Intensive culture of *Litopenaeus vannamei* without water exchange and with an artificial substrate

Cultivo intensivo de *Litopenaeus vannamei* sin recambio de agua y con un sustrato artificial

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ABSTRACT

Aiming to determine the effect of the periphyton growing on artificial substrates, juveniles (3 g initial weight and 440 g m^{-3} stocking biomass) of the whiteleg shrimp *Litopenaeus vannamei* (Boone, 1931) , were grown during 32 days in eight 1 m^3 cylindrical tanks with 3.7 m^2 of total submerged surface. Two culture treatments (with and without artificial substrate or control) were tested with four replicates each. Artificial substrate (Aquamats™) provided an additional surface area of 7.2 m^2. The mean dissolved ammonium (NH_4^+) and ammonia (NH_3) concentrations for the Aquamats group were 39 and 22% lower than the respective values obtained for the control cultures. The artificial substrate stimulated nutrient recycling among the biological components (shrimp, biofilm, bottom microfauna, etc.) since mean shrimp biomass yield was 13% higher for the Aquamats group, and it contained a significantly higher percentage of the total nitrogen and phosphorus inputs than the control treatment. The protein content of shrimp cultured with Aquamats was 21.4% higher than that obtained for the control group, which is explained by the higher availability (and diversity) of the natural food of the periphyton. In view of these results, the use of closed cultures added this artificial substrate seems a viable alternative for shrimp culture.

Key words: Shrimp production, closed culture, artificial substrate, nutrient budget, periphyton.

RESUMEN

Con el fin de verificar el efecto del perifiton presente en sustratos artificiales sobre la calidad del agua y el reciclaje de nutrientes, se cultivaron en sistemas cerrados durante 32 días juveniles de camarón blanco *Litopenaeus vannamei* (Boone, 1931) (peso inicial: 3 g y biomasa inicial de 440 g m^{-3}) en ocho estanques cilíndricos de 1 m^3 y 3.7 m^2 de superficie sumergida total. Se utilizaron dos tratamientos (con o sin sustrato artificial), cada uno con cuatro repeticiones. En las repeticiones de uno de los tratamientos se adicionaron 7.2 m^2 de sustrato artificial (Aquamats™). Los niveles medios de amonio (NH_4^+) y de amoniaco (NH_3) en los cultivos con Aquamats fueron equivalentes al 39% y al 22% respectivamente, de los valores registrados en los cultivos control. El tratamiento con sustrato artificial favoreció

el reciclaje de nutrientes, ya que la biomasa final fue 13% superior al control, y su contenido de nitrógeno y fósforo representó porcentajes significativamente mayores de los respectivos ingresos totales. El mayor nivel de proteína en la biomasa de camarón (21.4%) en los tratamientos con sustrato adicional, se explica por la mayor disponibilidad de alimento natural representado por el perifiton. En vista de estos resultados, el uso de cultivos cerrados y del sustrato artificial Aquamats™, parece una alternativa viable para el cultivo de camarón.

Palabras clave: Producción de camarón, cultivos cerrados, sustratos artificiales, balance de nutrientes, perifiton.

INTRODUCTION

The continuous growth and tendency to intensification of shrimp culture face several challenges: some are related to its impacts on the natural environment, such as the destruction of mangrove forests and the eutrophication of coastal areas (Naylor *et al*., 2000; Piedrahita, 2003), while others are due to the limits to intensification, mainly caused by the large amounts of costly formulated feed elaborated with animal protein, needed for intensive culture (Chamberlain, 1995; Sorgeloos, 2001). Additionally, formulated feed is responsible of water quality and pond bottom deterioration, due to the animal excretion, and sedimentation and lixiviation of uneaten food and feces (Burford & Williams, 2001; Avnimelech & Ritvo, 2003). This problem is magnified in closed intensive cultures, because food assimilation efficiency decreases in ponds with a high standing biomass (Martin *et al*., 1998; Zaki *et al*., 2004).

Thus, intensification of aquaculture is self-limiting, not only because of the high cost of formulated feed, but also for its poor utilization, which causes deterioration of the pond environment and poor growth, or generates even higher costs, because of the need to increase water exchange rates. This adds to the poor perception of aquaculture by the stakeholders, because it is perceived as an environmental threat (Tacon & Forster, 2003).

There are several techniques which allow the reduction of this threat, maintaining at the same time the water quality within acceptable levels (reviewed by Crab *et al*., 2007). Most are designed to remove waste products from the culture but with added costs because of the need of additional space for waste removal in settling ponds (van Rijn, 1996; Hargreaves, 2006) or through mechanical filters, generally followed by fine solid removal and foam fractionation, UV or ozone treatment and removal of dissolved organic waste in different types of biological filters (Greiner & Timmons, 1998; Malone & Beecher, 2000; Gutiérrez-Wing & Malone, 2006; Timmons *et al*., 2006a, b; Crab *et al*., 2007).

However, there are other alternatives based on the utilization of the dissolved waste products within the culture system —by autotrophic bacteria and algae—, or through direct heterotrophic conversion of organic and inorganic nitrogen species into microbial biomass (Ebeling *et al*., 2006; Linares & Sundbäck, 2006), which improve water quality, and at the same time, the microbial biomass becomes an important direct or indirect source of natural feed for the farmed organisms (Nunes & Parsons, 2000; Burford *et al*., 2004). It is achieved either using active suspension ponds —where strong aeration and mixing lead to the formation and growth of microbial flocs in the water column—, or through the addition to the pond of submerged substrates, which serve for the promotion of growth of mixed microalgae-bacteria mats (periphyton) (Avnimelech, 2006; Crab *et al*., 2007).

In this study, we evaluated the effects of one artificial substrate on water quality, and on survival, individual growth, biomass production and food conversion efficiency of the white shrimp *Litopenaeus vannamei* (Boone, 1931), cultured in mesocosm under intensive conditions with zero water exchange.

MATERIALS AND METHODS

The experiment was performed between October 3 and November 4, 2008, using eight 1000 l cylindrical high density polyethylene tanks (water depth: 0.9 m, bottom surface: 1.1 m^2, submerged surface of the walls: 3.7 m^2), located on the grounds of a commercial shrimp farm close to the Urías estuary (Mazatlán, Sinaloa, Mexico).

A 10-cm-deep layer of the superficial (upper 5 cm) bottom sediment of an operating pond of the farm, untreated and previously homogenized in a concrete mixer, was added to each tank. The tanks were filled with 1 m^3 of 300-µm filtered estuary water and, after one week, the juveniles (mean weight: 3.0 ± 0.2 g) were stocked at 133 shrimp m^{-2} $tank^{-1}$, equivalent to 440 g m^{-3} of stocking biomass.

One day before the experiment started, four tanks were provided with 7.2 m^2 of artificial substrate (Aquamats™, Meridian Applied Technology Systems, Calverton, Maryland, USA), which had been previously submerged in an operating shrimp pond during 5 days to allow the formation of the biofilm (Burford *et al*., 2004). Then, Aquamats™ colonized with microorganisms were placed vertically in a circular arrangement at a distance of about 10 cm from the tank walls, increasing by 150% the surface area available to the shrimps as substrate.

Shrimp were fed twice daily (08:00 y 18:00 hours) with 35% protein pelletized commercial food (Camaronina 35, Agribrands Purina Mexico®, Cuautitlán, Estado de México), supplied in feeding trays (36 cm in diameter) used for adjusting the daily food

ration according to the apparent consumption observed on the feeding trays (Clifford, 1997). The culture units were kept with zero water exchange, but replacing weekly the water lost by evaporation (estimated average loss, close to 4.8% of the total volume). Aeration (3-5 l min^{-1}; >1-mm air bubbles) was provided to all units with an air blower (Sweetwater 1 HP, Aquatic Eco-System, Inc., FL, USA) to keep adequate dissolved oxygen concentration, avoid thermal stratification and promote renovation of the water in contact with all submerged surfaces.

Temperature and dissolved oxygen were measured in each unit twice daily (08:00 and 18:00 hours) using an oxygen meter (YSI model 57, Yellow Springs, OH, USA). Salinity and pH were determined at 12:00 with an Atago S/Mill-E refractometer (Atago Co., Ltd., Tokio, Japan) and a portable field pH meter (Hanna HI 98150, Hanna Instruments, Woonsocket, RI, USA), respectively.

The concentrations of the dissolved nitrogen (N) and phosphorus (P) species (N-NO_3^-, N-NO_2^-, N-NH4$^+$, dissolved organic N, P-PO_4^{3-}, dissolved non reactive P) and of particulate N and P were determined with three replicates, by sampling 250 ml of the each tank. All water samples were filtered through Whatman GF-C filters, and the concentrations of dissolved N-NO_3^-, N-NO_2^-, N-NH_4^+, P-PO_4^{3-} and dissolved organic N were determined as in Strickland and Parsons (1972). Non reactive phosphorus was measured as in Rosales-Hoz (1979), and the concentration of unionized ammonia (NH_3) was calculated according to Spotte and Adams (1983).

The organic nitrogen and total phosphorus contents of the particles retained on the filters were determined as described by Holm-Hansen (1968) and Solorzano and Sharp (1980), respectively.

The initial and final organic nitrogen content of the sediment and the accompanying meiofauna (determined in triplicate samples after addition to each of the mesocosms, and of composite samples of the sediment obtained at the end of the experiment from the center and sides of each tank), shrimp biomass and the periphyton present on the artificial substrates (obtained scraping the substrate with a scalpel: Burford *et al.*, 2004) were determined with the Kjeldahl method (AOAC, 2005). This method was also used to determine the organic N of shrimp feed. The total phosphorus content was determined as dissolved reactive phosphate (Strickland & Parsons, 1972) after digestion, according to Jackson (1982).

The N and P budgets were calculated as:

Σ of the inputs = Σ of the outputs + losses not considered

Where:

Σ inputs: initial nutrient (N or P) content of the sediment and periphyton + amount supplied to each tank with shrimp feed + sum of the dissolved and particulate species of the relevant nutrient determined in the water used for tank filling and for the weekly water replacements + nutrient content of the shrimp stocked in each tank, and

Σ outputs: nutrient content of the shrimp biomass harvested + sum of the dissolved and particulate species determined in the water discharged + content of the sediment and periphyton at the end of the experiment.

The final survival and biomass yield were determined at the end of the experiment by counting and weighing the surviving shrimp of each tank respectively. The increases in weight followed an almost linear trend (R^2 = 0.985 ± 0.042 for the regression calculated with weights in grams, vs 0.971 ± 0.009 with ln-transformed data). Therefore, the daily specific growth rate (SGR) was calculated with the equation:

$$SGR = (FW - IW)/t$$

Where: IW and FW = mean initial and final individual wet weights, respectively; t = duration (in days) of the experiment.

The mass yield and the economic feed conversion ratios, FCR and ECR respectively, were calculated for each tank as suggested by Lawrence y Houston (1993) using the next two equations:

$$FCR = (BY - IB)/FS \quad (1)$$

$$ECR = MV/FC \quad (2)$$

Where: BY = biomass yield, IB = initial biomass and FS = total feed supplied, MV = market value of the biomass harvested and FC = cost of the feed supplied (average 2009 prices: 32 Mexican pesos kg^{-1} of head-on 9 to 10 g shrimp and 14.2 pesos kg^{-1} of formulated feed).

The mean protein content of the shrimp muscle was determined in triplicate (one sample of 10 shrimp for each tank) for each treatment, and the total concentration of proteins in the hemolymph, were determined at the end of the experiment using the Kjeldhal method (AOAC, 2005) and the bicinchoninic acid technique (Smith *et al.*, 1985), respectively.

The mean values of temperature, dissolved oxygen, pH, salinity and dissolved nutrient concentrations were compared using paired t tests or the equivalent Wilcoxon´s tests when the data were not normal or homoscedastic (Kolmogorov-Smirnov and Fisher´s F tests).

The mean values of final yields, survival, individual weights, SGR, and the feed and economic conversion ratios were compared using t or Mann Whitney´s tests, after arcsine square root transformation in the case of final survival. In all cases, the level of significance was α = 0.05 (Zar, 1999).

RESULTS

The NH_4^+ and NH_3 mean concentrations for the artificial substrate culture group were significantly lower ($p < 0.05$) compared with the control culture. There were no significant differences ($p > 0.05$) between the mean values for the rest of the water characteristics tested (Table 1).

The Aquamats™ culture obtained a mean final biomass yield of 1302 ± 16 g and survival above 81%. Both were significantly higher ($p < 0.05$) than the control group (1144 ± 106 g and 75% survival, respectively). The culture of shrimp without artificial substrate resulted in lower individual mean weights and specific growth rates, as well as significantly higher feed conversion efficiencies (Table 2).

The protein content of the hemolymph for both culture treatments was close to 50 mg ml^{-1}, but the muscle protein was higher ($p < 0.05$) in the culture with Aquamats (22.7 ± 1.3%) than in the control culture (18.7 ± 1.1%).

The nutrient balances are summarized in Table 3. The shrimp feed was the most important nitrogen input (78.4-79.6%) and the initial shrimp biomass represented between 16.4 and 16.9%. The nitrogen of water and sediment, as well as the initial biofilm on the Aquamats added in the experimental cultures (between 1.6 and 1.9%), were the least important contributions to the total N inputs.

At harvest, the nitrogen content of the shrimp biomass for the control culture was 40.5 g (43.4% of the total nitrogen inputs).

Table 1. Mean values (± standard deviation) of daily water physicochemical, and weekly nutrient concentrations in the culture of *L. vannamei* juveniles with and without (control) artificial substrate (Aquamats™).

Variable	Control	Aquamats™
T °C a. m.	28.6 ± 1.0	28.7 ± 1.0
T °C p. m.	31.8 ± 1.1	31.9 ± 1.2
O_2 a. m. (mg l^{-1})	4.1 ± 0.8	3.8 ± 0.9
O_2 p. m. (mg l^{-1})	3.7 ± 0.7	3.5 ± 0.7
pH	7.8 ± 0.5	7.8 ± 0.5
Salinity (‰)	35.6 ± 2.4	35.4 ± 2.5
N-NH_4^+ (mg l^{-1})	2.06 ± 0.94*	0.81 ± 0.26
N-NH_3 (mg l^{-1})	0.18 ± 0.01*	0.04 ± 0.02
N-NO_2^- (mg l^{-1})	0.08 ± 0.07	0.05 ± 0.03
N-NO_3^- (mg l^{-1})	1.12 ± 0.35	1.29 ± 0.10
Part. N (mg l^{-1})	0.68 ± 0.29	1.23 ± 0.42
P-PO_4^{3-} (mg l^{-1})	0.49 ± 0.049	0.47 ± 0.03
Part. P (mg l^{-1})	0.01 ± 0.009	0.01 ± 0.007

* Significant difference (t test for paired data, $\alpha = 0.05$).

Table 2. Mean values (± SD) of production variables in the culture of *L. vannamei* with and without (control) artificial substrates (Aquamats™).

Variable	Control	Aquamats™
Final yield (g)	1144 ± 106*	1302 ± 16
Survival (%)	75.0 ± 3.6*	81.4 ± 1.2
Weight (g)	7.7 ± 0.2*	8.1 ± 0.1
SGR (g/day)	0.147 ± 0.006*	0.159 ± 0.003
FS (g)	1344.0 ± 87.4	1368.0 ± 52.6
FCR	1.9 ± 0.2*	1.6 ± 0.1
ECR	0.8 ± 0.1*	0.6 ± 0.1

* Significant difference (t tests, α=0.05). SGR: Specific growth rate; FS: total feed supplied; FCR: mass feed conversion ratio; ECR: economic conversion ratio.

Table 3. Nitrogen and phosphorus balances in the culture of the white shrimp *L. vannamei* with and without (control) artificial substrates (Aquamats™). Values represent mean ± SD of the inputs and outputs (g) in 1-m^3 tank.

	Nitrogen		Phosphorus™	
	Control	Aquamats	Control	Aquamats
		INPUT		
Feed	74.2 ± 6.9	75.1 ± 4.5	13.7 ± 1.2	13.3 ± 0.8
Inicial biomass	15.8 ± 0.1	15.8 ± 0.1	1.8 ± 0.01	1.8 ± 0.01
Water	1.7 ± 0.1	1.7 ± 0.1	0.1 ± 0.001	0.1± 0.001
Biofilm	—	1.7 ± 0.2	—	0.8 ± 0.2
Sediment	1.5 ± 0.1	1.5 ± 0.1	4.0 ± 0.6	4.0 ± 0.6
Total	93.2	95.8	19.0	20.0
		OUTPUT		
Final yield	40.5 ± 4.4*	46.6 ± 0.7	4.6 ± 0.5*	5.3 ± 0.1
Sediment	40.8 ± 2.9*	23.4 ± 5.0	13.0 ± 1.0	12.0 ± 0.1
Biofilm	—	16.0 ± 4.7	—	1.0 ± 0.1
Water discharged	10.3 ± 1.2*	8.6 ± 0.8	0.7 ± 0.1	0.8 ± 0.01
Escaped shrimp	0.9	0.7	0.4	0.3
Total	92.5	95.3	18.8	19.5
Missing	0.6	0.5	0.2	0.5

*Significant difference between final values in each line (t test, α=0.05).

This was significantly lower ($p < 0.05$) than the 46.6 g of N (48.7% of the inputs) contained in the shrimp harvested in the cultures with artificial substrate. The N content of sediment and biofilm (23.4 ± 5.0 and 16.0 ± 5.7 g, 24.4 and 16.6%, respectively) for Aquamats™ was similar to the amount determined in the sediment of the control culture (40.8 ± 2.9 g: 43.8% of the total nitrogen inputs).

The mean total P input ranged between 19 and 20 g, close to 25% of this amount was harvested as shrimp biomass; as in the case of N, the amount recovered was significantly higher ($p < 0.05$) in the tanks provided with Aquamats™, in which an additional 5.2% was recycled as food still available in the biofilm.

Less than 4% of the P input was discharged at harvest, and between 60.3 and 68.8% of the P inputs remained stored in the sediments.

DISCUSSION

Although the shrimp groups were maintained in closed culture systems, the ammonia concentrations of both treatments remained below the safety levels for white shrimp at the size used in our experiment (6.5 mg NH_4^+ l^{-1}: Frías-Espericueta *et al.*, 1999; Frías-Espericueta & Paez-Osuna, 2001), and the mean concentrations of NH_3 were lower than 50% of the value which is likely to impair shrimp growth (0.45 mg NH_3-N l^{-1}: Wickins, 1976). For the control culture, this is partially explained by the biological activity of the biofilm present on the tank walls, which give a higher submerged surface to water volume ratios of the culture containers, in comparison to commercial ponds.

However, the biofilm of the additional submerged substrate maintained the mean ammonium ($NH4^+$) and ammonia (NH_3) concentrations close to 39% and 22% of the values obtained in the control tanks, and allowed an effective nutrient recycling, because the bacteria and periphyton present on the Aquamats™ maintained an adequate water quality and were an additional food source for the shrimps in culture (Thompson *et al.*, 2002; Avnimelech, 2006). This also improved the quality of the bottom environment, because a sizeable percentage of the nitrogenous wastes was recycled into shrimp biomass, rather than accumulated in the tank sediment.

There were no differences in the protein levels of the shrimp hemolymph between the culture groups, which were within the normal range for post-molt white shrimp (Racotta & Palacios, 1998; Sreenivasa-Rao *et al.*, 2008). This could indicate that there was no undue stress on the cultured organisms in spite of the lack of water exchange, which seems to confirm the positive effect of the high submerged surface to water volume ratios of the culture containers used in this experiment.

However, in agreement with the results obtained in shrimp cultures by other authors (Burford *et al.*, 2004; Fernandes da Silva *et al.*, 2008; Khatoon *et al.*, 2009), the significantly higher protein contents of the shrimp muscle for the culture with artificial substrate confirm the good quality of the protein-rich natural food which is associated to the biofilm of these substrates.

Our results confirm that the addition of submerged substrate may have several beneficial effects for shrimp culture. Some refer to production costs, because they maintain good water quality, and therefore permit low or zero water exchange rates (Milstein, 2005). Additionally, they significantly improve the shrimp growth and survival, and allow an important decrease of the mass and economic feed conversion ratios (Azim *et al.*, 2004, 2005).

One additional advantage in comparison to the traditional open culture systems, refers to the low amount of nutrients discharged to the surrounding environment, which is one of the several problems faced at present by the aquaculture industry worldwide. The obvious advantage in comparison to other treatment techniques for closed cultures, such as external ponds, biofiltration and solids removal systems, is the lower initial and operating cost, with the added advantage of a better utilization of the shrimp feed (Crab *et al.*, 2007; Avnimelech, 2009).

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