Research article

Periodic Fertilization Using Urea and Chicken Manure as Source of Natural Productivity in a Biofloc System during the Nursery of Pacific White Shrimp *Litopenaeus vannamei*

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Abstract The effect of periodic fertilization on natural productivity, growth and survival during the nursery of *Litopenaeus vannamei* under a zero water exchange set up was investigated. Nursery culture was carried out for 15 days in 2 m^3 indoor concrete tanks with stocking density of one post larvae (PL)·$liter^{-1}$ using PL 16 shrimp. Urea and chicken manure were initially applied a week before stocking and added weekly to maintain green water condition. There was no significant difference in the phytoplankton densities and microbial flocs in the two treatments. The results demonstrated that the weekly periodic application of urea in a zero water exchange set up was not viable while fertilization using chicken manure improved feed conversion rate as well as shrimp survival and production.

Keywords fertilizer, natural productivity, specific growth rate, post larvae

INTRODUCTION

World aquaculture has dramatically grown in the last 50 years. From a production of less than a million tonnes in the early 1950s, production in 2006 had risen to 51.7 million tonnes excluding aquatic plants (FAO, 2008). In terms of value, shrimp was by far the largest commodity representing 17% of the total value of international-traded fishery products in 2006 (FAO, 2009). From 2000 to 2005, its global market had expanded from less than \$1 billion to \$5.8 billion (FAO, 2008). Since then the increase in shrimp production has been brought about by so many factors, as it is the intensification of culture systems. Advancements in aquaculture engineering and biotechnology amongst others have triggered the transformation from extensive to intensive culture of shrimps. Introduction of new aquaculture species in certain regions is another major factor. An example is the introduction of the Pacific white shrimp *Litopenaeus vannamei* to Asia. In 1996, *L. vannamei* was introduced on a commercial scale in mainland China and Taiwan and subsequently spreaded to the Philippines and other neighbouring countries (FAO, 2004).

However, the intensification of shrimp aquaculture is coupled with pollution, environmental degradation, and occurrence of diseases caused by heavy effluent discharge and pathogen contamination of water supply. Thus, reducing waste outputs of shrimp aquaculture operations is essential to ensure a long-term sustainability. According to McIntosh et al., (2001), this could be achieved by improving feed and water management, application of water treatment to wastewater and adoption of zero or minimal water exchange. The latter is the soundest possible option because it does not only control effluent discharge but also reduces introduction of water borne pathogens by increasing biosecurity of the cultured organism (Gomez-Jimenez, 2005). The bioflocs technology is an important discovery in the zero water exchange or closed system of shrimp farming. Bioflocs technology (BFT) is a system wherein heterotrophic bacteria, algae, and other microorganisms are grown in flocs controlled conditions within the culture period (Jorand et al., 1995; Avnimelech, 2009). The microbial biomass or bioflocs utilize dissolved and particulate nitrogenous compounds in the water coming from the waste of the cultured organism; the assimilation of these compounds is stimulated by addition of carbon sources to the system (Avnimelech, 1999). The presence of bioflocs does not only improve water quality but also serves as additional food for the cultured organism. The BFT represents a sustainable way of producing food by the production of new biomass grown on the nutrient waste and is used as an alternative food source (Crab et al., 2007). Studies on zero water exchange during the nursery culture of *L. vannamei* showed several benefits. Baloi et al., (2010) reported higher survival of *L. vannamei* post larvae cultured using reclaimed water from a super intensive zero-water exchange pond in tanks without water exchange for seven days. Widanarni et al., (2010) demonstrated that the optimum carbon-nitrogen ratio 2:1 using the biofloc technology in *L. vannamei* nursery production obtained the best shrimp growth, yield and feed efficiency.

Another strategy to improve sustainability while increasing shrimp production is to promote natural productivity in the culture system. Natural food production in an aquatic system is enhanced by fertilization. Inorganic and organic fertilizers supply nutrients needed to increase primary productivity that supports the maintenance of other communities occupying higher trophic levels. Stahl (1979) reported that natural food alone consisting primarily of a mixture of organic detritus and soil, produced growth rates in freshwater prawn *Macrobrachium rosenbergii* post larvae better than commercial production ponds. Anderson et al., (1987) demonstrated that 53–77% of the growth of juvenile *L. vannamei* raised in earthen ponds was due to the assimilation of in situ natural pond biota. Porchas-Cornejo et al., (2011) reported that promotion of natural feed had a positive effect on weight gain, survival and final biomass of *L. vannamei*. Martinez-Cordova et al., (1998) found out that the growth and biomass of *L. vannamei* were higher in fertilized ponds despite lower feeding rate. *L. vannamei* grown extensively (without supplemental feeding) in fertilized ponds resulted to moderate to high survival rates (Jaspe et al., 2010). However, research on the use of different fertilizers in a zero water exchange set up during the nursery of *L. vannamei* is limited.

OBJECTIVE

This study determined the effects of periodic fertilization on natural productivity, growth and survival in the nursery of *L. vannamei* under a zero water exchange set up. Specifically, the following indices were measured: water quality – dissolved oxygen, pH, salinity; natural productivity – phytoplankton and zooplankton count and microbial floc; growth – specific growth rate in terms of weight and length, feed consumption rate; yield or production – survival.

MATERIALS AND METHODS

Experimental design and set up: The experiment utilized a complete randomized design with two treatments: inorganic fertilizer (urea) and organic fertilizer (chicken manure). Rearing of *L. vanammei* post larvae lasted for 15 days (from PL 16 to PL 30). It was conducted in the facilities of the Central Bangus Hatchery located in Pangangan Island, Calape, Bohol. The experiment used six (2 m^3) indoor concrete tanks assigned to the two treatments. The tanks were chlorinated and washed prior, which were provided with 7-10 cm mud substrate. Tea seed was added at 100 ppm to eradicate naturally occurring predators in the mud and lime was applied at 100 ppm subsequently. Chicken manure and urea were applied initially at 1250 $kg \cdot ha^{-1}$ and 100 $kg \cdot ha^{-1}$ a week before stocking and added weekly at a rate of 625 $kg \cdot ha^{-1}$ and 50 $kg \cdot ha^{-1}$, respectively to maintain green water condition. Tanks were filled with seawater with a salinity range of 30-35 ppt. Pond green water was inoculated in the tanks to provide initial algal population. Each tank was equipped with aeration line to provide sufficient dissolved oxygen level.

Stocking and feeding: *L. vannamei* post larvae (PL 14) were purchased from a private hatchery (Dobe Export International). The shrimps were acclimatized in a ten-tonner rectangular tank with same salinity and temperature of the hatchery water and were fed to satiation twice a day. Stocking in the experimental tanks was done early morning on the third day after obtaining the fry at a rate of 1 $individual \cdot liter^{-1}$. Initial weight and length of the shrimps were 17.85 mg and 1.49 cm. First feeding was done a day after stocking based on 2.5% body weight. Subsequent feeding was adjusted based on the actual body weight. Feeding ration was administered four times a day at 8 am, 10 am, 1 pm, and 4 pm.

Water quality and natural productivity monitoring: Phytoplankton and microbial-floc population in the rearing water were monitored weekly and physico-chemical parameters such as temperature, salinity, dissolved oxygen, and pH were checked daily. Temperature and dissolved oxygen was measured using portable DO meter, pH with portable pH meter, and salinity with a refractometer. Dominant phytoplankton, zooplanktons, and microbial flocs were counted weekly using a standard haemacytometer under a compound microscope.

Growth and survival monitoring: Weight of the shrimps was monitored weekly to assess growth and adjustment of the feed ration. The shrimps were harvested after 15 days and counted individually. One hundred (100) representative samples from each tank were weighed using 1.0 g precision electronic balance and length measured using Vernier caliper to the nearest 0.01 cm. To estimate the specific growth rate (SGR), food consumption rate (FCR), and survival rate (SR), the following formulas were used: SGR = [(ln final weight − ln initial weight) /days] ×100. Where: ln = natural logarithm of final and initial weight. FCR = weight of feeds consumed/weight gained and SR = (recovered stocks/total stocks) × 100.

Statistical analysis: To determine significant differences in the water quality parameters, microbial floc and phytoplankton counts, growth, survival, yield, and FCR between treatments, T-test for independent sample was used.

RESULTS AND DISCUSSION

Water Quality

Good water quality is essential in the success of shrimp nursery operation. Table 1 shows the minimum, maximum, and mean levels of these parameters all throughout the duration of the culture period. The mean daily values recorded during the experiment fall under the optimum range for culture of *L. vannamei*. Moreover, the results of all the water quality parameters showed no significant difference between the two treatments. The mean salinities of the two treatments were at 37.1 ppt and 36.7 ppt, respectively. Ponce-Palafox et al., (1997) found out that the optimum range for best survival and growth of *L. vannamei* was between 33-40 ppt. Dissolved oxygen is one of the most important water quality parameters, minimum DO standard for good growth of shrimps is at 3 ppm (Fast and Boyd, 1992). The mean dissolved oxygen concentrations of the two treatments were at 5.0 ppm and 5.1 ppm, respectively. The temperature readings were relatively stable throughout the culture period in all the experimental tanks, which fall under the optimum temperature for shrimps at 26°C to 33°C. Mean pH in the two treatments was at 7.6 and 7.5 with very minimal fluctuation until the end of the experiment. The recorded pH, fall under the optimum pH in aquaculture systems, at 7.5 to 8.5 values.

Table 1 Dissolve Oxygen, pH, temperature and salinity of water in Pacific white shrimp (*L. vannamei*) nursery culture with different fertilizers

Treatment/Parameters	Salinity (ppt)			Dissolved Oxygen			Temperature (^oC)			pH		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Inorganic Fertilizer (Urea)	33.7	40.0	37.1[a]	4.8	5.2	5.0[a]	26.7	29.0	27.6[a]	7.6	10.2	8.1[a]
Organic Fertilizer (Chicken Manure)	32.0	39.0	36.7[a]	4.5	5.7	5.1[a]	26.4	29.0	27.6[a]	7.5	10.2	7.6[a]

Means with different letters indicate significant differences between treatments (P<0.05).

Phytoplankton and Microbial Flocs

Phytoplankton play a significant role in stabilizing the whole pond ecosystem and in minimizing the fluctuations of water quality. A suitable phytoplankton population enriches the system with oxygen through photosynthesis during day light hours and lowers the levels of CO_2, NH_3, NO_2, and H_2S. During the first week of the experiment there was no significant difference in the phytoplankton densities between the two treatments (Table 2). Common phytoplankton observed were diatoms and *Chaetoceros* species. A day after the 3rd fertilization, the algae bloomed, manifested by dark green coloration of the water in the tanks applied with urea. Mean phytoplankton count in these tanks increased from 372,500 $\pm$ 2,500 cells·ml^{-1} to 513,333 $\pm$ 11,273 cells·ml^{-1} and was significantly higher compared to the tanks fertilized with chicken manure. Generally, the phytoplankton densities all throughout the experiment were relatively low compared to the healthy phytoplankton abundance reported by Cordova et al. (1998) in ponds applied with organic and inorganic fertilizer at 530,000-980,000 cells·m^{-1}. The denseness could be attributed to the use filtered seawater. Pond green water was only added in the tanks to provide initial algal population.

Phytoplankton aggregates with microorganisms in the aquaculture system to form microbial flocs. Bio-flocs or the microbial flocs are a mixture of microorganisms (bacteria, phytoplankton, and zooplankton), algae, and other particles (Jorand et al., 1995). Typical flocs are irregular by shape, have a broad distribution of particle sizes, which are fine, easily compressible, highly porous, and permeable to fluids (Lee Chen, 2004). Microbial floc or flocculated particles were observed to be increasing as shown in Table 2. The build-up rates of the floc on the 2nd week followed the standard community succession principle wherein from a clear water condition, algal bloom follows, and bacterial communities are established when the water turns to brown. Build up of flocculated particles in the two week time was relatively low.

The microbial flocs observed in the experiment were visibly large and distinct under the microscope. Optimum water quality conditions were important in maintaining healthy population of microbial flocs and these were met during the experiment. High dissolved oxygen in the system was not only essential for the shrimps but also for the metabolic activity of the cells and structure of the microbial flocs. A trend towards larger and more compact flocs at higher dissolved oxygen concentrations was noted by Wilen and Balmer (1999). Temperature was also important in microbial floc morphology. Krishna and Van Loosdrecht (1999) observed that higher temperatures (30-35°C) resulted in bulking of the sludge due to the excessive production of extracellular polysaccharide, thus intermediate water temperature of 20-25°C would be best to obtain stable microbial flocs. Similarly, pH has an effect on microbial floc; according to Mikkelsen et al., (1996) changes in pH determine the stability of bio-flocs present in the system.

Table 2 Mean phytoplankton and microbial floc count in the water of Pacific white shrimp (*L.vannamei*) nursery culture with different fertilizers

Treatment	Phytoplanktons (cell density ml^{-1})		Microbial Flocs (cell density ml^{-1})	
	Initial	Day 7	Initial	Day 7
Inorganic Fertilizer (Urea)	372,500 ± 2,500 [a]	513,333± 11,273[a]	723 ± 13[a]	797± 17[a]
Organic Fertilizer (Chicken Manure)	365,833 ± 3,819 [a]	423,333 ± 3819 [b]	732 ± 38[a]	836± 15[a]

Means with different letters indicate significant differences between treatments (P<0.05).

Growth and Survival

A day after the 3rd fertilization, the algae bloomed in the tanks applied with urea which resulted to mass mortality of all stocks. However, the fatality could not be attributed to the increase in phytoplankton density because it was relatively lower compared to a healthy phytoplankton bloom (Cordova et al., 1998). Furthermore, dissolved oxygen remained at its optimum level and apparently was not the cause of mortality. One possible reasons of the fatality could be the increase of NH_3 in the system triggered by the addition of urea. In the experiment conducted by Das et al., (2005) application of organic and inorganic fertilizer increased inorganic nutrients including ammonia. Application of inorganic fertilizer gave peak values of nitrogen species such as ammonia, nitrite and nitrate earlier during first to second week. Although natural food production was enhanced by applying organic and inorganic fertilizers, the results showed that weekly application of urea at 50 $kg \cdot ha^{-1}$ significantly affected survival, causing mass mortality of stocks. On the other hand, survival of shrimps in tanks fertilized with chicken manure was 92.68%; with specific growth rate ($\% \cdot day^{-1}$) of 14.05 in terms of weight and 18.17 in terms of length and total yield of 128.37 $g \cdot m^{-1}$. Lara-Anguiano et al., (2013) found out that *L. vannamei* culture in tanks with zero water exchange using organic fertilizer (molasses) had higher survival and production rates and reduced ammonia concentrations toward the end of the experiment while the use of inorganic fertilizer caused increased nitrogen and phosphorus.

CONCLUSION

Periodic fertilization using chicken manure at a rate of 625 $kg \cdot ha^{-1}$ improved feed conversion rate as well as shrimp survival and production within this experiment. Moreover, the results indicate that weekly periodic application of urea at a rate of 50 $kg \cdot ha^{-1}$ without any water replacement in tanks during the nursery of *L. vannamei* is not viable.

RECOMMENDATIONS

Further studies should design experiments that include the measurement of other water quality parameters such as NH_3, nitrites, nitrates, total suspended solids, and microbial evaluation such as total plate count and *Vibrio* count. It is also very interesting to determine the density of the zooplankton in the water vis-à-vis the feeding incidence.

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