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Preliminary Investigation into the Effect of Carbon Addition on Growth, Water Quality and Nutrient Dynamics in Zero-Exchange Shrimp (*Penaeus monodon*) Culture Systems

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Abstract

In this study we investigated the effect of adding carbon, in the form of molasses, to zero-exchange Penaeus monodon experimental culture systems. Shrimp growth was found to be significantly improved ($P < 0.05$) when carbon was added relative to a control (75.5 ± 10.9 mg.d^{-1}.shrimp^{-1} and 57.3 ± 2.4 mg.d^{-1}.shrimp^{-1} respectively). FCR was also found to be reduced when carbon was added (2.9 ± 0.3 and 4.3 ± 1.0 for carbon and control treatments respectively). Bacterial biomass was found to be higher in the treatment receiving carbon and we hypothesise that this lead to greater observed shrimp growth. Furthermore, we found that the addition of carbon shifted some nitrogen mineralisation from the sediment to the water column. The addition of carbon had no detectable influence on water column nutrient concentrations other than total organic carbon (TOC) which increased in response to molasses addition. TOC:TDN ratio did not exceed five despite continuous molasses addition to the carbon treatment, suggesting rapid uptake of labile carbon and accumulation of refractory DON compounds. We suggest that this trial be repeated with higher carbon carbon dosing rates for a longer period.

Introduction

The production of *P. monodon* in zero-exchange systems can potentially improve the sustainability and bio-security of shrimp farming in Thailand. However zero-exchange ponds rapidly become hyper-eutrophic compromising shrimp health and retarding growth (Burford, et al. 2003a; Thakur and Lin 2003). Avnimelech (1999) showed that increasing C:N ratios in aquaculture production systems is an effective means of facilitating bacterial uptake of dissolved inorganic nitrogen (DIN). Recent studies on closed shrimp culture at Belize Aquaculture Limited (BAL) showed that the stimulation of bacterial growth allowed high production of white shrimp *Litopenaeus vannamei* despite the onset of hyper-eutrophia (Burford, et al. 2003a; b). No water exchange is required but high aeration and mixing is supplied to counter the increased oxygen demand resulting from enhanced bacterial activity. Sedimentation of organic material is also minimised, increasing the organic load in the water and preventing sediment anoxia. Lining ponds ensures that pond scouring is avoided further preventing the build up of sediments. High bacterial loads were suggested to enhance breakdown of organic matter, reduce TAN and provide a food source for shrimp (Burford, et al. 2003a; b).

In Thailand, the promotion of bacteria is recognised as a mechanism for improving shrimp culture however it has not been demonstrated how bacteria can be promoted and whether improved water quality and shrimp growth can result. It is therefore our intention to assess the effect of adding molasses, a cheap available source of carbon, on water quality, microbial activity and shrimp growth in a zero-exchange experimental *P. monodon* culture system.

Material and Methods

This work was conducted at the Coastal Aquaculture Research Institute (CARIN) in Songkhla province, Thailand, and consisted of two experiments. The first aimed to determine how much N and C was released into the water column from shrimp feeding. This was used to calculate a carbon dosing rate, required to maintain the C:N ratio at >5. The second experiment was a tank trial in which juvenile *P. monodon* were cultured with or without the regular addition of carbon in the form of molasses.

Release of N and C from shrimp feeding

Forty juvenile *P. monodon* (10.3 g ± 1.2 std dev) shrimp were collected from an earthen culture pond and transferred to a 2 m^3 fibreglass holding tank containing

clean aerated seawater (1.5 m^3) at the same salinity (25 ppt). The shrimp were fed three times a day to satiation with an artificial diet of known CN content (40.3% C and 6.9% N). A half water exchange, including vacuuming of the tank bottom, was performed every second day. Shrimp were maintained for 8 days prior to the start of the experiment.

On the day of the experiment, twelve acid washed, rinsed, glass aquaria (10 l capacity) were filled with 3 l of autoclaved, filtered seawater (Whatman GF/F), a sample of which was collected for nutrient analysis. Each aquarium contained an aerator and was placed in a shallow tank containing seawater to maintain stable temperature. Shrimp were then individually weighed and added to eight of the aquaria at a density of two-three individuals per aquaria. The same artificial diet as that used during acclimation was added to four of the aquaria containing shrimp (0.5% of shrimp weight).

After 2 hr shrimp were carefully removed from each aquarium and returned to the holding tank. A water sample was then collected from every aquarium for total dissolved nitrogen (TDN), total organic carbon (TOC) and particulate organic carbon/nitrogen (POC/PON) analysis. The tanks were left to sit for 15 min at which time about half the water from each aquaria was carefully decanted to waste. The remainder was filtered to collect the particulate material, which was subsequently analysed for POC/PON. The experiment was performed a total of three times within a week.

The average amount of TOC and TDN, in the water from aquaria without feed but with shrimp, was subtracted from the values in the aquaria containing both feed and shrimp. The values were then corrected for the quantity of feed added. The ratio of TOC to TDN (both in $mg.l^{-1}.g\ feed^{-1}$) after feeding was then determined. Only aquaria where food had been consumed (ie. PON content at the end of the experiment had to be less than 20% of the input feed N) were used for the calculation. Results for the three experiments were combined.

Effect of carbon addition on growth, water quality and nutrient dynamics

The second experiment was conducted in eight identical concrete tanks (1.7 x 1.4 m, 2.4 m^2), each containing a large oval 'pad' of mud (≈ ¾ the tank area). The mud was collected from the outer edge of a *P. monodon* shrimp pond (Dhumrong Farm) that had been emptied a week earlier. The top 10-15 cm of the pond sludge was not collected. After collection the mud was air dried and broken into successively smaller clods over the course of two weeks. The dried dirt was mixed and measured (volumetrically) into eight similar mounds. Each mound was moved to a concrete tank and soaked overnight in enough seawater to form a firm pad..

The mud pad was left to dry for 2 weeks after which the tanks were filled with

0.5 m^3 of growout water (28 ppt) from a shrimp pond from Dhumrong farm. One m3 of coarse 50-100 µm filtered seawater (28 ppt) was also added giving a total volume of 1.5 m^3. Two uplift aerators, angled to create circulation, were placed in opposite corners of each tank. Regular air-stones were placed in the other corners. Two days after filling, and after a nutrient sample had been taken, juvenile *P. monodon* were stocked into the 8 tanks.

Shrimp were collected from Dhumrong farm four days prior to stocking. After collection the juvenile *P. monodon* (approximately 700 shrimp) were transferred to a large round concrete tank (10 m^2) containing seawater to a depth of 0.3 m. Salinity in the farm pond was the same as in the concrete tank (28 ppt). Shrimp were fed to satiation three times a day with an artificial diet (40.3 % C and 6.9 % N) and water was exchanged on the second day after collection. From the second day after stocking, the shrimp in each tank were fed with the same diet used for acclimation.

Four days after collection (4th December 2003), 75 shrimp were individually weighed and transferred to each of the concrete tanks (31 m^{-2}). Average weight at stocking was 1.74 g (± 0.12 g SD). Shrimp were fed on a feed tray four times a day (0900, 1300, 1700 and 2100 hrs). The first feed was calculated at 5% total body weight. Feed consumption was monitored 2 h after addition and quantities for the following feed were reduced (-5%) or increased (+5%) accordingly. Salinity was maintained at 28 ppt by the weekly addition of freshwater.

The initial concentrations of TOC and TDN in the water in the concrete tanks were found to be 6.7 mg.l^{-1} and 2.8 mg.l^{-1} respectively. To increase the TOC:TDN ratio to 5 an extra 7.3 mg.l^{-1} of TOC was required. Prior to the stocking of the concrete tanks with shrimp, 81 ml of a diluted molasses mixture (containing 340 g molasses. l^{-1} or 136 g C.l^{-1}) was added to 4 of the 8 concrete tanks (+Carbon treatment).

From the earlier experiment, the amount of TOC and TDN released into the water column following shrimp feeding was found to be 10.7 mg TOC.g feed -1 and 6.1 mg TDN. g $feed^{-1}$ respectively. For every gram of feed added an additional 19.8 mg C.g $feed^{-1}$ is required to lift the TOC:TDN ratio to 5. Therefore each evening after the last feed the diluted molasses mixture was also added to the +Carbon tanks at a rate of of 0.15 ml.g $feed^{-1}$ added for that day. After 4 and 6 weeks, water quality analysis showed that the TOC:TDN ratio in the +Carbon tanks was less than 5. Enough carbon was added to the +Carbon treatment tanks at both these times to increase the TOC:TDN ratio of the water column to >5 (approximately 70 ml and 50 ml to each +Carbon treatment tank at three and six weeks respectively).

Sample collection and analysis

Dissolved oxygen (DO), pH, temperature and salinity were measured daily in all eight tanks. Once a week at the same time, water samples were collected for

the following analysis: total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3), dissolved organic nitrogen (DON), TDN, PON, TOC, POC, total suspended solids (TSS), Chlorophyll a and bacterial abundance. For TAN, NO_2, NO_3 and TDN measurements, water samples were firstly filtered with a Whatman GF/C paper. Every third week total water column oxygen production and consumption were determined using electrometric methods described by Bratvold and Browdy (1998), DO change was monitored in light and dark BOD bottles containing filtered (120 µm) tank water, incubated just below the surface. Additional dark BOD bottles containing the nitrification inhibitor 2-chloro-6 (trichloromethyl) pyridine were also incubated (Bratvold and Browdy, 1998). For total oxygen production DO was measured twice over a 24 hr period in the light BOD bottles. Total water column oxygen consumption was determined after linear regression of repeated DO measurements (five in 24 hrs) in the dark BOD bottles with and without nitrification inhibitor.

Sediment oxygen demand (SOD) was measured by monitoring DO change over time in three incubated sediment cores from each tank. The cores were collected by plunging a PVC pipe (40 mm x 30 cm) into the sediment pad and carefully removing. A rubber stopper was placed in the sediment end of the pipe and the water within was decanted off, the core was filled with filtered (120 µm) water from that tank and the DO measured. The volume of water added was noted and a sample was also collected for TAN analysis. The core was then sealed with another rubber stopper and incubated in the treatment tanks, care was taken to ensure no air remained in the core. A duplicate set of control cores containing sediment but with GF/C filtered sterile seawater instead of tank water were also incubated. DO and TAN were measured in these control cores prior to incubation. SOD in all cores was determined after linear regression of repeated DO measurements (five in 24 hrs) in both sets of cores. At the completion of the incubation water samples were also collected for TAN analysis. TAN production between the cores filled with sterile seawater were subtracted from production rates obtained from the cores with tank water to give the rates in the water column. After 62 days (5th January 2004) the experiment was terminated, tanks were drained and shrimp collected and weighed.

Samples for TAN, NOx, TDN and TOC analysis, samples were immediately filtered (Whatman GF/C) then frozen. TAN, NO_3, NO_2 and TDN were analysed manually using the colorimetric methods described in Grasshoff (1983). DON was calculated as TDN subtract TAN, NO_3 and NO_2. Total nitrogen (TN) was calculated as TDN+PON. TOC was analysed with an Elementar Liquitoc Infra-red analyser as non-purgable organic carbon. PON and POC were collected on pre-combusted 25 mm Whatman GF/F filters. These were dried at 90°C for 48 hrs, rolled into a cylinder, wrapped in aluminium foil and analysed for CN content with a LECO CHN analyser. To determine feed and molasses moisture and CN content, weighed portions were dried at 90°C for 48 h then re-weighed and analysed on the CHN analyser.

Phytoplankton was filtered from the samples onto 0.45 µm cellulose acetate filter papers. Chlorophyll *a* was determined photometrically after acetone extraction (Grasshoff, 1983). Water samples for bacterial enumeration were immediately added to acid rinsed containers containing glutaraldehyde (5% final conc.). Bacteria samples were diluted with 0.2 µm filtered sterile seawater (1:10), sonicated (30 s at power level 3), rediluted (2:5) then filtered onto black 0.45 µm cellulose acetate filters (Erler, et al., 2004). Samples were counted by fluorescent microscopy using DAPI stain.

All parameters were compared using Students-*t* test after data was checked for normality. Oxygen production and demand were calculated using the methods of Bratvold and Browdy (1998). The data are presented as mean ± standard deviation.

Results

Release of N and C from shrimp feeding

The feeding of *P. monodon* on artificial pellets was found to increase the water column TOC and TDN (Fig. 1) by 10.7 ± 8.1 mg.g feed^{-1} and 6.2 ± 4.0 mg.g feed^{-1} respectively. This gave a TOC:TDN ratio of 1.73. The largest fraction of TDN was DON (3.2 ± 2.1 mg.g feed^{-1}) followed by TAN (2.8 ± 1.1 mg.g feed^{-1}).

Effect of carbon addition on growth, water quality and nutrient dynamics

Shrimp growth. Shrimp growth was found to be significantly higher in the +Carbon treatments ($P < 0.05$) relative to the control (75.5 ± 10.9 mg.d^{-1}.shrimp^{-1} and 57.3 ± 2.4 mg.d^{-1}.shrimp^{-1} respectively Fig. 2). FCR was also significantly improved ($P < 0.05$) in the +Carbon treatment relative to the control tanks (2.9 ± 0.3 and 4.3 ± 1.0 respectively). Survival was similar in both the +Carbon and control treatments (36 ± 3.2 % and 34 ± 33 % respectively).

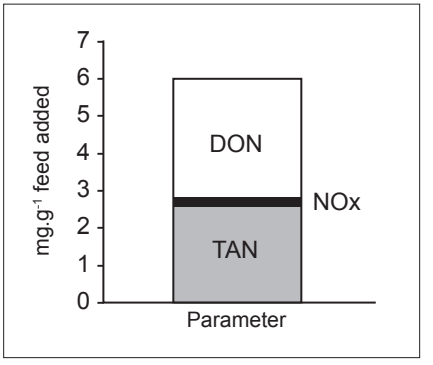

Fig. 1. Release of dissolved nitrogen into the water column following shrimp feeding

Water quality. In the +Carbon treatment there was a significantly higher biomass of bacteria relative to the control (1.27 ± 0.21 x 10^7 ml^{-1} and 0.95 ± 0.11 x 10^6ml^{-1} respectively) (Fig. 3). Phytoplankton abundance, as indicated by Chlorophyll a, was not significantly different between +Carbon and control. In both treatments Chlorophyll a values fluctuated

throughout the trial (Fig.3). Average TOC concentration was found to be significantly higher ($P < 0.05$) in the +Carbon treatment than in the control treatment (17.9 ± 3.1 mg.l^{-1} and 12.8 ± 2.0 mg.l^{-1} respectively). Average TOC:TDN ratio was also significantly different ($P < 0.05$) between +Carbon and control treatments (4.3 ± 1.1 and 2.8 ± 0.6), There were no other significant differences in water quality parameters between the two treatments (Table 1).

Nutrient dynamics. TAN flux in the water column was found to be significantly higher (13.2 ± 5.1 mg.l^{-1}.hr^{-1}) in the +Carbon treatment than the control (6.8 ± 2.2 mg.l^{-1}.hr^{-1}) (Table 2). By contrast, nitrification (expressed as mg DO consumed. l^{-1}.hr^{-1}) was significantly higher in the control (0.42 ± 0.03 mg.l^{-1}.hr^{-1}) than the +Carbon treatment (0.26 ± 0.01 mg.l^{-1}.hr^{-1}). DO production in

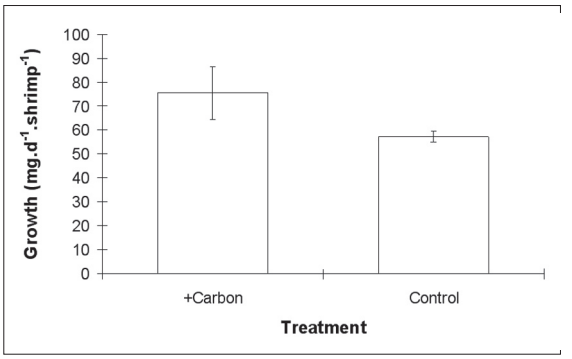

Fig. 2. Growth of shrimp in the +Carbon Control Treatments

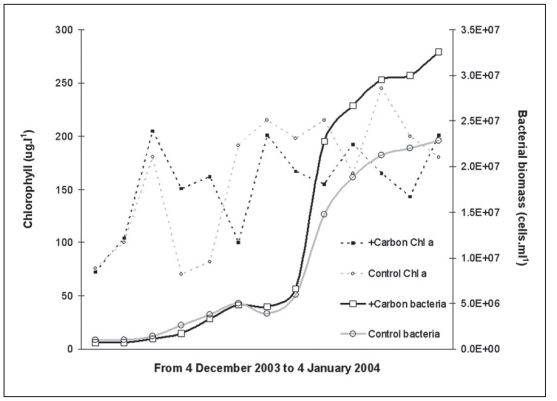

Fig. 3. Bacterial biomass and Chlorophyll *a* (Chl a) concentration in the +Carbon and Control treatments during the duration of the trial

	+Carbon	Control
TOC	17.9 (3.0)	12.8 (0.9)
TN	5.9 (1.7)	5.8 (1.6)
TDN	4.1 (2.3)	4.5 (1.2)
DON	2.6 (0.8)	2.5 (0.8)
PON	1.7 (0.9)	1.4 (0.9)
TAN	0.46 (0.21)	0.46 (0.21)
NO_3	0.57 (0.31)	0.78 (0.42)
NO_2	0.50 (0.22)	0.52 (0.22)
NOx	1.0 (0.5)	1.3 (0.6)

Table 1. Average nutrient concentrations (mg.l^{-1}) in the water column of the +Carbon and control treatments over the 62 day trial

Figure in parenthesis are standard deviations, TOC = total organic carbon, TN = total nitrogen, TDN = total dissolved nitrogen, DON = dissolved organic nitrogen, PON = particulate organic nitrogen, TAN = total ammonia nitrogen, NO_3 = nitrate, NO_2 = nitrite, NOx = NO_3 + NO_2.

the water column was marginally higher and respiration was marginally lower in the control tanks relative to the +Carbon tanks (Table 2).

Sediment oxygen demand was found to be significantly higher in the control tanks than the +Carbon tanks (53.5 ± 11.9 $mg.m^{-2}.hr^{-1}$ and 38.1 ± 4.4 $mg.m^{-2}.hr^{-1}$ respectively), as was flux of TAN (16.1 ± 4.2 $mg.m^{-2}.h^{r-1}$ and 9.9 ± 2.0 $mg.m^{-2}.hr^{-1}$ respectively) (Table 2).

Table 2. Oxygen and nutrient dynamics in the sediments and water column from the +Carbon and control treatments

		+Carbon	Control
Water Column	DO production (mg $DO.l^{-1}.hr^{-1}$)	0.41 (0.14)	0.45 (0.17)
	Total Respiration (mg. $DO.l^{-1}.hr^{-1}$)	0.16 (0.04)	0.13 (0.03)
	Nitrification (mg $DO.l^{-1}.hr^{-1}$)	0.26 (0.01)	0.42 (0.03)
	TAN production (µg $TAN.l^{-1}.hr^{-1}$)	13.2 (5.1)	6.8 (2.2)
Sediment	SOD (mg $DO.l^{-1}.hr^{-1}$)	38.1 (4.4)	53.5 (11.9)
	TAN production (mg $TAN.l^{-1}.hr^{-1}$)	9.9 (2.0)	16.1 (4.2)

DO = dissolved oxygen
TAN = total ammonia nitrogen
SOD = sediment oxygen demand

Discussion

This study demonstrated that the addition of carbon, in the form of molasses resulted in greater growth of the shrimp *P. monodon* and improved FCR. Avnimelech (1999) reported that increasing C:N results in greater availability of feed in the form of proteinaceous bacteria. This has also been suggested by Burford, et al. (2003a; b) in their studies of the BAL system. In this study we found significantly higher TOC:TDN ratios when molasses was added which coincided with significantly higher concentrations of bacteria. Therefore we postulate that increased bacterial biomass was a contributing factor to increased shrimp growth and lower FCR. The implication for Thai shrimp farmers is that cheap carbon sources can supplement the use of expensive artificial diets.

In addition to increasing food sources, the stimulation of bacteria also appears to have reduced the contribution of sediment to organic mineralization. The SOD and TAN production results, which were both higher when carbon was absent, support this hypothesis. Furthermore, TAN production was found to be higher in the water column when carbon was present, again suggesting a shift in mineralisation from the sediment to the water column. The promotion of water column mineralisation has been strongly encouraged in the BAL system by lining ponds and resuspending settled organic matter with vigorous aeration. The transfer of mineralisation activity to the water column implies that sediment quality may be improved through carbon

addition. This relates directly to shrimp health and may have been a significant factor in improving growth rates in the +Carbon system.

Despite the improvement in culture performance between treatments, survival, growth and FCR were poor compared to commercial practices. Possible reasons include stress resulting from capture and transport and a short acclimation time. It is suggested that this experiment be repeated with a longer acclimation time and with a flow through control treatment.

The preliminary feeding experiment conducted here allowed a calculation of carbon dosing rate, however, for the first half of the main experiment this dosing rate did not appear to increase TOC concentrations in the +Carbon treatment. The preliminary experiment may not have yielded a true estimate of excretion because again the acclimation time may have been too short to ensure the shrimp ate normally. It is suggested that a further trial be conducted on well acclimated animals.

Based on the measured TOC and TDN in the treatment tanks, molasses was added twice during the second half of the main trial to boost TOC:TDN ratios to >5. The regular addition of carbon did result in elevated TOC concentrations in the +Carbon treatment. It was interesting to note that despite addition of quantities of TOC that should have increased TOC:TDN to >5, the average ratio over the trial in the +Carbon treatment was 4.3. This suggests that in the +Carbon treatment the molasses was being rapidly assimilated by bacteria and phytoplankton as it is more labile than most of the organic material in shrimp ponds (Fenchel, et al., 1998). It also suggests that there is an accumulation of nitrogen through the culture cycle, most likely refractory DON, that reduces the TOC:TDN. The accumulation of refractory DON has been found by (Burford and Williams, 2001). We suggest that carbon be added to increase C:N ratio to >10 in future trials to account for DON accumulation.

The shrimp pond environment is a complex dynamic system where nutrient production and uptake is difficult to predict (Burford, et al. 2003a). This is particularly apparent in the phytoplankton abundance data which fluctuates between boom and bust cycles throughout the trial in both treatments. Phytoplankton have a significant influence on rates of nutrient transfer in zero-exchange shrimp ponds (Burford, 1997) and more than likely reduced the capacity to observe significant changes in nutrient concentrations. However over the 2 month trial the concentrations of TAN and NOx stayed well within acceptable culture limits. Zero-exchange culture of *P. monodon* appears therefore to be a viable option for Thai farmers, as has also been found by Thakur and Lin (2003).

In summary, we have demonstrated that the addition of carbon, in the form of molasses, to zero-exchange *P. monodon* culture systems can improve shrimp growth and reduce FCR. We hypothesise that increased growth resulted from greater food source availability in the form of higher bacterial biomass. We found that the addition of carbon shifted productivity from the sediment to the water column and therefore

improved sediment quality and shrimp growth. The addition of carbon was found to have no detectable influence on water column nitrogen concentrations. We suggest that rapid uptake of labile molasses and accumulation of refractory DON kept the TOC:TDN ratio below 5. We recommend further trials be conducted where carbon is added to achieve a TOC:TDN ratio of > 10.

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