in each pond and fed with a standard shrimp feed. Shrimp growth, physico-chemical parameters of water, bacteria, phytoplankton and zooplankton population, immune response and physical quality of shrimp were recorded during the period. Significant difference ($p < 0.01$) in shrimp growth (weight) was observed between control and treatment. Phytoplankton population and total haemocyte count were found to be increased and pathogenic bacteria population decreased in treatment ponds. Strong linear relationship was found between growth and biofloc content in treatment ponds. Shrimp grown in biofloc pond showed better colour and taste compared to control. Semi biofloc technique can be considered as an ideal culture method for bio secure production of white shrimp in semi arid lands.

1. Introduction

Nowadays aquaculture is growing rapidly and cutting edge technologies are being practiced to produce quality shrimp meat (Martini *et al.* 2015). In semi intensive culture, environment is disturbed by the discharge of waste water due to daily water exchange. One of the technologies that developed was an ecofriendly culture technique known as biofloc technology (BFT) (Avnimelech *et al.* 1989; Emerenciano *et al.* 2013; Martinez-Cordova *et al.* 2016). The consumption of biofloc by shrimp has demonstrated innumerous benefits such as improvement of growth rate (Wasielesky *et al.* 2006), decrease of feed conversion ratio (FCR) and associated costs in feed (Burford *et al.* 2004; Zokaeifar *et al.* 2013). BFT is being successfully expanded in shrimp farming units in almost all parts of world (Martinez-Cordova *et al.* 2015). Considering the significance of biofloc technique, a study was conducted to compare the growth performance of Indian white prawn, *Fenneropenaeus indicus* in semi intensive and semi biofloc culture method in high-density polyethylene liner ponds. Indian white prawn, *F. indicus is* an ideal candidate species for coastal aquaculture practice in the semi-arid lands of Saudi Arabia.

2. Materials and Methods

2.1. Experimental set up and pond preparation

The study was conducted for a period of 120 days in high-density polyethylene liner ponds (300 m^2) at King Abdulaziz University Fish Farm, Jeddah. Six culture ponds were limed and sundried for 1 week before water culture. Water culture was done as follows: On day 1, culture ponds were filled (30%) with seawater and manured by applying urea (400 g), molasses (1.5 L) and diammonium phosphate (200 g). During days 2 and 3, water level was increased up to 60% and the above dose was again applied on the 4th day. On days 5–7, pond water level was increased up to 100% (2 m) and on the 8th day, the third dose was applied. On day 11 required algal bloom was developed (40–50 cm Secchi disc). Two units of aspirator aerator (4 hp/pond) (Force-7, Acquaeco, Italy)

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were installed at 40 cm below water level with 35 cm angle downward in each pond.

There were control and treatment for the study and both were triplicated. Ponds in which water exchange was done at 10% and transparency maintained at 40–50 cm were considered as control (semi intensive). Whereas, ponds in which zero water exchange was done (topping up at 10%/week to maintain loss of water due to evaporation and transparency maintained between 30–40 cm) were designated as treatment (semi biofloc). To boost up heterotrophic bacterial growth, soya hull and molasses (2 kg each) were applied in treatment ponds as carbon sources once in 3 days. On 12th day of water culture, healthy and uniform size juvenile (1.84 ± 0.2 g:5.7 ± 1.8 cm) produced at KAU Fish Farm Hatchery were stocked at the rate of 100 pieces/m^2 in each pond (hapa survival >95%). A standard fish meal based pellet feed having 35% protein (from NAQUA, Jeddah) was supplemented to shrimp at the rate of 5%/body weight daily at 7 AM and 4 PM.

2.2. Water quality parameters

Water quality parameters such as pH, temperature and dissolved oxygen were recorded daily at 6 AM and 3 PM (Hana pH meter; YSI Incorporated Yellow springs, OH, USA). Salinity was tested every day at 3 PM with a Refractometer (ATAGO, Japan). Ammonia (unionized), nitrates (NO_3), nitrites (NO_2), orthophosphates (PO_4) and alkalinity (as $CaCO_3$) were recorded (Tropic Marine Test kit, Germany) weekly. The biofloc volume was determined everyday using Imhoff cones at 8 AM. For this, water sample (1 L) was collected from each pond (20 cm below from surface water) and transferred to a previously fixed Imhoff cones on stand. After settling the suspended organic solids for 20 minutes, the volume of biofloc was estimated and expressed in mL/L (Taw 2014).

2.3. Heterotrophic bacteria population and plankton community

Total plate count for colony forming units and plankton community (phytoplankton and zooplankton) in the pond water was examined every month (APHA 1995; Smith and Johnson 1996). The plankton sample was subjected to major group and species identification. Shrimp sampling (200 pcs/sampling) was done biweekly to assess the growth in weight. Feed quantity to be fed was readjusted based on growth after every sampling.

2.4. Immune response

Status on shrimp immunity was conducted a week before harvest. For this, shrimp from control and treatment ponds were brought to laboratory and subjected to a cold challenge test at 20°C for 24 hours. After the cold challenge, haemolymph sample was drawn from the heart of shrimp and estimated the haemocyte count using a compound microscope (Krupesha *et al.* 2009).

2.5. Growth analysis and physical quality test

Upon harvest, all shrimp from control and treatment ponds were collected, survival, biomass and pond bottom status (sludge deposition) were recorded. Specific growth rate was calculated as $\text{Log}_e\ W2 - \text{Log}_e\ W1/T2 - T1$ (where W2 is the weight of shrimp at time T2 and W1 is the weight of shrimp at time T1). Survival (%) was estimated as (total number of shrimp harvested/total number of PL stocked) × 100. A panel of experts (10 members) evaluated physical quality of shrimp such as colour, loose shell, soft shell and taste based on a grade chart and score (G3, good; G2, moderate; G1, bland).

2.6. Statistical analysis

One-way analysis of variance was used to find out the statistical difference between growth, water quality parameters, biofloc content, bacterial population and haemocyte count of control and treatment (Excel, 2007). Linear regression analysis was done to find out the relationship between biofloc content and shrimp growth (Excel, 2007).

3. Results

3.1. Growth performance

F. indicus grown in treatment ponds showed significant increase ($p < 0.01$) in growth when compared to control after 120 days of culture (Table 1 and Figure 1). Shrimp survival between control and treatment did not show significant difference ($p > 0.01$). FCR was found to be low in treatment ponds compared to control. Total biomass (kg/ha) production was high in treatment ponds. Significant relationship ($R^2 = 0.9019$) was observed between biofloc concentration (mL/L) and average body weight (g) in treatment ponds when compared to control (Figure 2).

3.2. Water quality parameters

Table 2 provides the result of water quality parameters recorded during the culture period. The parameters were found to be within the tolerable range required for shrimp growth. An increasing trend in salinity was observed in treatment ponds compared to control and it reached to a maximum of 42 ppt on 90th day of culture. This was due to evaporation and zero water exchange in the ponds. Alkalinity showed a decreasing trend in treatment ponds during culture days. Nitrate and nitrite content showed an increasing trend in treatment ponds compared to control and the difference was not found to be significant. Orthophosphate content increased in treatment pond compared to control. At the end of culture days, unionized ammonia was found to be high in treatment ponds. Biofloc concentration increased in treatment ponds in increasing days of culture with a maximum of 13 mL/L (Figure 3).

3.3. Heterotrophic bacteria population and plankton community

Significant difference ($p < 0.01$) in total plate count (TPC) for colony forming units was found between control and treatment ponds in each sampling day (Table 3). Total Yellow *Vibrio* and *Green Vibrio* colonies were found to be lowered in treatment ponds when compared to control.

Phytoplankton community was found to be decreased in both control and treatment ponds in increasing days of culture (Figure 4). However, it was high in treatment ponds compared to control ponds in every sampling. Species of genera such as *Tetraselmis, Peridinium, Trichodesmium, Nitzchia, Malassiosira, Cylotella,*

Table 1. Production details of shrimp after 120 days of culture

Parameters	Control	Treatment
	Mean ± SD	Mean ± SD
Initial weight (g)	1.84 ± 0.2	1.84 ± 0.2
Final weight (g)**	18.0 ± 3.3	20.5 ± 2.3
Net weight gain (g)	16.1 ± 2.9	18.6 ± 1.2
Average weekly growth (g)	0.9 ± 0.1	1.2 ± 0.1
SGR (%)	1.03 ± 0.02	1.09 ± 0.01
Survival (%)	83 ± 5.0	81 ± 6.0
FCR	2.90 ± 0.20	2.60 ± 0.10
Biomass/pond (kg)	184 ± 9.0	205 ± 3.0
Biomass (kg)/ha	6133 ± 309	6833 ± 100

$n = 300$ Values are the average of three ponds.
FCR = feed conversion ratio; SD = standard deviation; SGR = specific growth rate.
**$p < 0.01$.

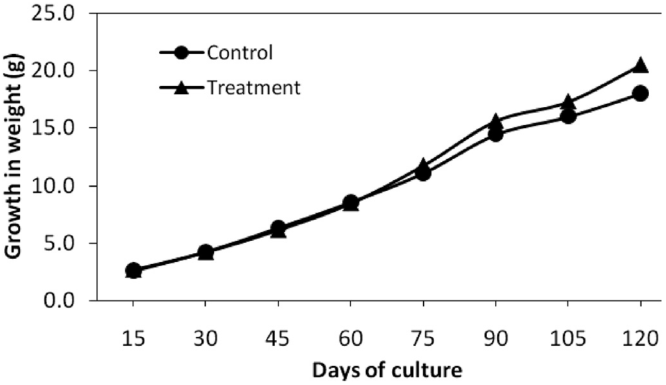

Figure 1. Growth of *F. indicus* during culture period.

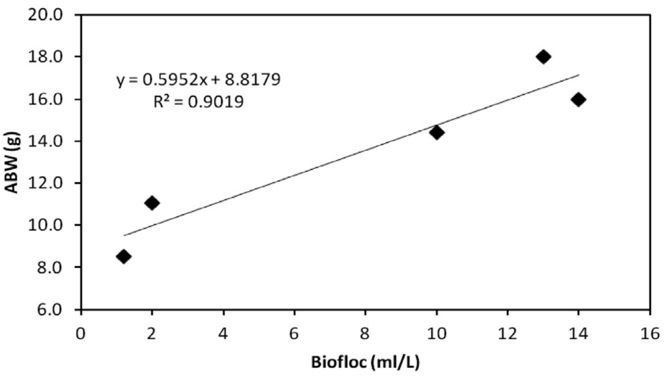

Figure 2. Linear relationship between biofloc and average body weight (ABW) in treatment pond.

Ceratium, Amphora, Navicula, Psuedonizchium and blue green algae were the dominant species in the community. Zooplankton community was found to be high in treatment and low in control ponds. Ciliates, protozoans, rotifers, crustacean larvae and copepods were observed in the group. Control ponds showed low levels of micro flora, organic substances and other organisms compared to treatment ponds.

3.4. Immune response

Shrimp immunity was expressed in total haemocyte count (THC/mL) of haemolymph (Figure 5). Significant difference ($p < 0.01$) in THC was found between control and treatment shrimp at the end of culture (baseline). Shrimp grown in treatment ponds showed high haemocyte count than that of control before and after cold challenge test.

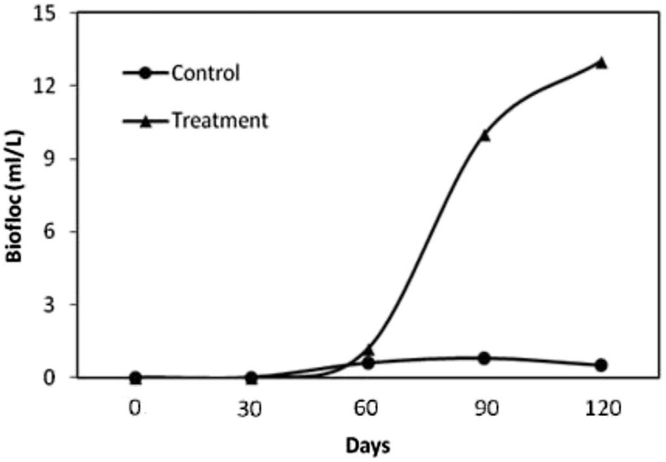

Figure 3. Biofloc content recorded in control and treatment pond.

3.5. Post-harvest status and shrimp quality

After harvest, sludge accumulation at 102 ± 12 kg was observed at the centre of pond bottom in all treatment ponds; whereas in control ponds it was 29.4 ± 5.8 kg. Physical quality test done by the panel of experts (10 members) revealed that shrimp grown in treatment ponds had better colour and taste than that of control. Loose and soft shell (%) was high in control ponds compared to treatment ponds (Table 4).

4. Discussion

Result of the present study shows that shrimp grown in semi biofloc ponds performed better than that of the shrimp reared in semi intensive culture method. Report shows that BFT enhances shrimp growth by maintaining good water quality, lowering FCR and maintaining a conducive culture environment (Ray 2014; Taw 2014). Biofloc is a medium rich in organic matter made of friendly bacteria, phytoplankton, protozoa, filamentous bacteria, nematodes, ciliates, flagellates and rotifers (Rivera *et al.* 2014). According to Burford *et al.* (2004) microorganisms found in the biofloc serve as natural food for white shrimp and thereby improves growth and survival rate. This observation is true in the case of pacific white shrimp, *Litopenaeus vannamei*, as this species has the ability to collect and use the suspended flocs in ponds as an additional feed. However, no study has hitherto been reported about the ability of *F. indicus* in consuming biofloc as additional feed. The high growth recorded in the treatment ponds of the present study may be due to the better pond environment as reported above.

In biofloc systems, the factors that control ammonia are algal uptake, bacterial assimilation and nitrification. The two-step

Table 2. Water quality parameters recorded in control and treatment pond

Parameter	Control		Treatment	
	Mean ± SD	Range	Mean ± SD	Range
Temperature (°C)	32.00 ± 1.2	30.4–33.8	32.30 ± 1.4	30.5–33.0
Dissolved oxygen (ppm)	4.20 ± 0.51	3.43–4.88	4.13 ± 0.47	3.62–4.90
pH	8.23 ± 0.10	8.13–8.37	8.22 ± 0.11	8.10–8.34
Salinity (ppt)	39.90 ± 0.45	39.00–40.60	41.20 ± 1.92	39.40–42.00
Alkalinity (ppm)	127.60 ± 7.3	122.70–141.3	134.00 ± 8.0	114.30–160.7
Nitrate (ppm)	0.03 ± 0.66	0.00–0.09	0.08 ± 0.04	0.00–0.17
Nitrite (ppm)	0.03 ± 0.01	0.01–0.04	0.52 ± 0.01	0.01–0.84
Orthophosphate (ppm)	0.21 ± 0.11	0.10–0.46	0.33 ± 0.12	0.12–0.56
Unionized ammonia (ppm)	0.05 ± 0.01	0.01–0.72	0.14 ± 0.07	0.01–0.39

Table 3. Total heterotrophic bacterial population in control and treatment ponds

Days	Ponds	TPC (CFU/mL)	TYV (CFU/mL)	TGV (CFU/mL)
30*	Control	10,000 ± 232	10,000 ± 432	12 ± 1
	Treatment	8500 ± 134	8500 ± 267	6 ± 2
60*	Control	12,333 ± 366	3833 ± 384	270 ± 21
	Treatment	10,833 ± 376	1333 ± 98	113 ± 13
90*	Control	63,000 ± 298	1093 ± 399	690 ± 28
	Treatment	47,333 ± 243	1066 ± 201	23 ± 9
120*	Control	69,000 ± 2301	976 ± 62	29 ± 5
	Treatment	6000 ± 123	316 ± 21	16 ± 2

$n = 9$. Analysis of variance showed significant difference between control and treatment in each sampling day.

CFU = colony forming unit; TGV = total green *Vibrio*; TPC = total plate count; TYV = total yellow *Vibrio*.

*$p < 0.01$.

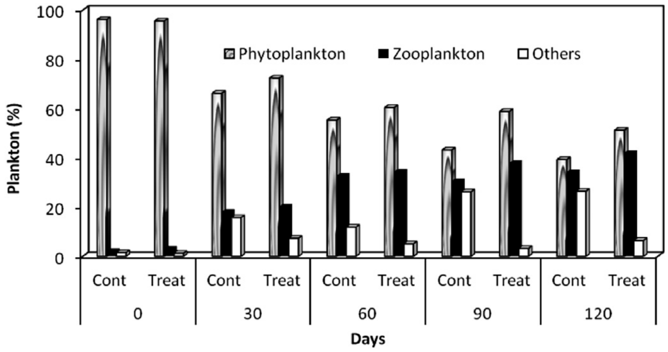

Figure 4. Plankton community in control and treatment ponds.

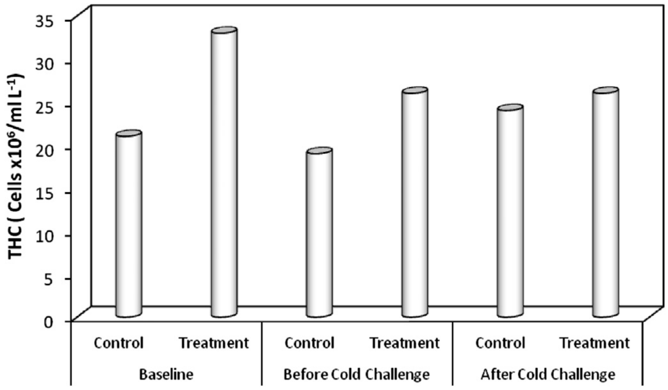

Figure 5. Total haemocyte count (THC) of shrimp in control and treatment pond.

oxidation of ammonia to nitrate is called nitrification. The bacterial process transforms a toxic form of nitrogen (ammonia) to one that is toxic only at high concentrations (nitrate). Over time, nitrate accumulates in low-exchange biofloc systems (Hargreaves 2013). This may be a reason for the accumulation of nitrate compounds in the treatment ponds in which zero water exchange was done.

Study shows that shrimp grow best and healthiest in aquaculture systems that have high levels of algae, bacteria and other natural biota (Kuhn *et al.* 2009). Natural production of some substances by bacteria in biofloc has been reported to inhibit growth of co-habiting pathogenic species such as *Vibrio harveyi* (Iyapparaj *et al.* 2013). Results of the present study show that yellow and green *Vibrio* colonies in biofloc ponds were found to be reduced in each sampling when compared to control ponds. This may be attributed to the inhibitory effect of substance in bioflocs as stated previously. The reduction of yellow *Vibrio* colonies found in biofloc water may be due to the antibiotic effect of biofloc as suggested by Bianchi (1979).

Recently, scientists have hypothesized possibilities of immunostimulatory features of the bioflocs leading to enhancement of immunity to provide broad-based resistance towards many infections (Crab *et al.* 2012). According to Wang *et al.* (2008), immunostimulants in shrimp culture ponds are group of live and synthetic compounds including bacteria and bacterial products, animal extracts, cytokines, lectins and plant extracts. Studies show that THC in haemolymph is considered as index of shrimp immunologic functions and a higher THC is responsible for high immune status (Krupesha *et al.* 2009). Results of the present study show that shrimp grown in biofloc ponds had high THC in blood compared to control ponds and the status was maintained even after the cold challenge test given at 20°C for 24 hours. Therefore, it can be suggested that shrimp grown in biofloc ponds have more immune capacity/response than those grown in control pond.

Crustaceans are able to change their colour or shading in response to environmental changes and can exhibit a great variety of pigment colours. The ability to change body colour to match environmental changes is brought about by pigment movements within the chromatophores in shrimp (Baloi *et al.* 2013). Decreases in light intensity will decrease the amount of reflected light and will probably cause dispersion of the dark pigments as the reduced light will make the background appear darker and it will make body colour more dark (Baloi *et al.* 2013). The bright colour noticed on the shrimp grown in biofloc ponds can be correlated to the dark pond environment due to less light penetration due to the suspended biofloc on water surface and algal cells in the pond. Le (2011) reported that salinity plays a significant role in flavour of shrimp meat which explains that shrimp grown in high saline water had better taste than those reared in low saline water. Results of the present study show that shrimp grown in biofloc ponds had better taste compared to control shrimp. The high saline environment in the biofloc ponds was due to the evaporation of water and zero water exchange in the pond. Therefore, it is suggested that the better taste of shrimp observed in biofloc pond was due to the influence of high saline condition as reported previously.

Results of the present study show that biofloc ponds had high sludge accumulation after harvest. This may be due to the deposition of dead organic matter and algae in the absence of water exchange in these ponds (Ebeling *et al.* 2006; Crab *et al.* 2012). If better management methods are implemented to control ammonia and sludge formation, BFT could certainly be an ideal method for farmers for the bio secure production of *F. indicus* in semi-arid lands of the Kingdom.

Table 4. Physical quality of shrimp (grade score: 3, good; 2, moderate; 1, bland)

Experiment	Appearance	Colour	Taste	Hard shell (%)	Loose shell (%)	Soft Shell (%)
Control	3 ± 0.21	2 ± 0.21	2 ± 0.15	78.5	8.5	13.0
Treatment	2 ± 0.31	3 ± 0.35	3 ± 0.12	89.1	5.5	5.4

Conflict of interest

There is no conflict of interest related to the research and the publication.

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