



Immuno-physiological response of *Litopenaeus vannamei* in oil palm kernel meal based biofloc systems

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ABSTRACT

A 45-day experiment was conducted to assess the immuno-physiological response of *Litopenaeus vannamei* in oil palm kernel meal based biofloc systems. *L. vannamei* of average weight (0.82 ± 0.02 g) were stocked in tanks at a stocking density of 300 PL/m³. Biofloc-based treatment obtained significantly better growth performance at the end of the experimental period. The stress parameters like SOD, CAT, LDH and GST were considerably lower in biofloc-based treatment as compared to the control. The oil palm based biofloc system showed lower AST and ALT values than the clear water control. The shrimp from biofloc treatment possessed significantly higher immune status as compared to control regarding the prophenol oxidase (proPO) activity. Biofloc treatment showed higher relative survival percentages than control. It indicates that oil palm kernel meal can be used as a potential carbon source for biofloc based shrimp culture.

Key words: CAT, LDH, *L. vannamei*, Oil palm kernel meal, proPO, SOD

Global population is expected to reach 9.7 billion by the year 2050 (UNDESA 2015) and providing the nutritional security for the growing population is the main challenge of the present day. This increase in population and rise in demands has put tremendous pressure on the food sectors of the world. Fish and fisheries sector provide the cheap protein source and livelihood for millions of people since ages.

Indian fisheries and aquaculture is a significant contributor to the nutritional security and agricultural exports. Nearly 14 million people are engaged in this sector in different activities. India contributes about 6.3% of the global fish production. India is the second largest producer of fish and also the second largest producer of freshwater fish in the world (DAHDF 2017).

Litopenaeus vannamei is the most preferred species for aquaculture because of its faster growth rate, slightly columnar habitat, availability of specific pathogen free (SPF) domesticated strains and culture feasibility in wide salinity range. *L. vannamei* production in India reached 406,018 MMT from 59,116 ha during the year 2015–16 (MPEDA 2016). However, *L. vannamei* farming in India is facing some serious issues like disease outbreaks (Lightner *et al.* 2012, Gunalan *et al.* 2014, Newman 2015). In addition to these, *L. vannamei* farming in India is also affected by

limited quarantine facilities, high feed cost, lack of quality seed and availability, and environmental impact and management (Babu *et al.* 2013).

The use of biofloc technology (BFT) in aquaculture can provide a solution to the problems faced by the shrimp industry. Microbial floc not only helps to improve the environmental control over production by reducing the nitrogen and ammonia from the culture water but also act as nutrient trappers, which can be useful in the feed management thereby reducing the feed cost. They are also helpful in enhancing the biosecurity and health. The BFT is achievable by using different types of organic carbon. Utilization of low-value carbohydrates or agro-industrial by-products for the production of biofloc can further reduce the cost of production in aquaculture by converting these agro-industrial wastes into the nutritious feed (Caipang *et al.* 2015, Ahmad *et al.* 2017). However, the effect of these unconventional carbon sources on the physiological response of the cultured organism is very much needed. Hence, the present study was carried out to examine these effects.

MATERIALS AND METHODS

Experimental site and rearing: The experiment was conducted at the Brackishwater Fish Farm of ICAR-CIFE Kakinada Centre located at 16°56' N latitude and 82°15' E longitude. Kakinada region consists of a tropical savanna (wet and dry) climate with a temperature range of 18–42°C and average rainfall of 110–115 cm. The specific pathogen

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free (SPF), *Litopenaeus vannamei* post larvae (PL15) were procured from Bay Fry Hatchery, Kakinada, Andhra Pradesh, India. Before the start of the experiment, the PL were acclimatized and reared in cement tank of 30,000 L capacity for 10 days with sufficient aeration and supplementary feeding.

Biofloc production and inoculation: Floc inoculums were developed as per Avnimelech (1999) using 20 g/l pond soil, 10 mg/l ammonium sulphate and 400 mg/l carbon source with aeration for 24 h. The FRP tanks of 300 L capacity were water of 10 ppt salinity before the addition of biofloc inoculum and each was provided with continuous vigorous aeration. After 24 h, the inoculums were distributed equally into the experimental tanks.

Experimental design and stocking: Completely randomized design (CRD) was followed in all the experiments. The experiment was designed with one clear water control and oil palm kernel meal based biofloc treatment with three replicates for each treatment. The PL25 *L. vannamei* of average weight 0.82 ± 0.02 g was stocked with 300 PL m^{-3} in 15-day-old biofloc treatments (Imhoff floc volume 5 ml/l) respectively. The shrimps were given feed at 5% of body weight 3 times daily at 7 h, 12 h and 17 h with a commercial pelleted feed (35% crude protein; 5% lipid; 27% carbohydrate). The feed ration was adjusted according to biomass changes 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. Carbon sources were calculated and added daily based on the quantity of feed added and the protein content in the feed used as per De Schryer *et al.* (2008).

Water quality: Water quality parameters were observed and analyzed as per standard procedures (APHA 1998). Water temperature, pH and dissolved oxygen were recorded daily whereas, alkalinity, TAN, nitrite and nitrate were estimated twice a week. Weekly estimation of the biofloc volume was done using Imhoff cones after 30 min sedimentation (Avnimelech and Kochba 2009).

Enzyme analysis: After the completion of the experiment, hepatopancreas of shrimps (5) were collected from each treatment tank for enzymatic analysis. A 20% tissue homogenate of hepatopancreas using 0.25 M chilled sucrose solution was prepared. Tissue debris was removed by centrifugation at 8,000 rpm for 10 min. The collected supernatant was stored at $-20^{\circ}C$ until further analysis.

The estimates were made of superoxide dismutase (Misra and Fridovich 1972) by measuring the optical density changes at 480 nm for 3 min in a UV-VIS spectrophotometer (Biochrom, UK); catalase activity (CAT) (Takahara *et al.* 1960); lactate dehydrogenase (LDH) activity (Wroblewski and LaDue 1955); glutathione-S-transferase (GST) activity (Habig *et al.* 1974); aspartate aminotransferase activity (AST) activity (Wootton 1964); ALT activity (Wootton 1964); and phenoloxidase activity (Söderhäll and Smith, 1983).

One-way analysis of variance (ANOVA) was done by

using SPSS (v-22.0). Post hoc comparison of means ($P < 0.05$) between different groups was analyzed by using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Water quality parameters were within the range reported for shrimp culture (Van Wyk and Scarpa 1999). All the stress parameters recorded lesser values in biofloc treatment (Table 1). Superoxide dismutase (SOD) is one of the most important antioxidant enzymes, which is preventing the formation of lipid peroxidation by catalyzing the disproportionation of the lipid peroxidation initiator (Tao *et al.* 2013). Catalase (CAT) is also an antioxidant enzyme in shrimp that acts as a critical role to protect organisms against oxidative stress by decomposing hydrogen peroxide (Tavares-Sanchez *et al.* 2004). GSTs are key components of various detoxification, antioxidant and stress tolerance pathways. Since they protect against injury induced by environmental chemicals, they have also been used as biomarkers to estimate chemical exposure in aquatic organisms (Van der Oost *et al.* 2003, Amado *et al.* 2006). LDH is the glycolysis pathway and their level increases during muscle glycolysis (Milligan and Girard 1993) under oxygen shortage in tissue (Murray *et al.* 2010). Different species and different experiment conditions respond differently and have different antioxidant abilities and different regulation mechanism. Castex *et al.* (2010) reported the decreased SOD and CAT activity in Pacific blue shrimp, *Litopenaeus stylirostris* fed a probiotic diet containing live *Pediococcus acidilactici*, which may cause lower free radical generation. According to Kim *et al.* (2015), SOD and CAT were notably decreased in a biofloc system using effective microorganisms, which suggest that the system may influence the reduction of ROS. The GST levels can be significantly increased by exposure to different environmental pollutants (chemicals or water quality parameters), suggesting that this increase is part of an adaptive response to stress and their action avoids damage to the biological systems. LDH activity increases in different types of stress like starvation (Vijayan *et al.* 1997), high density transportation (Chatterjee *et al.* 2006) to increase the production of lactate for gluconeogenesis (Moon and Foster 1995). Lower LDH activity in hepatopancreas

Table 1. Stress, metabolic and immune response of *L. vannamei* at the end of 45-day experiment

Treatment	Clear water control	Biofloc treatment
SOD (U/mg protein)	2.25±0.04 ^b	2.14±0.01 ^a
Catalase (U/mg protein/min)	4.94±0.18 ^b	3.51±0.19 ^a
GST (mU/mg protein/min)	2.80±0.15 ^b	1.77±0.11 ^a
LDH (U/mg protein/min)	0.027±0.003 ^b	0.019±0.001 ^a
AST (U/mg protein/min)	10.98±0.14 ^b	9.51±0.35 ^a
ALT (U/mg protein/min)	8.47±0.05 ^b	7.18±0.26 ^a
Prophenol oxidase (OD at 490 nm)	0.046±0.006 ^a	0.105±0.011 ^b

reflects a better physiological condition of the shrimp (Anand *et al.* 2017). Lowest values of the metabolic parameters were recorded in biofloc treatment (Table 1). The AST and ALT were the most important aminotransferases, which take part in amino acid metabolism. Their level in hepatopancreas is a direct indicator of shrimp health (Pan *et al.* 2003). Many of the earlier studies (Nakano *et al.* 1999) reported that dietary supplements in aqua feed reduced the level of AST and ALT in the liver and increased defensive potential against different kinds of stresses generated in the rearing system.

Highest prophenoloxidase (proPO) activity was recorded in biofloc treatment (Table 1), which can be due to the assimilation of microbial proteins assimilated by shrimp which are believed to attribute beneficial effects on well-being and shrimp immune status (Avnimelech 2015). Because of their lack of acquired immunity, marine invertebrates' defense against invading pathogens relies solely on innate immune mechanisms (Mitta *et al.* 2000). The survival rate of shrimp in heterotrophic system experienced enhancement as biofloc contains bacteria which contain peptidoglycan and lipopolysaccharide on their cell wall. Peptidoglycan and lipopolysaccharide are immunostimulant being capable of increasing nonspecific immunity of shrimp. The substances influence prophenoloxidase activity and phagocytosis of hyaline cells (Yeh *et al.* 2010, Supono *et al.* 2014).

Oil palm kernel meal is the by product of palm oil industries and the present study explored the potential use of this nonconventional carbon source for the biofloc development. Using the agro-industrial by-products/wastes for the production of aquaculture biofloc systems can help in the conversion of these materials into nutritious feed for the cultured aquatic species. The present study suggests that industrial waste and non-conventional carbon source (de-oiled oil palm kernel meal) can be used as a potential alternate cheap carbon source for commercial biofloc production systems which is evident by the results showing a better immune-physiological response in biofloc system.

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