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Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources

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ABSTRACT

The objective of this study was to document the immunological effects of growing shrimp in biofloc systems. The experiment consisted of four types of biofloc systems in which bioflocs were produced by daily supplementation of four different carbon sources, i.e. molasses, tapioca, tapioca-by-product, and rice bran, at an estimated C/N ratio of 15 and a control system without any organic carbon addition. Each biofloc system was stocked with Pacific white shrimp (*Litopenaeus vannamei*) juveniles that were reared for 49 days. The use of tapioca-by-product resulted in a higher survival (93%) of the shrimp as compared to the other carbon sources and the control. The highest yield and protein assimilation was observed when tapioca was used as the carbon source. After 49 days, phenoloxidase (PO) activity of the shrimp grown in all biofloc systems was higher than that of the shrimp from the control system. Following a challenge test by injection with infectious myonecrosis virus (IMNV), the levels of PO and respiratory burst (RB) activity in the shrimp of all biofloc treatments were higher than that of the challenged shrimp from the control treatment. An increased immunity was also suggested by the survival of the challenged shrimp from the experimental biofloc groups that was significantly higher as compared to the challenged shrimp from the control treatment, regardless of the organic carbon source used to grow the bioflocs. Overall, this study demonstrated that the application of biofloc technology may contribute to the robustness of cultured shrimp by immunostimulation and that this effect is independent of the type of carbon source used to grow the flocs.

1. Introduction

Disease remains a limiting factor for the aquaculture industry [1]. With respect to the shrimp culture industry, disease outbreaks have been the primary cause of production loss during the last two decades [1]. Disease outbreaks not only result from the mere presence of a pathogen in the system, a compromised health status of the cultured animals in combination with suboptimal environmental conditions are also factors facilitating disease outbreaks [2,3]. Therefore, disease prevention and control should not only focus on implementing biosecurity measures, but must be performed in an integral approach involving, among others, adequate nutrition, enhancing the immunity of the cultured animals and maintaining a good water quality.

Biofloc technology (BFT) has been studied at several occasions and contributes to the maintenance of good water quality in the system and to the nutrition of the cultured animals [4]. The basic principle of the biofloc system is to recycle waste nutrients, in particular nitrogen, into microbial biomass that can be used *in situ* by the cultured animals or be harvested and processed into feed ingredients [5–9]. Heterotrophic microbial aggregates are stimulated to grow by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source [4], so that the bacteria can assimilate the waste ammonia for new biomass production. Biofloc systems have been shown not only to maintain ammonia below toxic levels and to improve the feed nutrient utilization efficiency of the cultured animals [4,9,10], but also to provide extra nutrients [11] and exogenous digestive enzymes [12]. Biofloc application can also lead to increased growth, survival and reproductive performance of the cultured animals [13,14].

So far, very few studies [15–18] investigated the immunological potential of the biofloc technology although it is widely known that

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microorganisms, their cell components or their metabolites can act as immunostimulants that enhance the shrimp innate immune system and provide improved protection against pathogens [19,20]. Xu and Pan [16] reported that the total haemocyte count and phagocytic activity of the haemocyte of the shrimp from biofloc containing culture units were significantly higher than those of the shrimp in the non-biofloc control group. Furthermore, the authors also noted that shrimp grown in a biofloc environment harbored a higher total antioxidant capacity both in the plasma and hepatopancreas. A recent study reported that the expression of six selected genes (prophenoloxidase [ProPO1 and ProPO2], serine protease [SP1], prophenoloxidase activating enzyme [PPAE1], masquerade-like serine protease [mas] and Rat-sarcoma-related nuclear protein), directly and indirectly related to the shrimp immune response, were significantly upregulated in biofloc-grown shrimp [17]. Immune stimulation may thus be a very important feature in biofloc-grown shrimp contributing to disease control. It could for example (partly) explain the lower prevalence of acute hepatopancreatic necrosis disease (AHPND) observed in farms that apply BFT [21]. AHPND is currently causing very large problems in the culture of shrimp post larvae in Asia [22].

The objective of this study was to perform a study on biofloc-grown shrimp. The water quality was monitored over a 49-day period in biofloc systems supplied with different organic carbon sources (molasses, tapioca, tapioca by-products, and rice bran). The shrimp growth performance, immune responses and resistance to the infectious myonecrosis virus (IMNV) were also verified. The results of this study provide information on the immunostimulatory nature of biofloc for shrimp and how this varies depending on the carbon source supplied.

2. Materials and methods

2.1. Experimental design

Twenty glass tanks (90 cm × 40 cm × 35 cm) filled with 100 L seawater were used as the experimental culture units. Temperature in all tanks was maintained in the range of 27.3–28.3 °C during the entire experiment, aeration was provided in each aquarium using an air blower and the light regime was set at 12 h light/12 h dark. Inter-molt phase Pacific white shrimp juveniles, previously acclimatized collectively to the experimental room and conditions for 1 week, at an initial average body weight of 2.02 ± 0.05 g were randomly distributed in the tanks at a density of 30 shrimp/tank (83 shrimp m^{-2}). Four times daily, a commercial pellet containing 30% of crude protein (Feng Li, PT Matahari Sakti, Indonesia) was provided for 49 days to all tanks. The feeding level was determined at 7% on wet body weight per day and the daily feed amount was adjusted to the biomass in the tanks.

The experiment consisted of five treatments (four replicate tanks per treatment): one control treatment without organic carbon addition and with a weekly water exchange of 50%, and four treatments with different organic carbon sources added for biofloc development (molasses, tapioca, tapioca by-products, and rice bran, respectively). Freshwater was regularly added only to make up for water loss due to evaporation. All organic carbon sources were locally purchased. Organic carbon was added daily two hours after feeding at an estimated C/N ratio of 15 [5]. Proximate composition and organic carbon content in the different types of organic carbon source were determined according to Takeuchi [23] and Walkley and Black [24] (Table 1).

Table 1
Proximate composition and total organic carbon content of molasses, tapioca, tapioca-by-product, and rice bran (all values, except moisture, are expressed as percentage on dry weight).

	Molasses	Tapioca	Tapioca-by-product	Rice bran
Moisture (%)	31.9	10.0	13.8	9.6
Ash (%)	5.9	0.3	0.6	7.4
Protein (%)	3.8	1.6	nd	6.6
Lipid (%)	0.4	nd	nd	9.9
Fibre (%)	nd	nd	7.9	13.3
Nitrogen free extract (%)	58.1	88.1	77.7	53.4
Organic carbon (%)	38.0	50.3	48.8	43.5

nd: not detectable.

2.2. Water quality

Temperature, dissolved oxygen (DO), pH, and salinity were daily measured *in situ* using a portable DO meter (Lutron DO-5519, Taiwan), pH meter (Lutron YK2001PH, Taiwan) and refractometer (ATAGO 2491-MASTER S, USA). Biochemical oxygen demand (BOD), alkalinity, dissolved inorganic nitrogen (total ammonium nitrogen, NO_2–N, and NO_3–N), and total suspended solids (TSS) were determined weekly following the procedures in the Standard Methods for the Examination of the Water and Wastewater [25].

2.3. Zootechnical performance of the shrimp

Survival was expressed as the percentage of live shrimp on the final day of the experiment relative to the total initially stocked shrimp. Shrimp growth was monitored by weekly sampling and restocking of the measured animals. Specific growth rate was calculated according to Huisman [26] with the following formula:

$$\mathrm{SGR}\left(\% \Big/ \mathrm{day}\right) = \left(\sqrt[t]{\frac{\mathrm{wt}}{\mathrm{wo}}} - 1\right) \times 100$$

SGR = specific growth rate (%/day)
wt = final average shrimp body weight (g)
wo = initial average shrimp body weight (g)
t = experimental period (day)

The food conversion ratio (FCR) was expressed as the ratio of the total feed given relative to the shrimp biomass gain, whereas the input/output ratio was measured as the summed weight of feed and carbon source given per unit of biomass gain. These parameters were calculated for each tank at the end of the culture period.

2.4. Protein and lipid assimilation

Shrimp protein and lipid content were determined according to the Folch and Kjehdahl method as described in Takeuchi [23]. The assimilation of protein and lipid originating from the feed by the shrimp (%) were subsequently calculated according to the following formula [23]:

$$\text{Protein assimilation}(\%) = \frac{\text{Final protein content} - \text{Initial protein content}}{\text{Protein input}} \times 100$$