Effect of C:N Ratio Levels on Water Quality and Shrimp Production Parameters in *Penaeus monodon* Shrimp Culture with Limited Water Exchange Using Molasses as a Carbon Source

Pohan Panjaitan

Faculty of Animal Husbandry,The University of HKBP Nommensen, Jalan Sutomo No 4 Medan, Indonesia.
e-mail: pohanpanjaitan@yahoo.com.au, Phone: + 62 61 4522922; Fax: +62 61 4571426;
Mobile Phone: 081269371818

Abstrak

Hambatan utama di dalam pengembangan budidaya udang intensif adalah limbah tambak udang yang merusak lingkungan. Mengurangi dampak lingkungan buangan limbah, budidaya udang seharusnya dilakukan di sistem terbuka dengan pergantian air terbatas.Namun, masalah utama yang terakit dengan system pergantian air terbatas adalah penurunan kolam cepat akibat meningkatnya konsentrasi ammonia dan nitrit. Penambahan bahan karbon contohnya molasses ke tambak dengan mengatur level rasio karbon : nitrogen merupakan suatu stategi terbaik untuk mengontrol ammonia dan nitrite di budidaya udang dengan model pergantian air terbatas. Dengan demikian, tujuan utama studi ini adalah untuk mengevaluasi pengaruh level C:N ratio terhadap parameter kualitas air dan produksi udang di budidaya udang Penaeus monodon dengan menggunakan model pergantian air terbatas. Studi menunjukkan bahwa level C:N ratio berpengaruh nyata terhadap parameter kualitas air dan produksi udang di budidaya udang dengan model pergantian air terbatas.

Kata kunci: *Level rasio C:N, kualitas air, model pergantian air terbatas, molasses*

Abstract

The main obstacles in developing intensive shrimp culture is waste of shrimp farms which is detrimental to environment. Mitigating the environmental impacts of effluent discharge, shrimp culture should be implemented in closed system with limited water exchange. However, the major problem associated with limited water exchange system is the rapid deterioration in pond, resulting from increasing concentration ammonia and nitrite. The addition of carbon materials such as molasses into shrimp culture with manipulating the carbon : nitrogen ratio level is one of the best strategies of controlling ammonia and nitrite in shrimp culture with Limited Water Exchange Model (LWEM). Therefore, the principal aim of this study was to evaluate the effects of C:N ratio levels on water quality and shrimp production in Penaeus monodon shrimp culture with Limited Water Exchange Model (LWEM) using molasses as a carbon source. Study reveals that C:N ratio levels significantly effected on water quality and shrimp production parameters of shrimp culture with LWEM using molasses as carbon resource using molasses.

Key words: *C: N ratio level, water quality, limited water exchange model, molasses*

Intoduction

In conventional shrimp farming, water is mainly exchanged to prevent deterioration in water quality and control inorganic nitrogen. The operation of limited water exchange is one approach to improve sustainability and biosecurity in shrimp culture. However, the major problem associated with limited water exchange system is the rapid eutrophication in ponds, resulting from increasing concentrations of nutrients (ammonia and nitrite) and organic matters over the culture period (Thakur & Lin, 2003). Several studies on removing inorganic nitrogen by heterotrophic bacteria through addition of carbonaceous substrate into fish ponds has been conducted (Avnimelech, *et al*., l992, l994; Kochva *et al*., l994; Avnimelech, *et al*., l999; Allen, 2001) and into shrimp ponds (Arnold *et al*., 2009; Asaduzzaman *et al*., 2009).

In order to maximize the growth of heterotrophic bacteria a balanced ratio of carbon to

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nitrogen of the feed is required. Several authors (e.g. Tezuka, 1990; Anvimelech *et al*., 1992; 1994; Avnimelech, 1999; McIntosh, 2000; Panjaitan, 2010) reported that the feed C:N ratio level of 15.0:1 or more effectively removed inorganic nitrogen from water. Further, it was also observed that the application of molasses in shrimp culture with LWEM through controlling feed C:N ration levels is likely to be a novel strategy in improving management in shrimp culture and the best level of feed C:N ratio obtained was 20.0:1 in shrimp culture with zero water exchange model (Panjaitan, 2010). There are, therefore, several questions remaining unanswered: Does application of molasses have profit in shrimp culture with LWEM (5% of total volume weekly) in terms of water quality and shrimp production variables? What is the best level of C:N ratio in shrimp culture with LWEM? Can shrimp culture with LWEM using molasses be one alternative model in solving problem of current shrimp industry? It would be further beneficial information if study of effect of molasses through controlling C:N ratio levels on water quality and shrimp production variables is carried out in *Penaeus monodon* shrimp culture with LWEM because to date, there have been no studies of shrimp culture for black tiger shrimp *Penaeus monodon* with LWEM using molasses as a carbon source.

The aims of this study were to investigate in laboratory effect of feed C: N ratio levels on water quality, shrimp survival, growth rate, percentage weight gain, feed conversion ratio (FCR), water consumption rate and effluent discarded from shrimp culture with LWEM (5% of total volume weekly).

Meterial and Methods

The experimental shrimp culture

Experiment conducted in this study was shrimp culture with limited water exchange model at 5 % of total volume weekly (abbreviated as LWEM). The protocol used in the experiment were: (1) without molasses with C:N ratio=6.5 ($LWEM_{6.5}$), (2) using molasses with C:N ratio=15.0 ($LWEM_{15.0}$), (3) using molasses with C:N ratio=17.5 ($LWEM_{17.5}$), (4) using molasses with C:N ratio=20.0 ($LWEM_{20.0}$) and (5) using molasses with C:N ratio= 22.5 ($LWEM_{22.5}$).

The experiment was conducted in 160 L experimental tanks throughout the eight-week culture period. All treatments had three replicates and allocation for each treatment was completely randomized. Experimental tanks were mixed and aerated with two pieces of air stone suspended in bottom of water column in each tank. All tanks were filled with 160 L of sea water and water exchange was carried out in each tank (5 % of total volume weekly) during the study. The volume of water in each tank was maintained constant by adding 10 L of freshwater (tap water) weekly in order to replace discarded water and compensate evaporation. The other purpose of adding freshwater into each tank was to regulate the salinity requirement of *Penaeus monodon* shrimps. Water temperatures and salinity of the experiment over eight-week ranged 21.8-24.8° C and 25.50-33.80 ppt respectively.

Each experimental culture was stocked with 4 *Penaeus monodon* shrimps or equal to 30 shrimps m^{-2} (Allan & Manguire, 1992). The mean ± standard deviation of shrimp weight at stocking time of the experiment was 2.36 ± 0.03 grams. Shrimps were obtained from nursery of Charles Darwin University, Australia. Experimental shrimps were fed with commercial shrimp feed (Taiwan Company Product) with 38 % of crude protein content. The level of feed used in the experiment was 5 % of body weight per day during study (as recommended by the company) and it was applied twice daily (50 % of the total feed required each 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; Kochva *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, was calculated by conversion of that assumption while the carbon content of feed and molasses used in each study is 38.5% and 29.71%, respectively. The amount of molasses required per 1 gram of feed in each treatment, therefore, is 0.00, 1.68, 2.17, 2.67 and 3.16 gram for $LWEM_{6.5}$, $LWEM_{15.0}$, $LWEM_{17.5}$, $LWEM_{20.0}$ and $LWEM_{22.5}$, respectively. Furthermore, heterotrophic bacteria implemented in every treatment were from nature bacteria of sea water used (Avnimelech, 1999).

Water quality measurements and analyses

Water quality variables such as ammonia, nitrite, nitrate, temperature, salinity, pH, dissolved oxygen, and total bacteria were measured weekly.

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.

Levels of viable heterotrophic bacteria were determined by counting the colonies which grew on plates of Tryptone Soya Agar (TSA) with 10% of NaCl (Jorgensen *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).

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

The survival rate, growth rate, production and feed conversion ratio of shrimps were determined fortnightly for eight-week duration. The total body weight of shrimp (W) was measured for each experimental container. Similarly, the number of live shrimps (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) as used in common aquaculture studies (Tseng *et al*., 1998).

Data analysis

Data of laboratory study were analysed using Statistica Version 6.1 software and with one-way ANOVA (Steel & 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 & Torrie, 1980).

Result and Discussion

Levels of feed C:N ratio significantly effected the concentrations of ammonia in the experiment (Table 1). Generally concentrations of ammonia in the experiment decreased in response to increasing levels of feed C:N ratio and it was probably due to increase in the numbers of heterotrophic bacteria when the levels of feed C:N ratio increased. Further investigation revealed that concentrations of ammonia had an inverse correlation with numbers of heterotrophic bacteria. This trend is agreement with previous observation by several authors (Goldman *et al*., 1990; Tezuka, 1990; Anvimelech *et al*., 1992; 1994; Avnimelech, 1999; McIntosh, 2000) which revealed that there was a significant decrease in inorganic nitrogen with increasing feed C:N ratio levels.

Further investigation revealed that treatment without using molasses had highest concentrations of ammonia and nitrite. It was probably due to increasing the amount of molasses more effectively removed inorganic nitrogen than exchanging water in shrimp culture. It has been previously documented that addition of carbon in fish pond was more effectively in removing inorganic nitrogen than exchanging water (Anvimelech *et al*., 1992; 1994; Avnimelech, 1999).

The present study clearly shows that ammonia did not accumulate in the shrimp culture untreated with molasses in experiment. Nitrite accumulated in the shrimp cultures not using molasses for the experiment. This result indicates that water exchange around 5% of total volume weekly with no molasses can not control the accumulation of nitrite in shrimp culture water. The result also describes oxidation rate of ammonia by nitrifying bacteria was highest in shrimp cultured untreated with molasses in the experiment. It means that application of molasses inhibited activity nitrifying in shrimp culture. It has been reported that application of carbon decreased the activity of nitrifying bacteria (Bovendeur *et al*., 1990; Cheng & Chen, 1994; Ohashi *et al*., 1995; Zhu and Chen, 2001, Burford *et al*., 2003).

There was a significant effect on concentrations of dissolved oxygen in the experiment by levels of feed C:N ratio (Table 1). Furthermore, the results indicated that there was a significant reduce in concentrations of dissolved oxygen when levels of feed C:N ratio increased in shrimp culture with LWEM. Increase in the numbers of heterotrophic bacteria with levels of feed C: N ratio was likely to be responsible for this finding since concentration of dissolved oxygen in the experiment had an inverse correlation with the numbers of heterotrophic bacteria. This view is supported by further investigation revealing that numbers of heterotrophic bacteria in the experiment significantly increased as increasing the levels of feed C:N ratio (as discussed below). It has been stated that heterotrophic bacteria had an impact of decreasing dissolved oxygen in shrimp ponds (Visscher and Duerr, 1991; Avnimelech *et al*., 1992; Sun *et al*., 2001).

Lower concentrations of dissolved oxygen in treatment with higher levels of feed C:N ratio could be

Tabel 1. The multiple comparisons of means using Tukey HSD test for the effect of feed C:N ratio levels on water quality and shrimp production parameters in shrimp culture with LWEMW using molasses as a carbon source. Values are means and standard deviations of three replicates at the end of the eight-week experimental period. Values in every water quality variable within the same column that are followed by different superscript are significantly different ($p < 0.05$). Equality of variance and normality of each water quality variable level were checked by Cochran's test.

Parameters	Treatment	Mean ± Std .Dev.
1. Ammonia (mg/litre)	1. Without Molasses	0.2540 ± 0.0371^{a}
	2. Molasses with C/N Ratio = 15.0	0.0257 ± 0.0023^{a}
	3. Molasses with C/N Ratio = 17.5	0.0156 ± 0.0012^{b}
	4. Molasses with C/N Ratio = 20.0	0.0102 ± 0.0005^{c}
	5. Molasses with C/N Ratio = 22.5	0.0069 ± 0.0003^{d}
2.Nitrite (mg/litre)	1. Without Molasses	21.0733 ± 0.6649^{a}
	2.Molasses with C/N Ratio = 15.0	0.0000 ± 0.0000^{b}
	3. Molasses with C/N Ratio = 17.5	0.0000 ± 0.0000^{b}
	4. Molasses with C/N Ratio = 20.0	0.0000 ± 0.0000^{b}
	5. Molasses with C/N Ratio = 22.5	0.0000 ± 0.0000^{b}
3. Dissolved Oxygen (mg/litre)	1. Without Molasses	5.90 ± 0.06^{a}
	2.Molasses with C/N Ratio = 15.0	5.64 ± 0.12^{b}
	3. Molasses with C/N Ratio = 17.5	5.37 ± 0.02^{c}
	4. Molasses with C/N Ratio = 20.0	5.19 ± 0.01^{d}
	5. Molasses with C/N Ratio = 22.5	4.69 ± 0.02^{e}
4. pH	1. Without Molasses	8.11 ± 0.01^{a}
	2.Molasses with C/N Ratio = 15.0	7.96 ± 0.06^{b}
	3. Molasses with C/N Ratio = 17.5	7.85 ± 0.04^{c}
	4. Molasses with C/N Ratio = 20.0	7.77 ± 0.02^{d}
	5. Molasses with C/N Ratio = 22.5	7.69 ± 0.0123^{e}
5. Number of Heterotrophic Bacteria (CFU/ml)	1. Without Molasses	$1.05 \times 10^{9} \pm 6.08 \times 10^{7}$ ^{a}
	2.Molasses with C/N Ratio = 15.0	$2.74 \times 10^{9} \pm 1.86 \times 10^{8}$ ^{b}
	3. Molasses with C/N Ratio = 17.5	$5.59 \times 10^{9} \pm 1.95 \times 10^{8}$ ^{c}
	4. Molasses with C/N Ratio = 20.0	$6.49 \times 10^{9} \pm 1.40 \times 10^{8}$ d
	5. Molasses with C/N Ratio = 22.5	$9.44 \times 10^{9} \pm 3.60 \times 10^{8}$ ^{e}
6. Shrimp Survival Rate (%)	1. Without Molasses	100.00 ± 0.00^{a}
	2.Molasses with C/N Ratio = 15.0	100.00 ± 0.00^{a}
	3. Molasses with C/N Ratio = 17.5	100.00 ± 0.00^{a}
	4. Molasses with C/N Ratio = 20.0	100.00 ± 0.00^{a}
	5. Molasses with C/N Ratio = 22.5	100.00 ± 0.00^{a}
7. Growth Rate (gram/day)	1. Without Molasses	0.0718 ± 0.001^{a}
	2.Molasses with C/N Ratio = 15.0	0.1033 ± 0.001^{b}
	3. Molasses with C/N Ratio = 17.5	0.1007 ± 0.001^{b}
	4. Molasses with C/N Ratio = 20.0	0.1026 ± 0.001^{b}
	5. Molasses with C/N Ratio = 22.5	0.1029 ± 0.001^{b}
8. Gain Percentage (%)	1. Without Molasses	174.6226 ± 5.3175^{a}
	2.Molasses with C/N Ratio = 15.0	253.9269 ± 3.6116^{b}
	3. Molasses with C/N Ratio = 17.5	245.7076 ± 1.6369^{b}
	4. Molasses with C/N Ratio = 20.0	253.0838 ± 2.2337^{b}
	5. Molasses with C/N Ratio = 22.5	254.1819 ± 2.6533^{b}
9. Feed Conversion Ratio	1. Without Molasses	2.665 ± 0.040^{a}
	2.Molasses with C/N Ratio = 15.0	2.169 ± 0.026^{b}
	3. Molasses with C/N Ratio = 17.5	2.218 ± 0.006^{b}
	4. Molasses with C/N Ratio = 20.0	2.189 ± 0.003^{b}
	5. Molasses with C/N Ratio = 22.5	2.213 ± 0.010^{b}

to values of chlorophyll a which significantly decreased with increasing levels of feed C:N ratio. This explanation is supported by further analysis proves that concentrations of dissolved oxygen in the experiment were significantly positively associated with the levels of chlorophyll a. There is usually a strong positive correlation between dissolved oxygen and concentration of chlorophyll a at day time (Boyd and Tucker, 1998; Giri and Boyd, 2000; McGraw *et al*., 2001; Sun *et al*., 2001).

Even though there was water exchange in experiment (5% of total volume weekly), levels of dissolved oxygen decreased with experimental time. This finding was probably caused by accumulation rate of organic matter such as unconsumed feed, shrimp faeces, dead phytoplankton and zooplankton. This contention is supported by the present data showing an increase in organic carbon during experimental time in the experiment. Also shrimp size increased progressively with experimental period and could be responsible for decreasing levels of dissolved oxygen with experimental time.

Levels of feed C:N ratio had a significant effect on values of pH in the experiment (Table 1). Levels of pH significantly decreased in response to increasing levels of feed C:N ratio in the experiment. It has been suggested that treatment with higher level of feed C:N ratio produced higher concentration of inorganic carbon (CO_2) which reacted with water reducing level of water pH (Boyd, 1990).

Values of pH decreased with experimental time in all treatments. In treatments with molasses, decreasing pH may have been caused by increased application of molasses with experimental time due to increasing shrimp weight feed amount. Further, the declining pH levels during experimental time in treatment not using molasses was probably caused by activity of nitrifying bacteria indicated by a gradual increase in concentration of nitrite and nitrate with experimental period. Tacon *et al*. (2002) also reported that there was a decreased pH levels at the end of shrimp culture due to a gradual increase in nitrite with culture period.

Numbers of heterotrophic bacteria in the experiment were significantly effected by levels of feed C:N ratio (Tables 1). A significant increase in numbers of heterotrophic bacteria observed as the result of increasing levels of feed C:N ratio in shrimp culture in the experiment and this could be due to the higher amount of molasses applied in the treatment stimulated higher growth of heterotrophic bacteria. This investigation also is consistent with observation by Asaduzzaman *et al*. (2010) who documented that increasing C: N ratio from 10 to 20 raised biovolume of total heterotrophic bacteria in freshwater shrimp culture ponds. 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.

Generally the levels of heterotrophic bacteria in all treatment increased with experiment period, even though there was water exchange in the experiment. This could be because application of feed and molasses increased progressively with the experimental period, consequently, numbers of bacteria also increased. Further, accumulation rate of organic matter which was higher compared to rate of flushed organic matter from the experiment may be one reason for the increasing numbers of heterotrophic bacteria with experimental time

The survival rates of shrimp in all treatments of three experiments were 100% and were unaffected by the levels of feed C:N ratio whereas the levels of feed C:N ratio significantly affected growth rates, percentage weight gains and feed conversion ratios in all three experiments (Table 2).

The present study proved that the treatments using molasses in the experiment consistently had higher growth rate and percentage weight gain, and lower feed conversion ratio than treatment not using molasses. This contention is accordance with earlier investigation by several authors (Arnold *et al*., 2009; Asaduzzaman *et al*., 2009; 2010) revealed that the growth was significantly greater when carbon were implemented in shrimp culture with zero water exchange model. Furthermore, this result could be because heterotrophic communities in treatments using molasses developed flocs of composing bacteria cells (McIntosh, 2000), and flocculation of the cells can be alone or in combination with feed particles (Avnimelech *et al*., l989). Flocs containing high protein, amino acids and certain microelements, can be directly consumed by shrimps (Tacon *et al*., 2002; Kuhn *et al*., 2008;2009; Johnson *et al*., 2008; Vasagam *et al*.,2009).

In general, growth rates and percentage weight gains increased and feed conversion ratios decreased with increasing the levels of feed C:N ratio in the experiment. These results are supported by previous investigation proved 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 (Avnimelech, 1999). Moreover, these finding was probably due to concentrations of ammonia and nitrite having significantly decreased with increasing the levels of feed

C:N ratio because further analysis revealed that the growth rate and percentage weight gain had an inverse correlation with concentrations of ammonia and nitrite while feed conversion ratio had a positive association with levels of ammonia and nitrite in all three experiments. Reduction in fish growth and increased feed conversion ratio associated with high concentrations of ammonia in pond water has been widely documented (Rhyther and Goldman, 1974; Rogers and Klemetson, 1983; Soderberg *et al*., 1983; Millamena, 1990).

However, it is interesting to note that there was no significant difference in growth rates, percentage weight gains and feed conversion ratios, among $LWEM_{15.0,}$ $LWEM_{17.5,}$ $LWEM_{20.0}$ and $LWEM_{22.5}$ treatments. This result was probably caused by similarity among the treatments of each experiment in the concentrations of nitrite. In spite of a significant difference in ammonia concentrations among those treatments, ammonia was unlikely to effect on production variables due to levels of ammonia were still lower than safe level for *Penaeus monodon* shrimps.

Conclusions

Levels of feed C:N ratio significantly influenced water quality and shrimp production parameters of shrimp culture with LWEM using molasses as carbon resource. Further, based on water quality and shrimp production parameters during eight weeks of experimental period, the best level of feed C:N ratio obtained in shrimp cultures with LWEM using molasses was 15.0:1.

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References

Allan, G.L., & Manguire, G.B. 1992. Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds. *Aquaculture,* 107: 49-66.

Allens, R. 2001. Zero-exchange demostration farm in Nicaragua. In: Rosenberry, B. (Eds.). World Shrimp Farming 2001. *Shrimps News International*, 14: 29-33.

Arnold, S. J., Coman, F.E., Jackson, C.E., & Groves, S.A. 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.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Benerjee, S., Hasan, M.M. & Azim, M.E. 2010. Effects of addition of tilapia Oreochromis niloticus and substrates for periphyton developments on pond ecology and production in C / N – controlled freshwater prawn *Macrobrachium rosenbergii* farming systems. *Aquaculture,* 287: 371-380.

Asaduzzaman, M., Rahman, M.M., Azim, M.E., Islam, M.A, Wahab, M.A, Verdegem, M.C.J., & Verreth, J.A.J. 2010. Effects of C/N ratio and substrate addition on natural food comunities in freshwater prawn monoculture ponds. *Aquaculture,* 306: 127-136.

Avnimelech, Y., Lacher, M., Raveh, A., & Zur, O. 1981. A method for the evaluation of conditions in a fish pond sediment. *Aquaculture,* 23: 361-365.

Avnimelech, Y. & Mokady, S. 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., Mokady, S., & Schroeder, G.L.1989. Circulated ponds as efficient bioreactors for single cell protein production. *The Israel J. Aquaculture-Badmidgeh,* 41:58-66.

Avnimelech, Y., Diab, S., Kochva, M., Mokady, S.1992. Control and utilization of inorganic nitrogen in intensive fish culture ponds. *Aquaculture and Fisheries Management,* 23: 421-430.

Avnimelech, Y., Mozes., N., & Weber, B., 1992. Effects of aeration and mixing on nitrogen and organic matter transformations in simulated fish ponds. *Aquacultural Engineering,* 11: 157-169.

Avnimelech, Y., Kochva, M., & Diab, S. 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., Mozes., N., Diab, S., & Kochba, M. 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.

Bovendeur, J., Zwaga, A.B., Lobee, B.G.J., & Blom, J.H. 1990. Fixed-biofilm reactors in aquacultural water recycle systems: effect of organic matter elimination on nitrification kinetics. *Water Res*., 24: 207-213.

Boyd, C.E., & Tucker, C.S. 1998. Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, Massachusetts, USA.

Burford, M.A., Thompson, P.J., McIntosh, P., Bauman, R.H., & Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture,* 219: 393-411.

Cheng, S.S. & Chen, W.C.1994. Organic carbon supplement influencing performance of biological nitrification in a fluidized bed reactor. *Water Sci. Tech*., 30.

Giri, B.I., & Boyd, C.E. 2000. Effects of frequent, small doses of calcium carbonate on water quality and phytoplankton in channel catfish ponds. *North American Journal of Aquaculture,* 62: 225 -228.

Goldman, J.C., Caron, D.A., & Dennet, M.R. 1990. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnology Oceanography,* 32: 1239-1252.

Johnson, C.N., Barnes, S., Ogle,J., Grimes, D.J., Chang, Y.J., Peacock, A.D., & Kline, L. 2008. Microbial community analysis of water, foregut, and hidgut during growth of Pacific white shrimp, *Litopenaeus vannamei*, in losed system aquaculture. *J. World Aquacult. Soc.,* 39: 251-258.

Jorgensen, N.O.G., Kroer, N., Coffin, R.B., Yang.X.H., & Lee, C. 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.

Kochva, M., Diab, S., & Avnimelech, Y.1994. Modelling of nitrogen transformation in intensively aerated fish ponds. *Aquaculture,* 120: 95-104.

Kuhn, D.D.,Boardman,G.D., Craig, S.R., Flick,G.J., & McLean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, *Litopenaeus vannamei*, in recirculating aquaculture systems. *J. World Aquacult. Soc.,* 39: 72-82.

Kuhn, D.D., Boardman, G.D., Lawrence,A.L., Marsh, L., & Flick, G.J. 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture,* 296: 51–57.

McGram, W., Teichert-Coddington, D.R., Rouse, D.B., & Boyd, C.E. 2001. Higher minimum dissolved oxygen concentrations increase penaeid shrimp yields earthen ponds. *Aquaculture,* 199: 311-321.

McIntosh, R.P. 2000. Changing paradigms in shrimp farming: III. Pond design and operation considerations. *Global Aquaculture Advocate,* 3: 42-44.

Millamena, O.M. 1990. Organic pollution resulting from excess feed and metabolic build-up: Effect on *Penaeus monodon* poslarvae. *Aquacultural Engineering,* 9: 143-150.

Ohashi, A., Viraj de Silva, D.G., Mobarry, B., Manem, J.A., Stahl, D.A., & Rittmann, B.E.1995. Influence of substrate C/N ratio on the structure of multi-species biofilms consisting of nitrifiers and heterotrophs. *Water Sci. Tech.,* 32:75-84.

Panjaitan, P. 2010. The *Penaeus monodon* shrimp culture with zero water exchange model using molasses as carbon source. The Study Centre of Anima; Husbandry, Fisheries, Coastal and Marine Resource, Animal Husbandry Faculty, University of HKBP Nommensen, Medan Indonesia

Rogers, G.L., & Klemetson, S.L. 1983. Water quality maintenance for the three stages of Macrobrachium production. In Rogers, G.L., Day, R., Lim, A (Ed.). *Proceedings of the first International Conference on Warmwater Aquaculture Crustacea*, ed. Brigham Young University, Laie, Hawaii, USA, pp. 562-575.

Smith, P.T. 1998. Effect of removing accumulated sediments on the bacteriology of ponds used to culture *Penaeus monodon*. *Asian Fisheries Science,* 10: 355-370.

Soderberg, R.W., Flynn, J.B., & Schmittou, H.R. 1983. Effects of ammonia on growth and survival of rainbow trout in intensive static water culture. *Trans. Am. Fish. Soc*., 112: 448-451.

Sohier, L.P. & Bianchi, M.A.G.1985. Development of a heterotrophic bacterial community within a closed prawn aquaculture system. *Microbial Ecology,* 11:353-369.

Steel, R.G.D., & Torrie, J.H. 1980. Principles and Procedures of Statistics: Biometrical Approach, 2nd Edition. In: McGram-Hill (Ed.), New York.

Sun, Yao, Zhang, Shufang, Chen, Jufa, Song & 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., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P., & Decamp, O.E. 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Lipopenaeus 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. *Microbial Ecology,* 19: 227–238.

Thakur, D.P., & Lin C.K. 2003. Water quality and nutrient budget closed shrimp (*Penaeus monodon*) culture systems. *Aquacultural Engineering,* 27:159-176.

Tseng, K.F., Su, H.M., & Su, M.S. 1998. Culture of *Penaeus monodon* in a recirculating system. *Aquaculture,* 17: 138-147.

Vasagam, K.P.K., Suresh, A.V., & Chamberlain, G.W. 2009. Growth performance of blue shrimp, *Litopenaeus stylirotris* in self - cleaning microcosm tanks. *Aquaculture* 290: 236–242.

Visscher, P.T., & Duerr, E.O., 1991. Water quality and microbial dynamics in shrimp ponds receiving bagase-based. *J. World Aquacult. Soc*., 22: 65-76.

Zhu, S., & Chen, S. 2001. Effects of organic carbon on nitrification rate in fixed film biofilters. *Aquaculture Engineering*, 25:1-11.