

Original Paper

SHRIMP CULTURE OF *Penaeus monodon* WITH ZERO WATER EXCHANGE MODEL (ZWEM) USING MOLASSES

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ABSTRACT

One of the main obstacles to develop an intensive aquaculture is the accumulation of toxic inorganic nitrogen which should be kept very low by frequent water exchange or recycling of the water through a biofilter. This study describes another method of removing inorganic nitrogen using heterotrophic bacteria population of which was augmented by the addition of a carbonaceous substance, molasses, to increase the feed C:N ratio under laboratory condition. The principal aim of study was to establish correlation C:N ratio level with levels of ammonia, nitrite, dissolved oxygen, pH and shrimp growth in *Penaeus monodon* shrimp culture with Zero Water Exchange Model (ZWEM) using molasses as carbon resource. It was found that addition of molasses to shrimp farming with ZWEM had a role in removing ammonia and nitrite. Also, application of molasses to laboratory tanks increased the growth and percentage weight gain of shrimps and increased the population of heterotrophic bacteria.

Keywords: Inorganic nitrogen; heterotrophic bacteria; zero water exchange model; molasses; C:N ratio level

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INTRODUCTION

Water exchange is routinely used in mitigating ammonia, nitrite and organic matters concentrations and preventing algal blooms in conventional intensive shrimp culture. This water exchange management practice works only when the quality of inlet water into ponds is high. However, this is not the case for most shrimp ponds in Asian-Pacific region because the water quality of the water entering into pond is the effluent from outlet of the other farms (Landesman, 1994; Kautsky *et al.*, 2000) which already has low quality (Csavas, 1994) and contain parasites and pathogenic microorganisms (Csavas, 1994; Landesman, 1994). Also, problem in water exchange for improvement of water quality is frequently due to poor engineering design and management of ponds (Rivera-Monroy *et al.*, 1999) and the absence of drainage networks (Csavas, 1994).

The practice of flushing water from shrimp farms into water body is also detrimental to environment because this water is nutrient rich and cause eutrophication algae blooms

(Landesman, 1994; Hopkins *et al.*, 1995; Smith 1996). It also has high in suspended, particulate and dissolved organic (Hopkins *et al.*, 1993; 1995; Avnimelech *et al.*, 1994; Kochva *et al.*, 1994; Avnimelech *et al.*, 1998; Avnimelech, 1999), high levels of pathogenic microorganisms (Hopkins *et al.*, 1995; Smith, 1996; Chamberlain, 2001), high-biological oxygen demand and low available dissolved oxygen (Hopkins, *et al.*, 1995; Csavas, 1994; Smith, 1996) as well as high levels of chemicals such as hydrogen sulfide (Smith, 1996) and chemical residue which has potential to create resistant pathogenic microorganisms (Landesman, 1994; Hopkins *et al.*, 1995). Further, frequent water may not be possible due to environmental regulations prohibiting the release the nutrient rich water into the environment which may contain pathogens (Avnimelech, 1999).

In current years, shrimp production of brackishwater ponds sharply drops due to decrease in production rate and area cultivated.

Factors such as diseases, pollution, environmental degradation and poor pond management were identified as the causes of this decrease in shrimp production (Rosenberry, 1993). Furthermore, most shrimp-producing countries worldwide have been profoundly affected by diseases like whitespot due to degradation of water quality (Hopkins *et al.*, 1993; Tseng *et al.*, 1998; Chamberlain, 2001; Tacon, 2001; Burford *et al.*, 2003).

Mitigating the environmental impacts of effluent discharge and reducing the risk of disease contamination from externally polluted water supply, shrimp culture in recent years has evolved from open system with frequent water discharge to closed system with limited or zero water discharge. However, the major problem associated with closed system is the rapid eutrophication in ponds, resulting from increasing concentrations of nutrients (ammonia and nitrite) and organic matters over the culture period (Thakur and Lin, 2003). Likewise, Tacon *et al.*, (2002) revealed that closed zero-water exchange culture systems can only biologically support a certain level of nutrient input and shrimp biomass without the system 'crashing' and compromising shrimp growth and survival. Obviously, the balance between waste production and assimilation capacity in pond environment is of paramount importance for the success of closed system. Thus, shrimp cultures with zero water exchange through growing heterotrophic bacteria by using carbon material are essentially required to take full account of waste impact on growth of culture organisms, mortality and the overall expansion of total biomass in the production system.

Heterotrophic bacteria in aquaculture system may use nitrogen in uneaten whole feed, feed disintegrate in particles and faeces, which hereafter will be referred to as nitrogen in detritus (Wheeler and Kirchman 1986). The addition of monosaccharides such as glucose can stimulate NH_4^+ uptake by heterotrophic bacteria in marine waters (Hoch *et al.*, 1994).

The addition of carbon material can reduce inorganic nitrogen in fish experimental containers (Avnimelech, 1999), simultaneously produce single cells protein for fish (Avnimelech and Mokady 1988; Avnimelech *et al.*, 1989), reduce feeding and pumping costs (Avnimelech *et al.*, 1992; 1994; Kochva *et al.*, 1994; Avnimelech, 1999), the sources of carbon

matter used in ponds can be sorghum and wheat meal (Avnimelech *et al.*, 1994).

Thus, this study provides essential scientific information for several questions: Does application of molasses have benefit in shrimp culture with ZWEM? What is the best level of feed C:N ratio in terms of water quality and shrimp production variables? Is shrimp culture with ZWEM using molasses likely to be one novel technology in coping with major problems in present farming? Thus effect of feed C:N ratio levels on water quality and shrimp production variables in *Penaeus monodon* shrimp culture with ZWEM is substantially required in order to answer these questions above.

MATERIALS AND METHODS

The experimental shrimp cultures

Experiment was conducted in 160 litre experimental tanks with no water exchange during culture period (from 28 August 2001 until 23 October 2001) located at the aquaculture outdoor laboratory of Charles Darwin University, Australia. The water was aerated with air-stones and fertilized two weeks before stocking shrimps to stimulate phytoplankton growth. The fertilizers consisted of sodium nitrate (NaNO_3) at the rate of 1.67 mg L^{-1} , phosphoric acid (85 % H_3PO_4) at rate of $6 \times 10^{-4} \text{ ml L}^{-1}$ and 110 mg L^{-1} of soda ash (NaCO_3).

The protocol used in the experiment were: (1) without molasses with C:N ratio = 6.5 (ZWEM_{6.5}), (2) using molasses with C:N ratio = 15.0 (ZWEM_{15.0}), (3) using molasses with C:N ratio = 17.5 (ZWEM_{17.5}), (4) using molasses with C:N ratio = 20.0 (ZWEM_{20.0}) and (5) using molasses with C:N ratio = 22.5 (ZWEM_{22.5}). Each treatment had three replications and tank allocation for every treatment was completely randomized. Tanks had three pieces of air stone suspended in bottom of water column. The tank volume of 160 litres was maintained constant by adding 2 litres of freshwater (tap water) weekly to replace loss water due to evaporation.

Each tank was stocked with 4 shrimp or equivalent to shrimp density of 30 shrimp m^{-2} (Allan and Manguire, 1992). The mean individual weight at the stocking time was $5.014 \pm 0.336 \text{ gram}$ and shrimps were fed with

commercial shrimp feed (Taiwan Company Product) with crude protein content of 38 %. Feeding rate applied in the experiment was 5 % of body weight per day during study (as recommended by the feed company) and the amount of molasses applied in each tanks was adjusted in accordance with treatment (the level of feed C: N ratio) and daily feeding rate. Shrimp feed applied to each tank twice daily (50 % of the total feed required at 08.00 am and 06.00 pm) as conducted in fish culture ponds by Avnimelech *et al.*, (1992) while molasses was applied once daily at 08.00 am.

The levels of feed C:N ratio were calculated by dividing total input carbon with total input nitrogen used in shrimp cultures (Avnimelech *et al.*, 1989; Avnimelech *et al.*, 1992a; Avnimelech *et al.*, 1994; Kochba *et al.*, 1994; Avnimelech, 1999). The main carbon source of shrimp culture was from the feed and molasses, while the main nitrogen source was feed. Furthermore, the nitrogen content of feed was determined by the assumption that 30 % protein feed containing 4.65% nitrogen (Avnimelech, 1999). The nitrogen content of feed with more than 30 % protein, therefore, it was calculated by conversion of that assumption while the carbon content of feed and molasses used in each study was 38.5% and 29.71 % respectively. The amount of molasses required per 1 gram of feed in each treatment, therefore, was 0.00, 1.68, 2.17, 2.67 and 3.16 gram for ZWEM_{6.5}, ZWEM_{15.0}, ZWEM_{17.5}, ZWEM_{20.0} and ZWEM_{22.5} respectively. Furthermore, heterotrophic bacteria implemented in every treatment were from nature bacteria of sea water used (Avnimelech, 1999).

Water quality measurements and analyses

Salinity, temperature, pH and dissolved oxygen in water were measured using a Horiba water quality checker (U-10 Model). Before measurement of those parameters, the Horiba water quality checker was manually calibrated as described in the instruction manual.

Ammonia, nitrite and nitrate concentrations in water were measured photometrically using a Palintest Photometer, based on indophenol method, diazotization method, and cadmium reduction/diazotization method for ammonia, nitrite and nitrate respectively.

Levels of viable heterotrophic bacteria were determined by counting the colonies which grew on plates of Tryptone Soya Agar (TSA) with 10 % of NaCl (Johnsen *et al.*, 1993). Before plating each sample onto agar medium, serial dilutions were made in physiological saline solution composed of 9 % NaCl (Sohier and Bianchi, 1985). Levels of bacteria are quoted in colony forming units per ml of water (CFU ml⁻¹)(Smith, 1998).

The amount of water used (litre) to produced one gram shrimp (water consumption rate) was determined by dividing the total water amount used in every shrimp culture during growing period (litre) with the shrimp production (gram) at the end of study. Additionally, total concentrations of ammonia, nitrite and nitrate discarded at the harvest time from experiment tanks as primary importance variable in discharge regulation (Hopkins *et al.*, 1993) were determined by multiplying the concentration of those variables recorded at the end of study with total volume of experiment tanks.

Shrimp survival rate, growth, percentage weight gain and feed conversion ratio

Experiment of shrimp cultures in laboratory was conducted for eight-week duration. Every two weeks, the total body weight of shrimp (W) was measured for each experimental container. Similarly, the number of live shrimp (N) in each tank was counted. Further the amount of feed used in each tank (Wf) was recorded. The average body weight (W_a) were calculated by dividing W by N. The overall average values of survival rate (%), growth rate of shrimp (gram/day), percentage weight gains (%), and feed conversion ratios (FCR) were determined by the following equations below as used in common aquaculture studies (Balazs, 1973; Bages and Sloane, 1981; Tseng *et al.*, 1998).

$$1. \text{Survival Rate (\%)} = \frac{(N_o - N_t)}{N_o} \times 100 \%$$

$$2. \text{Growth Rate (gram/day)} = \frac{(W_{at} - W_{ao})}{t}$$

$$3. \text{Percentage Weight Gain (\%)} = \frac{(W_{at} - W_{ao})}{W_{ao}} \times 100\%$$

$$4. \text{Feed Conversion Ratio (FCR)} = (\sum Wf) / \Delta W$$

Where N_0 and N_t are the number of shrimps cultured in each tank at initial time (t_0) and time t ; W_{at} and W_{a0} are the average body weight of shrimps at initial time (t_0) and time t , t is period time of raising shrimps, $\sum W_f$ is the total amount of feed used in each tank, and ΔW is the increment of the total weight of shrimps in each tank for t time culture.

The water minimum, maximum and mean temperature of shrimp culture throughout the study were 25.9, 28.9 and 27.4 °C, respectively while water minimum, maximum and mean salinity of shrimp culture were 25.01‰, 27.90‰ and 27.20‰, respectively.

Data analysis

Data of laboratory study were analysed using Statistic Version 6.1 software and with one-way ANOVA (Steel and Torrie, 1980) to evaluate the effects of each treatment. The homogeneity of variance and normality of all data sets were tested using Cochran's test. Tukey test was used to differentiate among the treatment means of each experiment after ANOVA analysis (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of different treatments on concentrations of inorganic nitrogen

The levels of feed C: N ratio had significant effect on the concentrations of ammonia and nitrite (**Tables 1**). Concentrations of ammonia and nitrite in the experiment significantly decreased as the levels of feed C: N ratio increased except the levels of ammonia in ZWEM_{17.5} treatment were significantly higher compared to ZWEM_{15.0} ($P < 0.05$), and there was no significant difference in the concentrations of ammonia and nitrite between ZWEM_{20.0} and ZWEM_{22.5} ($P > 0.05$).

These results implied that molasses addition as a carbon source obviously had a role in inorganic nitrogen reduction through stimulating the growth of bacteria because numbers of bacteria increased in response to levels of C:N ratio inclined (as described below). Further, concentrations of ammonia and nitrite had a negative association with the numbers of bacteria. These findings are in

agreement to those results investigated by some authors (Tezuka, 1990; Hoch *et al.*, 1994) who established that addition of carbon diminished inorganic nitrogen due to increasing uptake of NH_4^+ by bacteria. Also it was reported earlier that zero water exchange ponds using carbon enable to control the accumulation of inorganic nitrogen through a balanced ratio of carbon to nitrogen of the feed (Avnimelech *et al.*, 1989; 1992; 1994; Avnimelech, 1998; 1999). In addition Stuart *et al.*, (2009) raised tiger shrimp *Penaeus monodon* in zero water exchange model using a daily carbon source (tapioca powder) to promote the microbial community and improve water quality.

Several previous studies also proved that carbon and nitrogen ratio obviously influenced rates of NH_4^+ uptake and inorganic nitrogen by bacteria in aquaculture systems (Avnimelech *et al.*, 1992, 1994; Hoch and Kirchman, 1995; Kochva *et al.*, 1994; Avnimelech, 1999; Montoya *et al.*, 2002), in experiments of Oceanography (Findlay, 1989; Kirchman *et al.*, 2000), and in Marine Ecology (Middleboe *et al.*, 1995). Furthermore, Goldman *et al.*, (1987) found no inorganic nitrogen at organic substrate having C : N ratio level of higher than 10.0:1. Similarly, Tezuka (1990) reported that the amount of nitrogen regenerated increased with decrease C:N ratio level of organic substrates and there was no regenerated ammonia when C:N ratio level of organic substrate was more than 15.0:1.

Effect of different treatments on concentrations of dissolved oxygen

The results shows that the levels of feed C: N ratio significantly affected the concentrations of dissolved oxygen (**Table 1**). Further statistical analysis indicates that the values of water dissolved oxygen decreased significantly with increasing levels of feed C: N ratio ($P < 0.05$). The concentrations of dissolved oxygen in experiment tended to decrease during the study period. The explanation for this result could be due to that increasing feed C:N ratio levels in shrimp cultures stimulated growth of bacteria that in turn required oxygen for their growth, subsequently, there was a decrease in dissolved oxygen concentrations with feed C:N ratio levels increased.

Table 1. The effect of feed C:N ratio levels on several water quality and shrimp production variables of shrimp culture with ZWEM using molasses.

Water Quality Variable	Treatment	Mean	Std. Dev
1. Ammonia (mg/litre)	1. Without Molasses with C:N Ratio = 6.5	1.1059	□ 0.0152 ^a
	2. Molasses with C:N Ratio = 15.0	0.3483	□ 0.0488 ^b
	3. Molasses with C:N Ratio = 17.5	0.9205	□ 0.0376 ^c
	4. Molasses with C:N Ratio = 20.0	0.0839	□ 0.0177 ^d
	5. Molasses with C:N Ratio = 22.5	0.0870	□ 0.0078 ^d
2. Nitrite (mg/litre)	1. Without Molasses with C:N Ratio = 6.5	45.0736	□ 0.0341 ^a
	2. Molasses with C:N Ratio = 15.0	2.8622	□ 0.0221 ^b
	3. Molasses with C:N Ratio = 17.5	0.1441	□ 0.0400 ^c
	4. Molasses with C:N Ratio = 20.0	0.0776	□ 0.0133 ^d
	5. Molasses with C:N Ratio = 22.5	0.0490	□ 0.0035 ^d
3. Dissolved Oxygen (mg/litre)	1. Without Molasses with C:N Ratio = 6.5	5.51	□ 0.04 ^a
	2. Molasses with C:N Ratio = 15.0	4.98	□ 0.04 ^b
	3. Molasses with C:N Ratio = 17.5	4.79	□ 0.12 ^c
	4. Molasses with C:N Ratio = 20.0	4.28	□ 0.20 ^d
	5. Molasses with C:N Ratio = 22.5	4.14	□ 0.04 ^e
4. Number of Heterotrophic Bacteria (CFU/ml)	1. Without Molasses with C:N Ratio = 6.5	2.31 X10 ⁹	□ 1.48 X10 ^{8a}
	2. Molasses with C:N Ratio = 15.0	8.48 X10 ⁹	□ 5.49 X10 ^{8b}
	3. Molasses with C:N Ratio = 17.5	1.09 X10 ¹⁰	□ 3.26 X10 ^{8c}
	4. Molasses with C:N Ratio = 20.0	1.27 X10 ¹⁰	□ 4.40 X10 ^{8d}
	5. Molasses with C:N Ratio = 22.5	1.74 X10 ¹⁰	□ 6.00 X10 ^{8e}
5. pH	1. Without Molasses with C:N Ratio = 6.5	7.21	□ 0.05 ^a
	2. Molasses with C:N Ratio = 15.0	8.16	□ 0.04 ^b
	3. Molasses with C:N Ratio = 17.5	8.01	□ 0.04 ^c
	4. Molasses with C:N Ratio = 20.0	7.86	□ 0.03 ^e
	5. Molasses with C:N Ratio = 22.5	7.73	□ 0.06 ^e
6. Shrimp Survival Rate (%)	1. Without Molasses with C:N Ratio = 6.5	83.33	± 7.63 ^a
	2. Molasses with C:N Ratio = 15.0	100.00	± 0.00 ^b
	3. Molasses with C:N Ratio = 17.5	100.00	± 0.00 ^b
	4. Molasses with C:N Ratio = 20.0	100.00	± 0.00 ^b
	5. Molasses with C:N Ratio = 22.5	100.00	± 0.00 ^b
7. Shrimp Growth Rate (gram/day)	1. Without Molasses with C:N Ratio = 6.5	0.133	± 0.035 ^a
	2. Molasses with C:N Ratio = 15.0	0.154	± 0.004 ^b
	3. Molasses with C:N Ratio = 17.5	0.161	± 0.004 ^c
	4. Molasses with C:N Ratio = 20.0	0.198	± 0.003 ^d
	5. Molasses with C:N Ratio = 22.5	0.172	± 0.004 ^e
8. Percentage Weight Gain (%)	1. Without Molasses with C:N Ratio = 6.5	108.105	± 8.322 ^a
	2. Molasses with C:N Ratio = 15.0	152.264	± 5.426 ^b
	3. Molasses with C:N Ratio = 17.5	164.214	± 11.581 ^c
	4. Molasses with C:N Ratio = 20.0	212.995	± 3.300 ^d
	5. Molasses with C:N Ratio = 22.5	176.946	± 7.931 ^e
9. Feed Conversion Ratio	1. Without Molasses with C:N Ratio = 6.5	3.398	± 0.257 ^a
	2. Molasses with C:N Ratio = 15.0	2.889	± 0.070 ^b
	3. Molasses with C:N Ratio = 17.5	2.715	± 0.087 ^c
	4. Molasses with C:N Ratio = 20.0	2.216	± 0.036 ^d
	5. Molasses with C:N Ratio = 22.5	2.388	± 0.118 ^e
10. Water Consumption Rate (Litre Water/Shrimp Production (gram))	1. Without Molasses with C:N Ratio = 6.5	15.2340	± 2.5692 ^a
	2. Molasses with C:N Ratio = 15.0	8.9898	± 0.6061 ^b
	3. Molasses with C:N Ratio = 17.5	5.3288	± 0.1862 ^c
	4. Molasses with C:N Ratio = 20.0	3.9223	± 0.0571 ^d
	5. Molasses with C:N Ratio = 22.5	5.3194	± 0.1541 ^c

Values are means and standard deviations of three replicates at the end of the eight-week experimental period.

This explanation can be supported by the results of present study proved that the concentrations of dissolved oxygen at the of study had a significant negative correlation with the numbers of heterotrophic (Sun *et al.*, 2001). It has been observed previously that bacteria contributed as much as 77 % of the total oxygen consumption in fish ponds (Olah *et al.*, 1987). Similarly, Visscher and Duerr (1991) investigated that microbial population consumed at high level of dissolved oxygen in shrimp ponds.

Effect of different treatments on the numbers of heterotrophic bacteria

The present study also observed that the levels of feed C:N ratio had significant affect ($P < 0.05$) on the total numbers of heterotrophic bacteria (**Table 1.**). There was a significant increase in the total numbers of heterotrophic bacteria with increasing levels of feed C: N ratio ($P < 0.05$). It revealed clearly that bacteria required carbon from molasses in order to multiply their cells. Azam *et al.*, (1983) well established that carbon such as glucose was utilized by natural bacteria. Further, the addition of glucose increased number of heterotrophic bacteria in water (Parsons *et al.*, 1981; Middleboe *et al.*, 1995). Likewise, some previous investigators (Avnimelech *et al.*, 1992; 1994; Kochva *et al.*, 1994; Avnimelech, 1999) who found that numbers of heterotrophic bacteria increased in response to increasing levels of C:N ratio. Moriarty (1986) also pointed out that there was increased number of bacteria as the result of increasing carbon in food input of penaeid prawn culture.

Effect of different treatments on the levels of pH

The levels of feed C:N ratio significantly influenced the values of pH (**Table 1**). There was a significant decrease in pH values with increasing the levels of feed C:N ratio ($P < 0.05$) except in ZWEM_{6.5} treatment. Further, the levels of pH in experiment decreased with experiment time. This result could be caused by bacteria which composed organic matters can increase the level of water inorganic carbon (CO₂) and subsequently decreased the values of pH. This view is agreement with the report documented by (Boyd, 1995; Ritvo *et al.*, 1998)

who observed that the pH usually declines as the redox potential declines as a result of microbial activity.

It could be reported that the lowest pH was in ZWEM_{6.5} treatment. The reason for this evidence was likely to be associated with the accumulation of nitrite in water. There was 45.0736 ppm of nitrite at the end of study in ZWEM_{6.5} treatment. It has been stated already that oxidizing each mole of ammonia to nitrite released two hydrogen ions which eventually reduced pH (Hargreaves, 1998). Also Tacon *et al.*, (2002) reported that a increase in nitrite caused decreased pH in shrimp culture.

Effect of different treatments on levels of shrimp survival and growth rate, percentage weight gain and feed conversion ratio

The levels of feed C: N ratio did not have significant effect on survival rates of shrimp in the experiment. The present study show that survival rates in all treatments were high (100 %) except the survival rate in ZWEM_{6.5} treatment of the experiment two where it was 83.33 %.

The levels of feed C: N ratio significantly affected ($P < 0.05$) shrimp growth rates, percentage weight gains and feed conversion ratios at the end of the experiment (**Table 1**). It was observed that in the experiment, the growth rates and percentage weight gains significantly increased and feed conversion ratios decreased with increasing the levels of feed C:N ratio except ZWEM_{20.0} treatment which had significantly higher growth rate and percentage weight gain and lower feed conversion ratio compared to those obtained in ZWEM_{22.5} treatment.

These results proved that the addition of carbonaceous material effectively removed inorganic nitrogen and produced single cell proteins in aquaculture system if available carbon sources have a carbon : nitrogen ratio of higher than 15.0:1 (Avnimelech *et al.*, 1992, 1994; Avnimelech, 1999). Further, Avnimelech (1999) reported that the growth of fishes in ponds treated with carbon : nitrogen ratio of 16.6:1 was significantly higher than those grown in ponds with carbon: nitrogen ratio of 11.1 : 1, while mortality of fishes and feed conversion and feed cost coefficient in

treatment with carbon: nitrogen of 16.6 :1 was significantly lower than in treatment with carbon : nitrogen of 11.1: 1. Also McIntosh (2000) has grown white shrimp successfully in ponds with zero water exchange model using grain-based pellet (18 % protein) with C: N ratio of 20.0:1.

These results could be due to heterotrophic communities in zero water exchange shrimp culture developed flocs of composing bacteria cells (McIntosh, 2000), and flocculation of the cells can be alone or in combination with feed particles (Harris and Mitchell, 1973; Avnimelech *et al.*, 1989). Flocs containing high protein, amino acids and certain microelements, can be directly consumed by omnivorous shrimp like white shrimps (Tacon *et al.*, 2002). Consumption of these flocs by shrimps (or fishes, see Schroeder, 1978) contributed to both the nutrition of the shrimp and efficient recycling of pond nutrient into shrimp biomass (McIntosh, 2000). Rosenberry (2001) stated that zero water exchange system produced ten times higher white shrimps than typical semi-intensive ponds and forty times higher white shrimps than typical extensive ponds. Also Stuart *et al.*, (2009) revealed that the growth of tiger shrimp *Penaeus monodon* was significantly greater when carbon were implemented in shrimp culture with zero water exchange model.

Effect of different treatments on levels of water consumption and effluent at the end of culture period

It was shown that water consumption rates was affected by feed C:N ratio levels in the both experiments (**Table 1**). Generally, the water consumption rates tended to decrease with feed C:N ratio levels increased. Similarly, there was a significant decrease in the total mass of ammonia, nitrite, nitrate, total suspended solids and organic carbon (mg per gram shrimp) discarded from harvest time with increasing feed C:N ratio levels in the two experiments excepts shrimp culture treated with C:N ratio level = 20.0:1 have the lower concentration in effluent wasted from tanks compared to those obtained in shrimp culture treated with C:N ratio level = 22.5:1 (**Table 1**).

CONCLUSIONS

The concentration of ammonia and nitrite decreased significantly with increasing level of feed C:N ratio in shrimp cultures with ZWEM using molasses as carbon resource. While growth rates and percentage weight gains significantly increased and feed conversion ratios decreased with increasing the levels of feed C:N ratio. These results imply that application of molasses as a carbon resource had a role in removing inorganic nitrogen and increasing growth rate and percentage weight gains of shrimps.

Based on water quality and shrimp production variables during eight weeks of experimental period, the best level of feed C:N ratio obtained in shrimp cultures with ZWEM using molasses was 20.0:1.

REFERENCES

- Avnimelech, Y., M. Lacher, A. Raveh, and O. Zur. 1981. A method for the evaluation of conditions in a fish pond sediment. *Aquaculture* 23, 361-365.
- Avnimelech, Y. and S. Mokady, 1988. Protein biosynthesis in circulated fishponds. In: Pullin, R.S.V., Bhukaswan, T.Tonguthai, K. Maclean, J.,L. (Eds.), *The second International Symposium on tilapia in aquaculture*, pp. 301 - 309.
- Avnimelech, Y., S. Mokady, and G.L. Schroeder, 1989. Circulated ponds as efficient bioreactors for single cell protein production. *The Israel. Aquaculture-Badmidgeh* 41, 58 - 66.
- Avnimelech, Y., S. Diab, M. Kochva, and S. Mokady, 1992. Control and utilization of inorganic nitrogen in intensive fish culture ponds. *Aquaculture and Fish Manag.* 23, 421 - 430.
- Avnimelech, Y., M. Kochva, and S. Diab, 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. *The Israel J. Aquaculture-Badmidgeh* 46, 119 - 131.

- Avnimelech, Y., N. Mozes., S. Diab, and M. Kochva, 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. *Aquaculture* 134, 211 - 216.
- Avnimelech, Y. 1998. Minimal discharge from intensive fish ponds. *J. World Aquacult. Soc.* 29, 32-37.
- Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* 176, 227 - 235.
- Azam, F., T. Fenchel, J., G. Field, L.A. Meyer-Reil, and F., Thingstad, 1983. The ecological role of microbes in the sea. *Mar. Ecol. Prog. Ser.* 10, 257 - 263.
- Bages, M. and L. Sloane, 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* (Fabricius) postlarvae. *Aquaculture* 25, 117 - 128.
- Balazs, G.H., 1973. Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture* 2, 369 - 377.
- Boyd, C.E., 1995. Proceeding Special Session on Shrimp Farming. In: Browdy, C.L., Hopkins, J.S. (Eds.), *Aquaculture*, Sandiego, USA, pp. 183 - 199.
- Burford, M.A., P.J. Thompson, P. McIntosh, R.H. Bauman, and D.C. Pearson, 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture* 219, 393 - 411.
- Chamberlain, G.W., 2001. Managing zero water -exchange ponds. In: Rosenberry, B.(Eds.). *World shrimp farming 2001*. Published Annually Shrimps News International 14, 11-18.
- Csavas, I. 1994. Important factors in the success of shrimp farming. *J. World Aquacult. Soc* 25, 34 - 56.
- Findlay, R.H., G.M. King, and L. Watling, 1989. Efficacy of phospholipid analysis in determining microbial biomass in sediments. *Applied and Environmental Microbiology* 55, 2888 - 2893.
- Goldman, J.C., D.A. Caron, and M.R. Dennet, 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnd Oceanogr* 32, 1239 - 1252.
- Gottschalk, G. 1986. Bacterial metabolism. Springer.
- Hargreaves, J.A. 1998. Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture* 166, 181-212.
- Harris, R.H. and R. Mitchell, 1973. The role of polymers in microbial aggregation. *Ann. Rev. Microbiol.* 27, 27-50.
- Hoch, M.P., M.L. Fogel, and D.L. Kirchman, 1994. Isotope fractionation during ammonium uptake by marine microbial assemblages. *Geomicrobiol* 12, 113 - 127.
- Hoch, M.P. and D.L. Kirchman, 1995. Ammonium uptake by heterotrophic bacteria in the Delaware estuary and adjacent coastal waters. *Limnd Oceanogr* 40, 886 - 897
- Hopkins, J.S., R.D. Hamilton, P.A. Sandifer, C.L. Browdy, and A.D. Stokes, 1993. Effect of water exchange rates on production, water quality, effluent characteristics, and nitrogen budgets of intensive shrimp ponds. *J. World Aquacult. Soc* 24, 304 - 320.
- Hopkins, J.S. M.R. DeVoe, and A.F. Holland, 1995. Environmental impacts of shrimp farming with special reference to the situation in the Continental United State. *Estuaries* 18, 25 -42.
- Johnsen, R.I., O.G. Nielsen, and B.T. Lunestad, 1993. Environmental distribution of organic waste from a marine fish farm. *Aquaculture* 118, 229 - 244.
- Jorgensen, N.O.G., N. Kroer, R.B. Coffin, X.H. Yang. And C. Lee. 1993.

- Dissolved free amino acids, combined amino acids, and DNA as sources of carbon and nitrogen to marine bacteria. *Mar. Ecol. Prog. Ser.* 98, 135- 148.
- Kautsky, N., P. Ronnback, M. Tedengren, and M. Troell, 2000. Ecosystem perspectives on management of disease in shrimp pond farming. *Aquaculture* 191, 145 - 161.
- Kirchman, D.L., B. Meon, M.T. Cottrell, D.A. Hutchins, and D.W.B. Weeks, 2000. Carbon versus iron limitation of bacterial growth in the California upwelling regime. *Limnol Oceanogr* 45, 1681 - 1688.
- Kochva, M., S. Diab, and Y. Avnimelech, 1994. Modelling of nitrogen transformation in intensively aerated fish ponds. *Aquaculture* 120, 95 - 104.
- Landesman, L. 1994. Negative impact of coastal aquaculture development. *J. World Aquacult. Soc.* 25, 12 - 17.
- McIntosh, R.P., 2000. Changing paradigms in shrimp farming: III. Pond design and operation considerations. *Global Aquaculture Advocate* 3, 42 - 44.
- Middelboe, M., N.H. Borch, and D.L. Kirchman, 1995. Bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary: effects of carbon and nitrogen limitation. *Mar. Ecol. Prog. Ser.* 128, 109 - 120.
- Montoya, R.A., A.L. Lawrence, W.E. Grant, and M. Velasco, 2002. Simulation of inorganic nitrogen dynamics and shrimp survival in an intensive shrimp culture system. *Aquaculture Res.* 33, 81 - 94.
- Moriarty, D. J.W. 1986. Bacterial productivity in ponds used for culture of penaeid prawns. *Microb.Ecol* 12, 259-269.
- Olah, J., R.P. Sinha, S. Ayyappan, C.S. Purushothaman, and S. Radheyshyam, 1987. Sediment consumption in tropical undrainable fish ponds. *Internationale Revue gesammte Hydrobiologie* 72, 297-305.
- Parsons, T.R., L.J. Albright, F. Whitney, C.S. Wong, and M.P.J. Williams, 1981. The effect of glucose on the productivity of sea water: An experimental approach using controlled aquatic ecosystems. *Mar. Environ. Res.* 4, 229 - 242.
- Ritvo, G., J.B. Dixon, A.L. Lawrence, T.M. Samocha, W.H. Neill, and M.F. Speed 1998. Accumulation of chemical elements in Texas shrimp pond soils. *J. World Aquacult. Soc.* 29, 422 - 430.
- Rivera-Monroy, V.H., N. Bahamon, L.A. Torres, F. Newmar, and R.R. Twilley, 1999. The potential use of mangrove forest as nitrogen sinks of shrimp aquaculture pond effluents: The role of denitrification. *J. World Aquacult. Soc* 30, 12 - 24.
- Rosenberry, R. 1993. Production drops 16 % in 1993. In: *World Shrimp Farming 1993*. Aquaculture Dig., (December) : 1-19.
- Rosenberry, B. 2001. New shrimp farming technology: Zero-exchange, environmentally friendly, super-intensive In: *World shrimp farming 2001*. Published annually shrimps news International 14, 5-10.
- Schroeder, G.L. 1978. Autotrophic and heterotrophic production of microorganisms in intensively - manured fish ponds, and related fish yields. *Aquaculture* 14, 303 - 325.
- Smith, P.T. 1996. Physical and chemical characteristics of sediment from farms and mangrove habitats on the Clarence river, Australia. *Aquaculture* 146, 47 - 83.
- Smith, P.T. 1998. Effect of removing accumulated sediments on the bacteriology of ponds used to culture *Penaeus monodon*. *Asian Fish. Sci.* 10, 355 - 370.

- Sohier, L.P., and M.A.G. Bianchi, 1985. Development of a heterotrophic bacterial community within a closed prawn aquaculture system. *Microb. Ecol.* 11, 353 - 369.
- Stuart, J., E. Frank, Coman, J. Chris, Jackson, and A.G. Sarah, 2009. High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. *Aquaculture*, 293, 42-48
- Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics: Biometrical Approach, 2nd Edition. In: McGraw-Hill (Ed.), New York.
- Sun, Y., S., Zhang, J. Chen, Song, and Junli. 2001. Supplement and consumption of dissolved oxygen and their seasonal variations in shrimp pond. *Mar. Sci. Bull.* : 3, 89-96.
- Tacon, A.G.J. 2001. Minimizing environmental impacts of shrimp feeds. *Global Aquaculture Advocate* 4, 34 - 35.
- Tacon, A.G.J., J.J. Cody, L.D. Conquest, S. Divakaran, I.P., Forster, and O.E. Decamp, 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition* 8, 121-137.
- Tezuka, Y., 1990. Bacterial regeneration of ammonium and phosphate as affected by the Carbon : Nitrogen: Phosphorus ratio of organic substrates. *Microb. Ecol.* 19, 227 - 238.
- Thakur, D.P. and C.K. Lin 2003. Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Eng.* 27, 159 - 176.
- Tseng, K.F., H.M. Su. and M.S. Su, 1998. Culture of *Penaeus monodon* in a recirculating system. *Aquaculture* 17, 138 - 147.
- Visscher, P.T. and E.O. Duerr, 1991. Water quality and microbial dynamics in shrimp ponds receiving bagasse-based feed. *J. World Aquacult. Soc.* 22, 65-76.
- Wheeler, P.A. and D.L. Kirchman, 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.* 31, 998 -1009.

