Effects of control of C/N ratio by low-cost carbohydrate addition on water quality and pond ecology in freshwater prawn *Macrobrachium rosenbergii* post-larvae nursing system

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Abstract

An experiment was conducted to evaluate the effects of control of carbon/nitrogen ratio (C/N ratio) by addition of low cost carbohydrate to the water column on water quality and pond ecology in freshwater prawn Macrobrachium rosenbergii post-larvae nursing system. In this experiment, two level of dietary protein 20% and 35% without carbohydrate addition ('P20' and 'P35') and with carbohydrate addition ('P20+CH' and (P35+CH') were compared in small ponds of 40 m² area stocked with 20 post-larvae $(0.021 \pm 0.001 \text{g})$ per m². Maize flour was used as low cost carbohydrate and applied to the water column followed by the first feeding during the day. The addition of carbohydrate significantly reduced (p< 0.05) ammonia-nitrogen (NH₃-N) and nitrite-nitrogen (NO₂-N) of water in P20+CH and P35+CH treatments. It significantly increased (p < 0.05) the total heterotrophic bacteria (THB) population both in water and sediment. Fifty nine genera of plankton were identified belonging to the Bacillariophyceae (11), Chlorophyceae (21), Cyanophyceae (7), Dinophyceae (1), Rotifera (7) and Crustacea (9) without any significant difference (p>0.05) of total phytoplankton and zooplankton among the treatments. Survival rate of prawn was significantly lowest (p < 0.05) in P20 and no significant difference (p>0.05) was observed between P20+CH and P35 treatments. Control of C/N ratio by the addition of low-cost carbohydrate to the pond water column benefited the freshwater prawn nursing practices in three ways (1) increased heterotrophic bacterial growth supplying bacterial protein augment the prawn post-larvae growth performances, (2) reduced demand for supplemental feed protein and subsequent reduction in feed cost and (3) reduced toxic NH₃-N and NO₂-N levels in pond nursing system.

Key words: C/N ratio, Carbohydrate, Freshwater prawn, Nursing, Heterotrophic bacteria

Introduction

Macrobrachium rosenbergii nursery system is an intermediate stage between larval rearing and grow-out, in which post-larvae (PL) are cultured at high densities from metamorphosis to juveniles. Nursing of newly raised *M. rosenbergii* PL for 1-3 months

period, prior to stocking in the grow-out pond, is an important step in freshwater prawn aquaculture (Alam *et al.* 1997). In Bangladesh, prawn nursing is important because climatic conditions and intermittent water availability restricts the length of growing season and prevent continuous culture to market size (New 1990). Lack of technological knowledge and adequate research on prawn nursing system lead to higher mortality, therefore, reducing benefit of the farming system. There are very limited research reports on prawn nursery system in Bangladesh. Some authors such as Angell (1994), Alam *et al.* (1997), Barman *et al.* (2003) and Asaduzzaman *et al.* (2005) reported prawn nursery system in Bangladesh.

One of the potential management measures to improve the nursing system may be the addition of organic carbon rich substrate (glucose, cassava, sorghum meal or cellulose and tapioca flour) to control the C/N ratio. The C/N ratio has been widely used as an index of the rate at which organic matter decompose (Alexander 1961). The manipulation in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system (Avnimelech 1999, Browdy et al. 2001). The heterotrophic bacterial population utilizes the inorganic N to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by carp, tilapia (Schroeder 1987, Beveridge et al. 1989, Rahmatulla and Beveridge 1993) or shrimp (Burford et al. 2004, Hari et al. 2004) and may also by freshwater prawn, thus lowering the demand for supplemental feed protein (Avnimelech 1999). The non-utilized protein is excreted as ammonium that often limit prawn post-larvae growth and even lead to mortality. This problem can be overcome in heterotorphic systems by addition of carbonaceous substrates and the induction of accelerated ammonium uptake and build up of microbial proteins that can be used as an additional protein source for prawn post-larvae. Recent research (Hari et al 2004) demonstrated that a 25% protein diet supplemented with a low cost carbohydrate yielded the same production as a normally used 40% protein diet. With this point of view, the present research has been designed primarily to understand some practical information on the effects of C/N ratio control by addition of low cost carbohydrate to the water column on (i) Water quality (ii) water and sediment bacterial quality and (iii) plankton production in freshwater prawn post-larvae nursing system.

Materials and methods

The experimental ponds were divided into 4 treatments and 4 randomly allocated ponds were used for each treatment. The experiment had the two levels of dietary protein (20% and 35%) without carbohydrate addition as first and second treatments and two level of dietary protein with carbohydrate addition to the water column as third and fourth treatments. The treatments without carbohydrate addition are abbreviated as 'P20' and 'P35', while the treatments with carbohydrate addition are abbreviated as 'P20+CH' and 'P35+CH'.

Experimental setup

The field trial was carried out for a period of 60 days from 29 July to 28 September 2006 at the Fisheries Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh. A long pond (83×8.9 m) was drained completely and partitioned by galvanized iron sheets into 18 small ponds of 40 m² each. Among these 18 small ponds, 16 ponds were used for this experiment. The ponds were prepared following the usual pre-stocking procedure which includes draining up the ponds, cleaning aquatic weeds and strengthening of dike and peripheries. The surroundings of all the ponds were fenced by 1m height nylon net to prevent the entry of predators like snakes, frogs and others. All unwanted fishes and other aquatic organisms were eradicated by rotenone application at the rate of 30 gm/40m²/ft. Quicklime (CaCO₃) was applied to the pond bottom at the rate of 250 kg/ha. Water depth was maintained 1 m throughout the experimental period. The ponds were fertilized with semi-decomposed cattle manure, urea and triple super phosphate (TSP) at the rate of 1,000, 50 and 50 kg/ha, respectively. A shelter built by bamboo kanchi with palm leaves consists of three floors was installed in each pond before prawn PL stocking to provide shelter for the prawn PL. Twenty days old post-larvae (PL 20) of M. rosenbergii (0.021±0.001g) purchased from the Bangladesh Fisheries Research Institute (BFRI) hatchery, Mymensingh were stocked at a density of 20 PL/m^2

Two types of experimental diets were formulated to contain 20% and 35% protein using fishmeal, soybean meal, mustard oil cake, wheat flour, and rice bran. At the early stage, homogenously mixed ingredients were directly supplied to the ponds without making pellet feed. After 20 days of culture, homogenously mixed ingredients were made into dough and finally made into pellets using a kitchen type pellet machine. Maize flour collected from the local market was used as carbohydrate source. The quantity of carbohydrate (CH) added was calculated following Eq. (1) (Avnimelech 1999), and assuming that the added carbohydrate contains minimum 50% carbon, the CH addition needed (Δ CH) to reduce total ammonia nitrogen concentration by 1 g N /m³ is 20g/m³.

(1) $\Delta CH = \Delta N/0.05$

It can be assumed that the ammonium flux into water, ΔNH_4^+ , directly by excretion or indirectly by microbial degradation of the feed residues, is roughly 50% of the feed nitrogen (Avnimelech 1999):

(2) ΔN = Quantity of feed \times % N in feed \times % N in excretion

The amount of carbohydrate addition needed to assimilate the ammonium flux into microbial protein is calculated using Eqs. (1) and (2):

(3) $\triangle CH = Quantity of feed \times \% N in feed \times \% N in excretion/0.05$

According to the Eq. (3), 0.32 kg maize flour was administrated per kg of the 20% protein diet and 0.56 kg maize flour per kg of the 35% protein diet fed.

Feeding rate was calculated from the average weight of prawn during fortnightly sampling. Prawn's daily feeding rates were 25% body weight at the start of the experiment, and declined gradually to 10% at the end of the culture period. Feed was distributed evenly over the pond's surface, twice daily at 08.00 and 18.00 hours. The preweighed maize flour was mixed in a beaker with pond water and uniformly distributed over the pond's surface followed by the first feeding. At the end of the experiment prawn juveniles were harvested draining the ponds completely.

Water and sediment quality parameters

Throughout the experimental period, the water and sediment quality parameters were recorded fortnightly. Temperature (YSI model 58), transparency (Secchi disc), dissolved oxygen (YSI model 58) and pH (Jenway, model 3020) were measured between 9.00 and 10.00 am at the pond site. Water samples were collected using a horizontal water sampler from three locations of each pond and pooled together. Sediment samples were collected from three locations using PVC pipe (4 cm diameter). Composite water column samples were filtered through a GF/C Whatman glass fiber and the filtrate was analysed for nitrate nitrogen (NO₃-N), nitrite nitrogen (NO₂-N), ammonia nitrogen (NH₃-N) and phosphate phosphorus (PO₄-P) by HACH Kit (DR/2010, a direct reading spectrometer). Chlorophyll-a in non-filtered water column samples was estimated following the standard method (APHA 1995). Total heterotrophic bacteria (THB) count in water and sediment was estimated following the standard procedures (APHA 1995) and expressed as colony forming units (cfu).

Assessment of plankton

Plankton samples were collected fortnightly from each pond. Ten liters of water sample was taken from different places and depths of the pond and passed through fine $(25 \ \mu)$ mesh plankton net. Filtered samples were taken into a measuring cylinder and carefully made up to a standard volume of 50 ml. Then the collected plankton samples were preserved in 5% buffered formalin in small plastic bottles. From each 50 ml preserved sample, 1 ml sub-sample was examined using a Sedge Wick-Rafter cell (S-R cell) and a binocular microscope (Olympus CH-40) with phase contrast facilities. One ml sub sample from each sample was transferred to the cell and then all planktonic organisms present in 10 squares of the cell chosen randomly were identified and counted. Plankton identification was performed following APHA (1992) and Bellinger (1992). For each pond, mean number of plankton was recorded and expressed numerically per liter of water. The quantitative estimation of plankton was done using the following formula :

$$N = \frac{A \times 1000 \times C}{V \times F \times L} \quad (Rahman 1992)$$

Where, N = Number of plankton cells or units per liter of original water,

A = Total number of plankton counted,

C = Volume of final concentrate of the sample in ml,

- V = Volume of field in cubic mm,
- F = Number of field counted and
- L = Volume of original water in liter.

Statistical analysis

For the statistical analysis of the data, one-way ANOVA and DMRT were done by using the SPSS (Statistical Package for Social Science) version-10.0. Significance was assigned at the 0.05% level. Duncan's test was used to tests the results of multiple ranges for comparisons of averages.

Results

Water and sediment quality parameters

The mean temperature, pH, dissolved O_2 , nitrate-nitrogen, phosphate-phosphorus and chlorophyll-a of the treatments were in the range of 28.17 to 28.92 °C, 7.51-7.91, 5.95-7.01mg/l, 0.02-0.031 mg/l, 0.445-0.599 mg/l and 190.88-235.44 mg/l, respectively. There was no significant differences (p>0.05) in any of the above water quality parameters among the treatments. Water quality parameters are summarized in Table-1. Carbohydrate addition significantly reduced (p<0.05) the NH₃-N and NO₂-N in the experimental ponds.

Table 1. Means $(\pm SE)$ of fortnightly water quality parameters recorded from different treatments.
Values of each treatment are means of 5 sampling dates and four ponds (N=20)

Properties	Treatments				
	P20	P20+CH	P35	P35+CH	ANOVA
Transparency (cm)	27.85 ±1.2ª	27.10±1.2ª	21.70 ± 0.9^{b}	28.00±1.7ª	NS
Temperature (°C)	28.17 ± 0.43	28.22 ± 0.42	28.92 ± 0.28	28.75 ± 0.38	NS
pН	7.91	7.51	7.63	7.63	NS
DO (mg/l)	7.01 ± 0.4	5.95 ± 0.5	6.41 ± 0.4	6.78 ± 0.5	
NO ₃ -N (mg/l)	0.020 ± 0.002	0.024 ± 0.002	0.031 ± 0.009	0.021 ± 0.002	NS
$PO_4 - P (mg/l)$	0.479 ± 0.053	0.445 ± 0.052	0.599 ± 0.046	0.504 ± 0.059	NS
NO ₂ -N (mg/l)	0.053 ± 0.005^{a}	0.017 ± 0.002^{b}	0.058 ± 0.005^{a}	0.015 ± 0.006^{b}	*
NH ₃ -N (mg/l)	0.418 ± 0.034^{b}	$0.203 \pm 0.019^{\circ}$	0.569 ± 0.042^{a}	$0.223 \pm 0.021^{\circ}$	*
Chlorophyll <i>a</i> (µg/l)	206.58 ± 28.5	190.88 ± 21.58	235.44 ± 18.11	205.15 ± 24.68	NS

* p<0.05, NS - not significant. Means with the different superscripts are significantly different (p <0.05)

The total number of bacteria varied from 17.1 to 58.3 ($\times 10^{6}$ cfu/ml) in water column and 10.9 to 68.4 ($\times 10^{8}$ cfu/g) in pond bottom sediment during the culture period. The highest number of bacterial load was observed in treatment P35+CH on 25 September, 2006 and the lowest number of bacterial load in water column was observed in treatment P35 on 29 July, 2006. The result of the ANOVA showed that the addition of the carbohydrate source had a significant (p < 0.05) effect on the total heterotrophic bacterial (THB) count and it promoted the growth of THB population in water column and pond sediment (Table 2). The THB count in the water increased (P < 0.05) with culture period both in water (Figure-1a) and sediment (Figure 1b).

Table 2. Mean \pm S.E of fortnightly variation in the bacterial load (APC) in water column and bottom sediment of the different treatments

Sampling Treatments					
Period	P20	P20+CH	P35	P35+CH	ANOVA
Bacterial load in	n water column (2	×10 ⁶ cfu ml ⁻¹)			
29 July	18.67 ± 0.91	20.12 ± 0.84	19.27 ± 0.95	21.20 ± 1.01	NS
13 August	$20.22 \pm 0.49^{\circ}$	25.37 ± 1.65^{ab}	23.52 ± 0.86^{bc}	25.42 ± 1.17^{a}	*
28 August	21.57 ± 0.59^{a}	25.85 ± 2.52^{b}	24.87 ± 0.97^{bc}	34.65 ± 1.66^{a}	*
12 September	22.27±0.43ª	33.32 ± 2.66^{b}	27.32±0.73 ^{bc}	44.10 ± 3.61^{a}	*
25 September	23.77±0.64°	36.72 ± 2.98^{b}	30.17±0.76°	54.27 ± 2.85^{a}	*
Bacterial load in	n bottom sedimer	nt (×10 ⁸ cfu g ⁻¹)			
29 July	13.00 ± 0.55^{ab}	12.57±0.59 ^b	11.80 ± 0.39^{b}	$14.45 \pm 0.27^{\circ}$	*
13 August	17.40±1.71°	21.75±1.41 ^b	18.20 ± 0.82^{bc}	26.15 ± 0.80^{a}	*
28 August	22.67 ± 1.95^{b}	27.50±1.64 ^b	24.67±0.85 ^b	35.80 ± 1.88^{a}	*
12 September	26.95 ± 2.07^{b}	32.60 ± 2.10^{b}	30.35 ± 1.35^{b}	48.10 ± 3.12^{a}	*
25 September	33.50 ± 2.74^{b}	39.02±1.68 ^b	38.02±1.71 ^b	61.25 ± 3.53^{a}	*

* p<0.05; NS - not significant. Means with the different superscripts are significantly different (p<0.05)

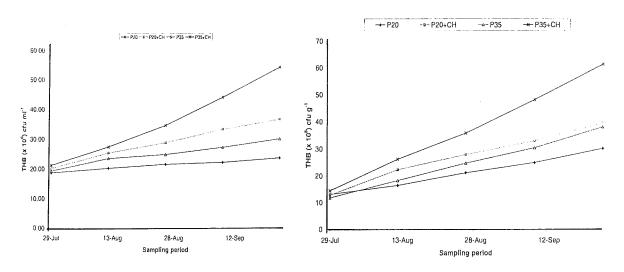


Fig. 1a. Variation of THB in the pond water.

Fig. 1b. Variation of THB in the sediment.

Plankton production

Plankton populations in the water of the experimental ponds were enumerated and identified upto genus level. It consists of 59 genera belonging to 7 planktonic groups.

Forty three genera of phytoplankton belonging to Bacillariophyceae (11), Chlorophyceae (21), Cyanophyceae (7), and Euglenophyceae (3) and Dinophyceae (1) were isolated from the experimental ponds. Sixteen genera of zooplankton were also identified which belonged to Rotifera (7) and Crustacea (9). Mean abundance of different phytoplankton and zooplankton group were not significantly different (p > 0.05) among the treatments.

Groups	Treatments				
	P25	P25+CH	P35	P35+CH	ANOVA
Bacillariophyceae	11.6±1.6	10.9±1.7	15.7 ± 0.7	13.7±1.2	NS
Chlorophyceae	44.2 ± 3.2	51.9 ± 3.1	52.7 ± 3.9	54.0 ± 5.0	NS
Cyanophyceae	18.2 ± 1.1	18.3 ± 1.3	21.0 ± 1.2	19.2 ± 1.3	NS
Euglenophyceae	2.4 ± 0.3	2.2±0.3	2.4 ± 0.3	2.1 ± 0.3	NS
Dinophyceae	6.9 ± 0.9	5.7 ± 0.5	6.4 ± 0.6	4.7 ± 0.4	NS
Total phytoplankton	457.0 ± 32.1	526.3 ± 33.0	529.9±37.4	546.6 ± 49.6	NS
Crustacea	4.6 ± 0.5	5.8 ± 0.6	7.2 ± 0.6	7.0 ± 0.5	NS
Rotifera	6.2 ± 0.7	6.4 ± 0.6	6.2 ± 0.5	6.4 ± 0.5	NS
Total zooplankton	18.5 ± 2.0	19.2 ± 1.8	18.4 ± 1.5	19.2 ± 1.6	NS

Table 3. Mean abundance ($\times 10^3$ cells/l) of phytoplankton and zooplankton with their groups among the treatments

NS - not significant

Growth performances of prawn PL

At the begining of the study, the mean initial weights (g) were same (0.021 g) but the mean final weight of the harvested prawn juveniles were 2.51 ± 0.08 , 4.42 ± 0.11 , 4.34 ± 0.17 and 5.73 ± 0.10 g in treatment P20, P20+CH, P35 and P35+CH, respectively. The mean weight gain found to vary from 2.29 to 2.66 g, 4.09 to 4.62 g, 3.98 to 4.65 g and 5.47 to 5.95 g in treatment P20, P20+CH, P35 and P35+CH, respectively. The mean survival rates were found at 54.87 \pm 3.40, 66.55 \pm 2.23 and 68.60 \pm 5.06 and 70.77 \pm 4.70% in treatment P20, P20+CH, P35 and P35+CH, respectively. Survival rate was significantly higher (p<0.05) in P35+CH than P20, P20+CH and P35 treatments, with no significant differences (p>0.05) between treatment P20+CH and P35.

Growth	Treatments				
parameters	P25	P25+CH	P35	P35+CH	ANOVA
Initial weight (g)	0.021 ± 0.0001	0.021 ± 0.0001	0.021 ± 0.0001	0.021 ± 0.0001	*
Final weight (g)	$2.51 \pm 0.08^{\circ}$	4.42 ± 0.11^{b}	4.34 ± 0.17^{b}	5.73 ± 0.10^{a}	NS
Weight gain (g)	$2.5 \pm 0.08^{\circ}$	4.4 ± 0.11^{b}	4.3 ± 0.17^{b}	5.7 ± 0.10^{a}	NS
Survival (%)	54.9±3.4°	66.6±2.2 ^b	68.6 ± 5.06^{b}	70.8 ± 4.7^{a}	NS

Table 4. Growth performances (Mean ± S.E) of prawn post-larvae in different treatments

* p<0.05; NS - not significant. Means with the different superscripts are significantly different (p< 0.05)

Discussion

In the present study, the addition of carbohydrate to the water column increased the C/N ratio. Higher dietary protein levels resulted in significantly higher NH3-N and NO₂-N concentrations in the water column. Li and Lovell (1992) reported that the ammonia concentration increased with increasing dietary protein concentration and protein feeding rate. By adding CH to the nursing ponds, NH₃-N and NO₂-N concentrations in the water column were significantly reduced. This agrees with Avnimelech and Mokady (1988), Avnimelech et al. (1989) and Avnimelech (1999) who reported that the addition of carbohydrate to intensively well-mixed production systems will reduce the total ammonia nitrogen (TAN) concentration through immobilization by bacterial biomass. In the present study, extensively managed small ponds were used, which means a minimum water exchange and no mixing of the water column. Under such extensive conditions, CH addition to the water column also resulted in a significant increase in the THB count, together with observed lower NH₃-N concentrations in water. CH addition also caused a significant reduction in NO2-N concentration in the water column, which can be attributed to low availability of NH₃-N as substrate for nitrification and hence the production of NO₂-N (Avnimelech 1999, Hari et al. 2004). The higher nitrite values recorded in the P35 treatment also revealed the possibility of more nitrification. The nitrification ultimately leads to the formation of NO₃-N, so one would expect also to find differences in nitrate concentration as a result of CH addition. However, the nitrate level was not influenced by CH addition, and the underlying mechanism needs further study.

In freshwater prawn nursing systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon,1982, Diab and Shilo, 1988). In the present study no significant differences were observed in chlorophyll-a concentration between treatments. Thus the reduction in NH_3 -N and NO_2 -N levels observed in CH added treatments could only be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari *et al.* 2004).

The comparable growth performances in treatments P20+CH and P35 shows the possibility of reducing the dietary protein level in favor of addition of CH to the water column without any significant reduction in growth performances (Hari *et al*, 2004). The use of low protein feed led to a significant reduction in the feed based inorganic nitrogen accumulation in the pond (Li and Lovell 1992). Furthermore the addition of CH enhances the THB in the pond, which in turn result further reduction in inorganic N. Thus the low toxic inorganic N levels in the pond (Wahab *et al.* 2003) and utilization of microbial cells as feed act as favorable factors for the augmented better growth performances of prawn post-larvae in P20+CH treatment (Avnimelech 1999, Burford *et al.*, 2004). Better survival rates in P20+CH and P35+CH treatments showed that water quality were favorable for *M. rosenbergii* cultivation (Hariati *et al.* 1996), and suggest that differences in growth and survival are related to food quality and food availability. Allan *et al.* (1995) recorded faster growth of prawns in well-prepared ponds with an abundant meiofanua. In the present study, a similar phytoplankton biomass was present

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in all the treatments as indicated by chlorophyll-a concentrations, and qualitative and quantitative study of phytoplankton, but THB counts both in the water column and the sediment, were higher in CH added ponds. Utilization of microbial protein depends upon the ability of the target animal to harvest the bacteria and to digest and utilize the microbial protein (Avnimelech 1999; Burford *et al.* 2004, Hari *et al.* 2004). The higher growth performances in the CH added treatments of the present study showed that *M. rosenbergii* PL can well utilize the additional bacterial protein as a result of CH addition.

The higher bacterial population and reduced level of inorganic nitrogen in the carbohydrate added treatments revealed that the maize flour is a good source of organic carbon as it was well utilized by the heterotrophic bacterial population. In previous studies, several other carbohydrate sources like glucose and cassava meal cellulose powder (Avnimelech and Mokady 1988, Avnimelech *et al.* 1989, Avnimelech *et al.* 1994 and Avnimelech *et al.* 1999), molasses (Burford *et al.* 2004) and tapioca flour (Hari *et al.* 2004) in fish and shrimp pond to reduce the TAN. In the present study, a more practical approach was made by selecting the maize flour as carbohydrate source, due to its low cost (Tk. 10 kg⁻¹), easy availability, low protein content and wide acceptance by the farmers as one of the potential feed ingredients.

In summary, Carbohydrate addition to freshwater prawn nursery ponds reduced the levels of potentially toxic NH_3 -N and NO_2 -N in the water columnt. The protein level in the diet can be reduced from 35% to 20%, without compromising growth performance and survival of prawn post-larvae, if CH is added to the water column to enhance heterotrophic bacterial protein production. C/N ratio control improved the water quality of freshwater prawn nursing system through (1) reduced demand for feed protein and (2) reduced concentrations of potentially toxic NH_3 -N and NO_2 -N in the system.

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