

Original Research Article

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Effect of De-Oiled Oil Palm Kernel Meal Based Biofloc System on Growth and Digestive Enzyme Activity of Pacific White Shrimp, *Litopenaeus Vannamei*

Karthireddy Syamala^{1*}, W.S. Lakra², N.K. Chadha³, N.P. Sahu⁴ and K. Pani Prasad⁵

¹ICAR-Central Institute of Fisheries Education, Kakinada centre, Kakinada, Andhra Pradesh-533001, India

²OSD Blue Revolution, ³Division of Aquaculture, ICAR-Central Institute of Fisheries Education, PanchMarg, Yari Road, Andheri (w), Mumbai, Maharashtra-400 061, India

⁴Fish Nutrition, Biochemistry and Physiology Division, ⁵Aquatic Environment and Health Management Division, ICAR-Central Institute of Fisheries Education, PanchMarg, Yari Road, Andheri (w), Mumbai, Maharashtra-400 061, India

*Corresponding author

ABSTRACT

Keywords

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A 40-day experiment was conducted to study the effect of de-oiled oil palm kernel meal based biofloc system on growth performance and digestive enzyme activity of *Litopenaeus vannamei*. Juveniles of *L. vannamei* with an average weight of (0.82±0.02 g) were stocked in tanks with a water volume of 150 L at a stocking density of 300 PL m⁻³. Highest weight gain percentage (519.05±9.52), protein efficiency ratio (1.78±0.02) and survival rate (88.67±0.88) were observed in oil palm kernel meal based biofloc treatment. Lowest FCR value (1.61±0.02) has been recorded in biofloc treatment. Although the digestive enzymes had not shown any significant difference, a slight increase in the protease and amylase activities have been observed in biofloc treatment. So the present study suggests that non-conventional carbon source – de-oiled oil palm kernel meal can be utilized for the development of biofloc.

Introduction

Limitation of aquaculture expansion area has given rise to the concept of aquaculture intensification. The major setback of the aquaculture intensification is the accumulation of nitrogenous wastes and environmental pollution which will result in disease condition or death of the farmed animals (Yang and Qiu, 2006; De Schryver and Verstraete, 2009). The concept of

aquaculture intensification has raised many environmental and social concerns. Aquaculture development needs the support of aquaculture intensification coupled with resource usage, environmental, social and economic sustainability (Naylor, 2000; Avnimelech, 2009). Biofloc technology has the capability to meet all the prerequisites as mentioned earlier.

The Pacific white shrimp *Litopenaeus vannamei* is the most widely cultivated species in the world. Recently, *L. vannamei* farming is facing some issues and problems in the culture which needs a serious contemplation. Diseases such as black gill disease, White Spot Syndrome Virus (WSSV), Infectious Hypodermal Hematopoietic Necrosis Virus (IHHNV), Monodon Baculo Virus (MBV), White muscle disease, White gut disease, Muscle cramp syndrome, Runt Deformity Syndrome (RDS), Early Mortality Syndrome (EMS) and Enterocytozoon hepatopenaei (EHP) associated with severe growth retardation have been reported in *L. vannamei* (Lightner *et al.*, 2012; Gunalan *et al.*, 2014; Newman, 2015). In addition to the disease problems, other issues like limited quarantine facilities, production cost towards feed, quality seed availability, feed quality and availability and environmental impact and management are also affecting the *L. vannamei* culture in India (Babu *et al.*, 2013).

Extensive studies on the biofloc based nursery and grown out phases of *L. vannamei* in the recent years had shown improvement in the growth performance, feed utilization and disease resistance (Burford *et al.*, 2004; Hari *et al.*, 2004; Hari *et al.*, 2006; Wasielesky *et al.*, 2006; Samocha *et al.*, 2007; Ju *et al.*, 2008a; Kuhn *et al.*, 2008; Xu *et al.*, 2012, 2013). The carbon sources most widely used for biofloc are molasses, cassava, wheat flour, sorghum, corn flour, starch, glucose, cellulose and dextrose (Emerenciano *et al.*, 2013). However, all these carbon sources already have potential value and application in other aspects which question their usage in the biofloc production (Crab *et al.*, 2012). Given these, new research on biofloc production using the low-cost non-conventional carbon sources from agro-industrial residues is very essential. Utilization of non-conventional carbon sources for biofloc production can

convert these agro-industrial wastes into the nutritious feed (Crab *et al.*, 2012; Caipang *et al.*, 2015; Ahmad *et al.*, 2017).

The present study evaluated the use of non-conventional carbon source, de-oiled oil palm kernel meal (*Elaeis guineensis*), which is a low-cost palm oil industry waste for biofloc production. The effects of de-oiled oil palm kernel meal on water quality, growth and digestive enzyme activity of *L. vannamei* were studied to assess the viability of this carbon source in biofloc systems.

Materials and Methods

Experimental design and rearing

The present study was carried out at the Brackish water Fish Farm of Central Institute of Fisheries Education, Kakinada Centre, Kakinada, East Godavari District, Andhra Pradesh, India. An experiment of 40 days with one control (clear water system) and one treatment (oil palm kernel meal based biofloc) with three replicates each following completely randomized design (CRD) was conducted. *L. vannamei* juveniles with an average weight of $(0.82 \pm 0.02\text{g})$ were stocked in fibre reinforced plastics (FRP) tanks with a water volume of 150 L at a stocking density of 300 PL m^{-3} .

The shrimps were fed at 5% of body weight thrice a day (07.00 hrs 12.00 hrs and 17.00 hrs) with a commercial pellet feed (35 % crude protein; 5 % lipid; 25 % carbohydrate). The feed was adjusted according to the changes in the biomass during the experimental period. The control tanks were fed with only commercial feed whereas the biofloc treatment tanks were supplied with both feed and carbon source. No water exchange was carried out in biofloc treatment tanks.

Preparation of biofloc and carbon source addition

The carbon source used in the present study, Oil palm kernel meal was obtained from Ruchi soya industries limited, palm oil division at Peddapuram, East Godavari District, Andhra Pradesh, India. Avnimelech (1999) methodology with 20 g L⁻¹ pond soil, 10 mg L⁻¹ ammonium sulphate, and 400 mg L⁻¹ carbon source was used for the preparation of biofloc inoculum. The suspension was well aerated for 24 hrs and later mixed into experimental tanks. Oil palm kernel meal was added regularly to maintain a C: N ratio of 15:1 following De Schryver *et al.*, (2008). The pre-weighed oil palm kernel meal was mixed with water and uniformly distributed in the biofloc treatment tanks.

Water quality

Water quality parameters such as salinity, temperature, dissolved oxygen, pH, total alkalinity, total ammonia nitrogen (TAN), nitrite and nitrate were observed and analyzed as per standard procedures (APHA, 1998). Water temperature, pH and dissolved oxygen were determined daily. Alkalinity, TAN, nitrite and nitrate were estimated weekly twice. Biofloc volume was determined weekly using Imhoff cones after 30 minutes sedimentation (Avnimelech and Kochba, 2009).

Proximate analysis

The crude protein, ash, crude lipid, fibre and carbohydrate of the carbon source and biofloc were analyzed using standard methods (AOAC, 1995). Biofloc samples were collected water through a 10 µm mesh nylon bag (Xu *et al.*, 2012) and were dried in an oven at 105 °C until constant weight was achieved. Protein, lipid and ash contents were estimated by kjeldahl, soxhlet and muffle

furnace methods, respectively. Nitrogen content was evaluated by the Micro-Kjeldahl method (Pearson, 1976) and crude protein level was calculated by multiplying the percentage nitrogen with 6.25. Lipid was determined by acid hydrolysis (Nielsen, 2010). Carbohydrate content was determined by the difference between 100% and the sum of values of moisture, protein, fibre, lipid and ash. The percentages of the crude protein, ash, crude lipid, fibre and carbohydrate were calculated on dry weight basis of the carbon source and biofloc.

Zootechnical performance

The growth performance of shrimps, length and weight of the shrimps (n=30) were recorded at a regular interval of 10 days. The parameters evaluated included, Specific Growth Rate (SGR, %/day⁻¹) = [(ln Final weight – ln Initial weight) / Number of days] X 100; Feed Conversion Rate (FCR) = Feed given (dry weight) / Body weight gain (wet weight); Feed Efficiency Ratio (FER) = Body weight gain (wet weight) / Feed given (dry weight); Protein Efficiency Ratio (PER) = Body weight gain (wet weight) / Crude Protein in feed; Survival (%) = (Total number of animal harvested / Total number stocked) x 100.

Digestive enzyme activity

At the experiment end, hepatopancreas of shrimps (n=5) were collected from each treatment tank for enzymatic analysis.

A 20% tissue homogenate of hepatopancreas was prepared with 0.25 M chilled sucrose solution. The tissue solution was homogenized and centrifuged at 8000 rpm for 10 minutes for removal of tissue debris. The supernatant was collected and stored in -20°C until further analysis.

Amylase activity of samples was determined using the DNS method by measuring the absorbance at 540nm (Bernfeld, 1955). One unit of amylase is defined as micromoles of maltose produced per min at 37 °C. The Protease activity of samples was estimated by casein digestion method, and the final absorbance was measured at 280 nm (Liu *et al.*, 1991). The protease activity was determined from the tyrosine standard curve, and one unit of protease activity was defined as micromoles of tyrosine released per min per mg protein at 37 °C.

Statistical analysis

The statistical analysis of growth, digestive and proximate parameters was analyzed by one-way analysis of variance (ANOVA) using SPSS (version - 22.0). Duncan's multiple range tests were used for post hoc comparison of mean ($p < 0.05$) between different groups. All the data presented in the text, figures and tables expressed are mean \pm standard error and statistical significance for the test was set at $p < 0.05$.

Results and Discussion

Water quality

Water quality parameters observed in the present study are given in table 1. The water temperature was recorded between 26-28°C and had not shown any significant difference ($p > 0.05$) between the treatments. Salinity was observed at 7ppt and showed no significant difference ($p > 0.05$). Dissolved oxygen had not shown any significant difference ($p > 0.05$) between treatments and mean DO value was between 5.50-5.57 mgL⁻¹. pH value varied significantly ($p < 0.05$) among the treatments and lowest pH was recorded in biofloc treatment. Alkalinity values had shown significant difference among treatments and the mean ranged from 153.10-161.90 mg

CaCO₃L⁻¹. A significant difference ($p > 0.05$) was not observed in the TAN values among the treatments, but the highest TAN value of 1.5 mgL⁻¹ was recorded in biofloc treatment. A significant difference ($p < 0.05$) was recorded in nitrite and nitrate values. The highest nitrite and nitrate values of 2 mg L⁻¹ and 40 mg L⁻¹ were recorded in biofloc treatment.

Proximate analysis

The proximate analysis of de-oiled oil palm kernel meal and biofloc are given in table 2. The de-oiled oil palm kernel meal contained 15.58% crude protein, 6.8% crude lipid, 3.53% ash, 30.29% carbohydrate and 35.83% fibre. Biofloc comprised of 22.96% crude protein, 3.8% crude lipid, 4.7% ash, 26% carbohydrate and 14% fibre.

Zootechnical performance

The zootechnical performance of *L. vannamei* recorded at the end of 40 days is given in table 3.

The initial individual weight has not shown any significant difference ($p > 0.05$) among the treatments. The final individual weight, specific growth rate, weight gain, feed conversion efficiency and survival rate of the control was significantly lower than the biofloc treatment. The PER and FCR had shown a significant difference ($p < 0.05$) among the treatments and the highest value of PER (1.78 \pm 0.02) and lowest FCR (1.61 \pm 0.02) were recorded in biofloc treatment.

Digestive enzyme activity

Protease and amylase activities of the treatments are given in figure 1. Although no significant difference ($p > 0.05$) was observed, slightly higher protease and amylase activities were found in biofloc treatment.

Water quality

All the water quality parameters observed in the present study were in the optimum range for *L. vannamei* culture (Van Wyk and Scarpa 1999; Cohen *et al.*, 2005; Mishra *et al.*, 2008; Fast and Lester, 2013). pH value has shown significant difference among the treatments. The reduction of pH value in the biofloc treatments may be attributed to the respiration of the microbes in the biofloc and accumulation of carbon dioxide concentration (Wasiolesky *et al.*, 2006). Significantly lower alkalinity values were observed in biofloc treatments, which may be due to the oxidation of ammonia into nitrate and respiration of microbes in the heterotrophic system (Ebeling *et al.*, 2006). No significant difference was observed in the TAN values among the treatments. Lesser TAN values in the present study indicate the active nitrification process and the presence of heterotrophic bacteria (Vinatea *et al.*, 2010). Significantly highest nitrate value of 40 mg L⁻¹ was recorded in biofloc treatment. Reduction in TAN, nitrite, alkalinity and accumulation of nitrate in the study indicates the presence of nitrifiers (Cohen *et al.*, 2005; Ebeling *et al.*, 2006). The

results of water quality indicate the efficiency of the de-oiled oil palm kernel meal based biofloc system.

Proximate analysis

Alcohols, sugars, starches and fibres are the suggested sources of carbon for biofloc production (Martinez-cordova *et al.*, 2015). Carbon source used in the present study has a considerable amount of carbohydrate as well as fibre which together contribute about 66%. The added advantage of these complex carbon sources (starch and fibre) is that it acts as a substrate for the aggregation of bacteria and helps in maintaining the organic carbon release for longer periods (Martinez-cordova *et al.*, 2015). De-oiled oil palm kernel meal has a crude protein level of 15.5% which can act as an additional protein source for the cultured animal (if ingested directly or through biofloc). The nutritional composition of biofloc was in range as already reported by many researchers in fish as well as crustacean based biofloc technology (Ekasari *et al.*, 2010; Megahed, 2010).

Fig.1 Specific activities of protease (unit's min⁻¹ mg⁻¹ protein) and amylase (unit's min⁻¹ mg⁻¹ protein) in the hepatopancreas of *L. vannamei* juveniles at the end of 40 days experiment

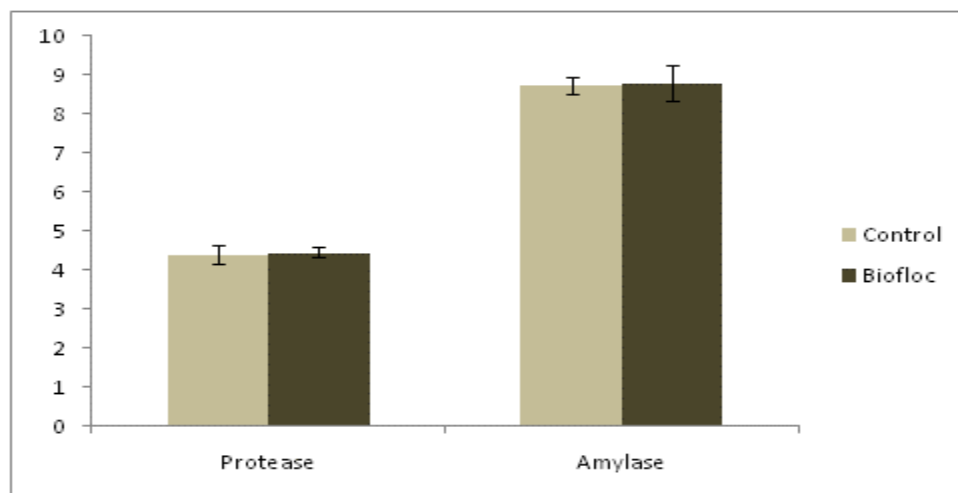


Table.1 Water quality parameters (mean ± S.E and range) observed in the present study

Parameters Treatments	DO (mgL ⁻¹)	pH	Alkalinity (mg CaCO ₃ L ⁻¹)	TAN (mgL ⁻¹)	NO ₂ ⁻ (mgL ⁻¹)	NO ₃ ⁻ (mgL ⁻¹)	Temperature (°C)	Salinity (ppt)
Control	5.57±0.10 (4.7, 6.5)	7.99±0.04 ^b (7.4, 8.2)	161.90±2.14 ^b (140, 180)	0.27±0.04 (0, 0.5)	0.19±0.03 ^a (0.1, 0.5)	1.37±0.08 ^a (1, 2)	26.57±0.13 (26,28)	7
Biofloc	5.50±0.13 (4.5, 6.5)	7.70±0.10 ^a (6.7, 8.2)	153.10±1.40 ^a (140, 160)	0.44±0.09 (0, 1.5)	0.85±0.11 ^b (0.3, 2)	25.00±1.65 ^b (10, 40)	26.52±0.13 (26,28)	7

Table.2 Proximate composition of De-oiled Oil palm kernel meal and Biofloc expressed as % dry matter

	De-oiled Oil palm kernel meal	Biofloc
Crude protein	15.58 ± 0.36	22.96 ± 0.30
Crude lipid	6.8 ± 0.19	3.8 ± 0.11
Ash	3.53 ± 0.24	4.7 ± 0.19
Carbohydrate	30.29 ± 0.35	26 ± 0.27
Fibre	35.83 ± 0.32	14 ± 0.41

Table.3 Growth performance and feed utilization of *L. vannamei* juveniles at the End of 40 days experiment

	Treatments	
	Control	Biofloc
Initial individual weight (g)	0.83±0.02 ^a	0.81±0.02 ^a
Final individual weight (g)	3.55±0.08 ^a	4.99±0.08 ^b
Weight gain (%)	329.20±7.09 ^a	519.05±9.52 ^b
SGR (%/day)	3.64±0.04 ^a	4.56±0.04 ^b
FCR	1.86±0.01 ^b	1.61±0.02 ^a
FCE	0.54±0.004 ^a	0.62±0.006 ^b
PER	1.54±0.01 ^a	1.78±0.02 ^b
Survival (%)	84.67±1.45 ^a	88.67±0.88 ^b

Zootechnical performance

In the present study, utilization of non-conventional carbon source de-oiled oil palm kernel meal had shown a significant effect on the growth performance and feed utilization of *L. vannamei*. The increase in growth performance and feed utilization of the shrimps reared in biofloc were reported by several researchers (Kuhn *et al.*, 2010; Megahed., 2010; Emerenciano *et al.*, 2011; Krummenauer *et al.*, 2011; Ray *et al.*, 2011; Emerenciano *et al.*, 2012; Xu and Pan, 2012; Kim *et al.*, 2014; Ray and Lotz, 2017). In addition to this, biofloc acts as a supplemental feed source which results in the better zootechnical performance (Burford *et al.*, 2004; Kuhn *et al.*, 2008; Megahed, 2010). The nutritional requirement of *L. vannamei* is 30-36% crude protein, 6-8% lipid, 15-20% carbohydrate (Zhu *et al.*, 2010; Shahkar *et al.*, 2014; Wang *et al.*, 2014; Li *et al.*, 2015). Biofloc is known to provide additional protein, lipid, vitamin and mineral for shrimp growth (Izquierdo *et al.*, 2006; Wasielesky *et al.*, 2006; Ju *et al.*, 2008b).

According to Lin *et al.*, (2007), the exogenous enzymes in the diets increase the efficiency of the digestion process. The microbes associated with biofloc are assumed to produce exogenous enzymes which help in the breakdown of proteins, carbohydrates and other nutrients into smaller units which improved the feed digestibility and absorption (Xu and Pan, 2012). The microbes in the biofloc once ingested by shrimp acts as an extracellular enzyme source and improve the digestive function. So as a supplemental feed source and external enzyme source, biofloc might have assisted in enhancing the growth performance and feed utilization in the present study. Studies reported low FCR values in the biofloc treatments which may be due to the additional contribution of the biofloc to the shrimp nutrition (Wasielesky *et*

al., 2006). Present study recorded a survival rate of 88.67 ± 0.88 in the biofloc treatment which is significantly higher than control as reported in an earlier study (Mishra *et al.*, 2008).

Digestive enzyme activity

The main site of digestion and absorption in shrimps are intestine and stomach with the help of digestive enzymes secreted by digestive gland (Dall *et al.*, 1990). The digestive enzyme activity in hepatopancreas has not shown any significant difference. However, slight increases in protease and amylase activities were observed in biofloc treatment. A similar result was reported by Xu and Pan, 2012 in *L. vannamei*, where a slight increase in the amylase and protease activities of the biofloc treatments without any significant difference was observed. Biofloc is assumed to be cellular nutrition source due to the presence of various bioactive compounds and unknown factors (Ju *et al.*, 2008b). Shrimps grown in biofloc are speculated to change digestive enzyme activities in response to the changes in the diet conditions (Le Moullac *et al.*, 1996; Xu and Pan, 2012). The biofloc is presumed to enhance the production or activity of the digestive enzymes which in turn contributed to the better growth and feed utilization (Moss *et al.*, 2001; Xu *et al.*, 2012, 2013).

The present study explored the potential of non-conventional carbon source, de-oiled oil palm kernel meal in the biofloc development. As the research is progressing in the biofloc systems in aquaculture, the need and search for the cheaper carbon sources is increasing. Utilization of carbon sources which already have an application in others fields will not be a befitting option for aquaculture biofloc systems. Using the agro-industrial by-products/wastes for the production of aquaculture biofloc systems can help in the

recycling of these materials into useful, nutritious feed for the aquatic organisms. So the present study suggests that oil palm industry waste and non-conventional carbon source (de-oiled oil palm kernel meal) can be used as a potential alternative cheaper carbon source for commercial biofloc production systems.

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