CULTURE OF TIGER SHRIMP PENAEUS MONODON FABRICIUS USING PROBIOTICS IN BRACKISH WATER PONDS IN PERAK, MALAYSIA

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ABSTRACT

The growth of tiger shrimp *P. monodon* and physicochemical factors of pond water and sediment were observed in tropical climate using bacterial products called probiotics. No improvement in shrimp growth and water quality was noted compare to other reported studies. The organic matter (OM) and total sulfur (TS) of pond soil tend to decrease over culture period. Daily growth rate of shrimps was recorded 0.20 g/day in 116 days culture period. Dissolved oxygen concentrations, pH, temperature and salinity were found fluctuated mostly between 6.8-10.3 mg/l, 7.22-8.44, 30-32ºC and 16.0-28.2‰, respectively. Soil texture of the culture ponds was sandy. Concentration of organic matter was low in the ponds probably due to decomposition by microbes during mineralization process. Comparatively, the concentration of soil TS was found lower in the culture pond suggesting that heterotropic bacteria utilized the superficial soil sulfate compounds, which converts into sulfur and its related compounds. The higher concentrations of major macronutrients in soil could be attributed partly to nutrient loading and accumulation from soil pore water during drying over time and pond age. Surprisingly, the concentrations of soil Mg (16541.7±3007.8 ppm) was almost uniform probably due to the use of agricultural limestone dolomite (8132 kg/h) into the pond. Noticeable differences were not found for concentrations of soil Cu, Cd, Zn, Pb, Fe, Mn, Al, Co, V, Cr, Ti, As, Ag and Sb in the ponds. None of the elements accumulated in the ponds were reached to harmful concentrations for culture species.

Keywords: aquaculture, probiotics, *Penaeus monodon*, macro-micronutrients and Malaysia

INTRODUCTION

The culture potential of black tiger shrimp *P. monodon* is well known due to its production performance and economic points of view. The growth, production and survival of any culture species like shrimp depends on the culture system practiced such as extensive and semi intensive. The physico-chemical factors of the culture pond and their individual or synergetic affect play an important role on the production of shrimp and pond ecology. The ecosystem and biota of the culture ponds may also influence on the production performance of shrimp culture. Studies suggested that the growth and survival of shrimps are affected by temperature, salinity and dissolved oxygen concentration (Subrahmanyam, 1973; Verghese *et al.*, 1975; 1982 and Liao, 1977). The growth of shrimp also depends on the water management followed by depth of pond water and quality of supplementary feed. Millamena (1990) found that the survival rate of *P. monodon* post larvae is directly influenced by organic content and dissolved oxygen concentration in the culture ponds.

Several studies have aimed to increase production of tiger shrimp through manipulating of stocking density, fertilization, artificial feeding, opening of new lands for culture and combination of other species into the culture system (Verghese *et al*., 1975; Chakraborti *et al*., 1985). Currently, in advance aquaculture technology systems, farmers used water reservoirs for sedimentation, green water, microbial products i.e. probiotics and compressed air line for bottom aeration together with paddle wheels (Shishehchian, 2000). Biomanipulators i.e. tilapia, milkfish and sea bass were also stocked inside the net cages in the reservoirs, while some of the species especially tilapia can directly help shrimp when they produce enzymes or slime that inhabit the growth of luminous bacteria (SEAFDEC, 2000). Recent development in aquaculture science has improved vastly in certain areas of tiger shrimp *P. monodon* production but the results still remains inconsistent. Therefore, in this context, an investigation was undertaken to observe the ecological factors with the growth of *P. monodon* in the culture pond using microbial products and compared with the other studies elsewhere.

MATERIALS AND METHODS

This study area is situated in the District Perak, West coast of Malaysia (5° 45' N and 101° 37'E). The ponds were about 8 years old and considered an aged ponds. Three culture ponds were selected initially, but White Spot Syndrome Virus (WSSV) infected two ponds during the middle of cycle period leaving only one pond remaining uninfected. Therefore, sampling was carried out in one pond (4673 m^2) till end of 116 days culture period. Since the pond is fully managed by the owner, the present study is considered as a case study, which compared with the other parallel study with different pond management system. All data were collected between April 2001 and July 2001 during the whole culture period. The culture management and preparation of sampling pond was described in Abu Hena *et al.* (2003). Water quality parameters were measured *in situ* in every three-week interval. Dissolved oxygen (DO) was measured using DO meter (YSI model 57). Water salinity and temperature by SCT meter (YSI model 33). Water pH by pH meter (EDT model FE 253). Transparency by Secchi disk in nearest cm. Ekman grab sampler was used for collection of soil samples. Three samples were collected in a diagonal direction (corner to corner) from each pond by using a small boat. Samples were brought back to laboratory for further analysis within 2-4 hrs. In the laboratory, soil samples were dried in room temperature and powdered. Later on, it was sieved through 200 μm mesh screen. Organic matter of soil was detected by ignition method (Boyd, 1995a). Soil texture was analyzed following procedure described by Bouyoucos (1962). Total sulfur by Tandon (1990) and macro-micro nutrients by Allen (1972) using ELAN 6000 ICPMS.

RESULTS AND DISCUSSION

The details of the physico-chemical factors and nutrients of culture pond water are given in Table 1. The water quality is the most important limitation of the commercial viability of aquaculture operation, especially in semi intensive culture condition (Boyd and Watten, 1989). The physico-chemical factors of water were not significantly difference within the ponds using probiotics and none probiotics throughout the culture period. The concentrations of water nutrients increased with time and reached maximum stage at the end of culture period. Ammonium (NH_4^+) concentration was found higher in the culture pond using probiotics (Table 1) when compared with previous study (Abu Hena *et al*., 2003). Higher stocking density of shrimp and their metabolic products, and/or partly microbial activity is the probable cause. As expected ammonium concentrations increased throughout the grow-out period as the shrimp biomass increased. Compared with other reported study, the pond water nutrients of the present studied pond are similar to or comparable with other culture ponds of *P. monodon* in Australia (Burford, 1997) with the value for NO_3^- (0.08 ppm), NH_4^+ (0.30 ppm) and $PO_4^=$ (0.05 ppm). This comparison suggests that the expected improvement of water quality was not apparent in the pond using microbial products. The result of present study was in agreement with the statements of Boyd and Gross (1998). Shariff *et al*., (2001) also observed that the improvement of overall pond water parameters did not achieve between treated and untreated ponds with microbial products and the mechanisms by which the bacteria improved survival is unknown.

Table 1: The Range of Water Quality Parameters of Culture Ponds Using Probiotics and None Probiotics.

Parameters (Range)	This study	Pond without probiotics[1]
DO (mg/l)	6.8-10.3	7.2-12.2
Temperature (ºC)	30.0-32.0	26.2-32.0
pH	7.22-8.44	7.53-8.17
Salinity (‰)	16.0-28.2	19.0-27.0
Transparency (cm)	16.0-37.0	20.0-70.0
Total suspended solid (g/l)	0.10-0.17	0.08-0.18
BOD_3 (mg/l)	0.15-0.65	4.80-7.90
Chlorophyll *a* (mg/l)	0.05-0.25	0.03-0.24
NO_3^- (ppm)	0.01-0.02	0.01-0.04
NH_4^+(ppm)	0.28-1.99	0.14-0.41
$PO_4^=$(ppm)	0.01-0.16	0.01-0.11
TS (ppm)	1010-2486	1205-4375

[1]Abu Hena *et al.* (2003)

The textural classes of the soil samples and different physical and chemical variables of soil are shown in Table 2. The soil of the pond using probiotics was sandy. Soil texture is an important factor in construction of pond, and shrimp farmers tend to favor sites with high clay content. The wet pH values were not significantly differences between the sampling ponds. The dry soil pH was not found significantly differences among the present studied ponds through out the culture period (Table 2) and almost near to the wet pH values. Culture pond soils become aerobic when dried. In acidic soils the exchangeable aluminum concentrations controlled pH value, while calcium carbonate and other minerals in neutral and slightly alkaline soils. Therefore, dry soil pH measured in distilled water may be higher or lower than the pH values of wet pond soils (Munsiri *et al*., 1995). The dry pH values in this study (Table 2) were comparable (7.1-8.6) with the study ponds by Ritvo *et al*. (1998) and higher than found (3.68-6.2) by Das *et al*. (2002).

The cation exchange capacity (CEC) of the present investigated ponds is shown in Table 2. Cation exchange capacity is the ability of colloids in a soil to adsorb cations (Boyd, 1995a). The soils from probiotics and none probiotics pond differ markedly in CEC and the ranges were between 4.22-6.56 and 7.55-22.06-meq/100 g, respectively. This difference may be due to natural variation of soil textural composition between the two ponds. Munsiri *et al.* (1995) stated that cation exchange properties of soils result from clay and organic matter fractions. The range of clay percentage of the present studied ponds was 5.99-7.01% and 54.10-64.58%, whereas the organic matter was 2.70-5.47% and 4.17-9.27% for the shrimp ponds using probiotics and none probiotics, respectively. The soil contained 4.5-6.0% organic matter and 30-50% clay, and the comparatively high CEC of soil resulted from organic matter (Munsiri *et al.*, 1995), which support the present finding (Table 2). McNutt (1981) stated that sediment containing 40% clay and 5% organic matter has a CEC of 14.1 meq/100 g. This value is within the range of CEC values reported for none probiotics pond. Organic matter concentration of soils was found significantly higher (>7.0%) in the shrimp pond none probiotics compare to the culture ponds with probiotics (>3.0%). The culture pond soils using probiotics contains lower percentage of organic matter and total carbon probably due to decomposition of organic materials during the mineralization process (Moriarty, 1986). The mean value of soil TS (1.58±0.33% dry wt) decreased in the culture pond using probiotics, while it was higher in none probiotics pond (Abu Hena unpublished data). The lower amount of soil TS in the culture pond with probiotics may be due to utilization sulfate compounds by heterotropic bacteria (Boyd 1990, 1995b), which converted to sulfur and its related compounds into the pond environment. This may be the case for the present investigated pond using probiotics. The sulfur oxidizing bacteria in the ponds treated with microbial products suggesting efficient conversion of H_2S to sulfur compounds (Devaraja *et al.*, 2002) and increased the TS of pond water.

Table 2: Range of Concentration for Different Physical and Chemical Variables of Soil from Shrimp Culture Ponds.

Variable	This study	Pond without probiotics[1]
Sand (%)	86.12-89.24	5.48-7.56
Silt (%)	4.51-5.89	36.25-38.24
Clay (%)	5.99-7.01	54.10-57.15
pH (wet)	7.37-9.32	7.87-8.51
pH (dry)	7.65-8.79	7.92-8.33
Cation exchange capacity (meq/100 g)	4.22-6.56	7.55-22.06
Organic matter (%)	2.70-5.47	4.93-9.93
Organic carbon (%)	1.42-2.88	2.59-5.22
Total carbon (%)	2.20-6.82	5.11-7.55
Total sulfur (% dry wt)	0.77-3.63	1.98-2.48

The concentrations of macro-micronutrient of pond soils are presented in Table 3. The concentrations of Ca, Mg, K and Na were found significantly higher in the culture pond using probiotics than none probiotics pond (Table 3). The observed higher concentrations of major macronutrients in soil could probably attribute to nutrient loading from water, feed, uneaten feed, faeces and accumulation from soil pore water during drying over time and pond age. Ritvo *et al*. (1998) stated that the tentative explanation for the higher content of major cations in the incoming water could be due to precipitation. It is surprising to record that the concentrations of soil Mg in probiotics pond (16541.7±3007.8 ppm) was almost uniform probably due to application of dolomitic agricultural limestone (8132 kg/t) into the pond. This concentration was very high according to the classification of soil characteristics done by Boyd *et al*. (1995b) for culture pond. The concentration of other elements was medium characteristics for K (567.1±103.23 ppm) and Ca (3961.9±705.3 ppm), and very low characteristics for Na (1303.7±78.47 ppm) in the pond using probiotics. Soils from none probiotics pond have very low characteristics for Ca, Mg and Na, whereas concentration of K was medium (836.1±39.2) according to Boyd *et al.* (1995b) classification. The overall concentrations of Ca, Mg, K and Na did not increase significantly through out the culture period in both ponds. It indicates that in these ponds, part of nutrient load was either converted to organic form (Ritvo *et al.*, 1998), or major macronutrients did not washed out during water exchange.

Table 3: Range of Concentration for Different Chemical Variables of Soil from Shrimp Culture Ponds.

Variable (ppm)	This study	Pond without probiotics[1]
Calcium	1969-81510	206-586
Magnesium	4900-28412	231-445
Potassium	360-1240	466-1060
Sodium	946-1545	142.0-342.8
Iron	7.0-28.5	163.0-359.0
Manganese	1.46-4.68	47.4-146.1
Zinc	0.05-0.17	32.0-205.3
Copper	0.01-0.04	3.73-7.89
Aluminum	19.6-76.3	413.3-751.0
Lead	1.57-6.89	8.31-17.5
Cobalt	<0.001	2.08-3.51
Cadmium	0.003-0.09	0.02-0.55
Vanadium	0.53-2.63	11.2-18.6
Chromium	0.05-0.09	13.0-23.1
Titanium	2.32-4.92	21.4-151.4
Arsenic	0.05-0.21	10.9-17.7
Silver	0.01-0.98	0.02-0.40
Antimony	<0.02	0.02-1.96

[1]Abu Hena Unpublished Data

Not much variation in micronutrients of culture pond soils was observed during the culture period. Differences in concentrations are most likely related to the properties of different types and ages of pond bottom soils, and probably due to inputs in uneaten feeds, used fertilizer, water quality and other products. Munsiri *et al*. (1995) and Smith (1996) stated that the supplied feed could have contributed to the concentrations of Cu, Zn, Co and Cd in the culture ponds. Ritvo *et al*. (1998) observed that the accumulation of Fe, Mn, Cu and Zn was due to precipitation of sulfur compounds under anaerobic conditions that develop in the flooded soils. The concentrations of Zn and Co were not found higher in the aged ponds by Munsiri *et al*. (1995), which support this finding for almost all micronutrients and other elements. Cadmium concentrations were found almost similar (<1 ppm) with Smith (1996).

Daily mean growth rate of culture shrimps were observed 0.20 g/day and 0.23 g/day for the culture pond using probiotics and none probiotics, respectively. The lower daily growth rate of shrimps in probiotics pond probably due to several interrelated factors i.e. water quality, natural food availability, stocking density and others environmental forcing. A lower growth rate of shrimps was also reported as stocking density increased (Apud *et al*., 1981). Probably it may be case for probiotics pond with the stocking density of 48 PL/m^2. Apud *et al*. (1981) also concluded that the amount and quality of food was adequate for survival of shrimps, but probably not suitable for promoting faster growth when ponds are stocked at higher densities of PL. Compare to the reported studies elsewhere the growth rate of present studied pond (0.20 g/day) was relatively lower (Liao, 1977; Chen *et al*., 1989; Lumare *et al*., 1993) probably due to variable of pond ecosystem and culture management.

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