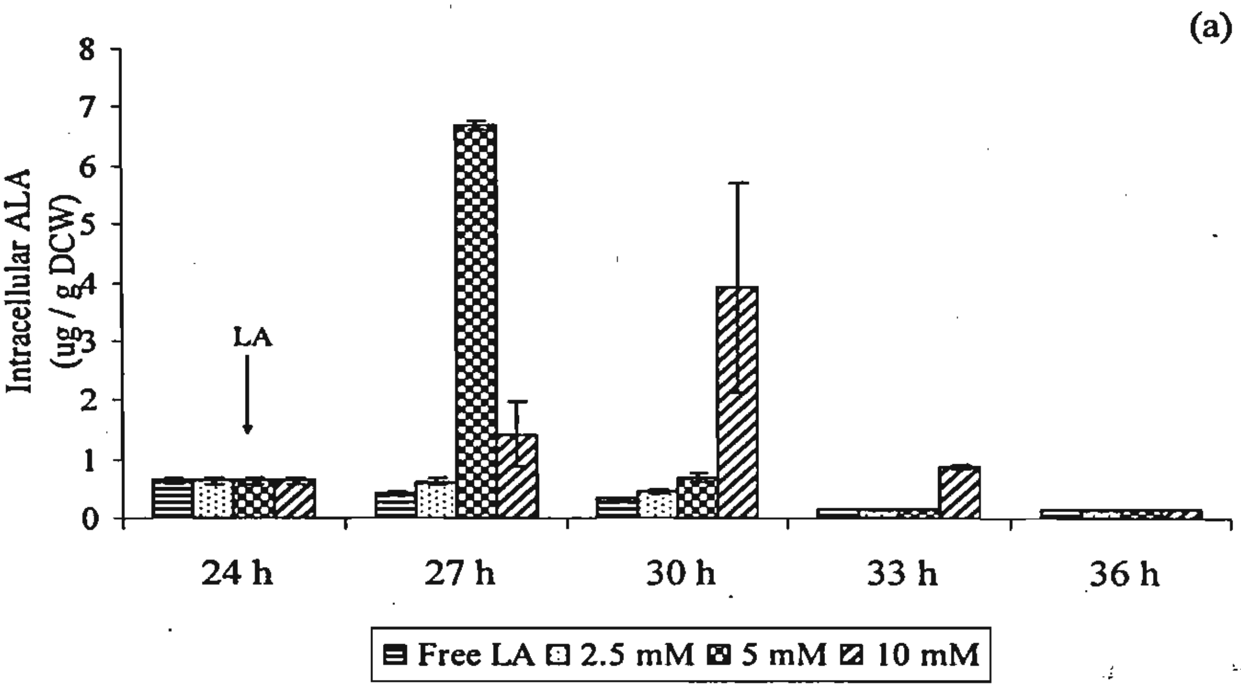


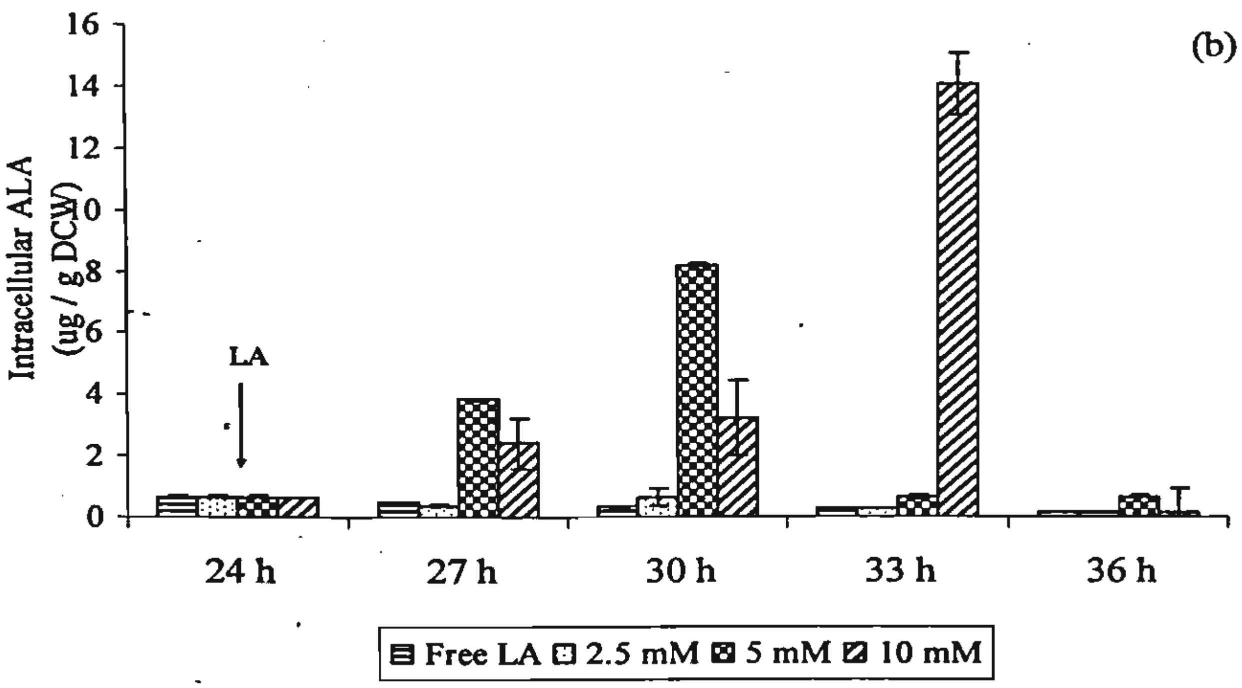


Fig.4 Effect of levulinic acid (LA) concentration on intracellular ALA accumulation in *Rhodobacter sphaeroides* SH5 cultivating in MGG medium containing 20 mM glucose (a), and 50 mM glucose (b) under microaerobic-dark condition (200 rpm) at 37°C

2.4 Effect of Fe supplementation

Supplementation of 30 µM iron in MGG medium (50 mM glucose) and addition of 10 mM LA at 24 h cultivation could increase the intracellular ALA of *Rhodobacter sphaeroides* SH5 to 226 µg/gDCW, with the productivity of 7.54 µg/gDCW/h (Fig. 5). This was 370 folds higher than the normal level of intracellular ALA accumulation at 24 h cultivation in MGG medium (50 mM glucose). Iron was selected to induce intracellular ALA accumulation because it is necessary for the conversion of coproporphyrinogen III to protoprophyrinogen IX and for the formation of heme compound in photosynthetic pathway. Moreover, Iron may suppress the secretion of ALA in the culture medium and inhibit ALA synthetase activity in the cell free extract. It can be considered that intracellular ALA synthetase activity may be inhibited by iron if it is accumulated in the cell at high concentration (Sasaki *et al.*, 1991).

From these results, the reason why extracellular ALA was not observed in the presence of iron may due to the fact that ALA synthetase was inhibited and small amount of intracellular ALA formed in the cells by residual activity of ALA synthetase (from iron inhibition) may be utilized for tetrapyrrol synthesis to maintain cellular growth. Supplementation of Fe^{2+} and/or Fe^{3+} to the culture of *R. sphaeroides* IFO 12203 enhanced intracellular ALA synthetase but ALA secretion could not be observed as it did in the absence of Fe^{2+}, even though the ALA dehydratase inhibitors (LA) was added (Sasaki *et al.*1989).

When the medium with supplementation of iron and addition of LA, the strain *R. sphaeroides* SH5 preferred to accumulate ALA inside the cells.

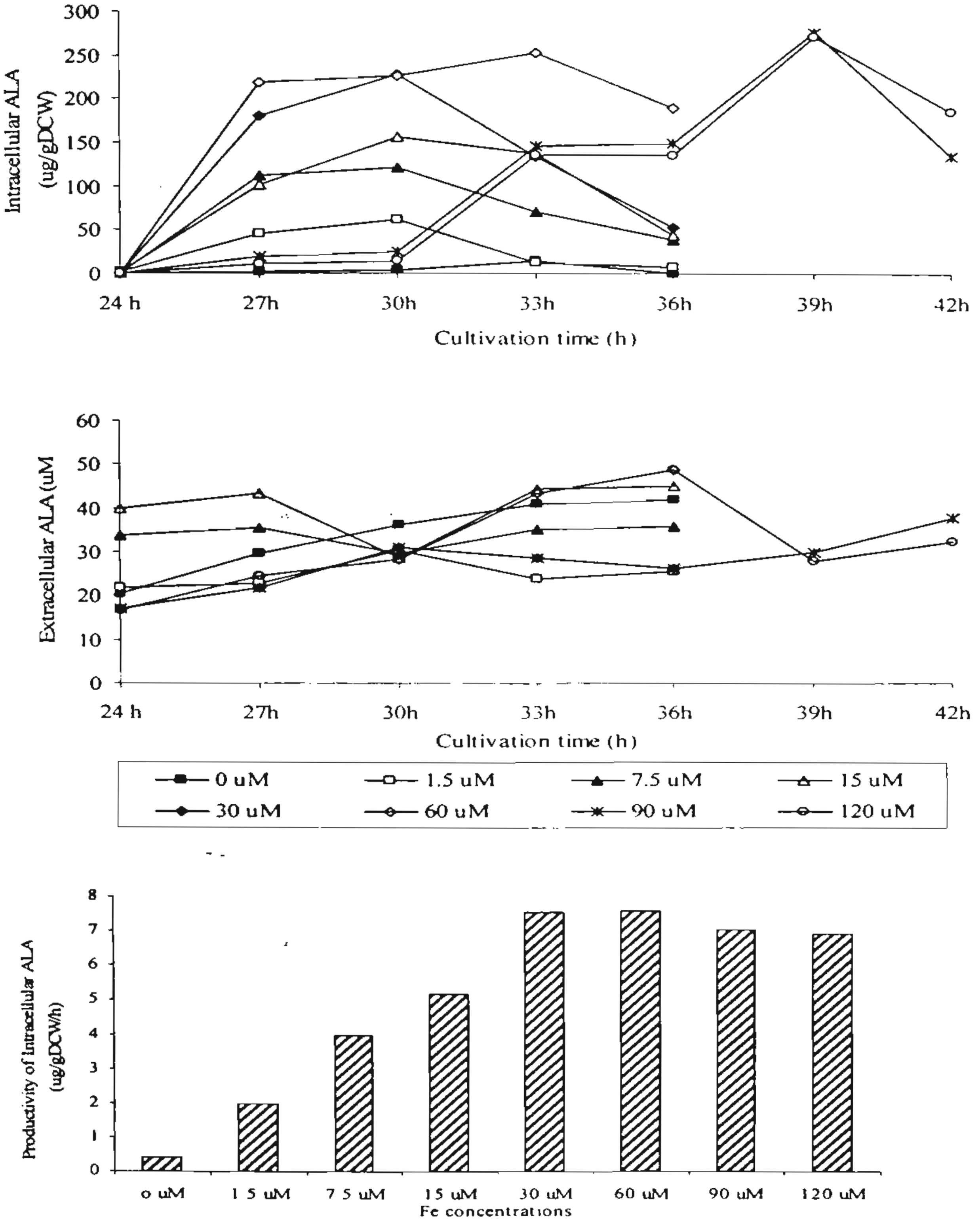


Fig.5 Effect of iron supplementation and LA addition on ALA production from *Rhodobacter sphaeroides* SH5 in MGG medium (50 mM glucose) under microaerobic-dark condition (200 rpm) at 37°C

3. Shrimp feeds preparation

The intracellular ALA content in dry cells of broken-biomass containing normal level of ALA (feed no.3), non-broken-biomass containing high level of ALA (feed no.4) and broken-biomass containing high level of ALA (feed no.5) were 0.17±0.03, 70.59±2.87, 67.32±3.41 (μg/g DCW), respectively. It had been evident from previous investigations that photosynthetic bacterial cells (PSB) contain high protein and other physiologically active substances for inclusion in aquaculture feedstock. In China, the studies on probiotics in aquaculture were focused on photosynthetic bacteria. Three strains of photosynthetic bacteria were used in prawn (*P. chinensis*) diet preparation and their effect was studied (Qiao, *et al.*, 1992). Addition of the photosynthetic bacteria in the food or culture water was found to improve prawn growth as well as water quality. Protein content of PSB, 60-70%, is relatively high compared with that of yeast protein (40-60%). This study therefore using biomass of PSB to substitute fish meal in diet ingredients. In addition, PSB contain various vitamins, especially a high content of vitamin B_{12}. In Japan, PSB had been widely fed as sources of vitamins and trace mineral for cultivation (Sasaki *et al.*, 1991). Nutrient content of the diets in term of crude protein, crude lipid, crude ash and moisture content were given in Table 4.

Table 4 Nutrient content of the experiment feeds

Nutrient content (%dry basis)	Feed no.				
	1 (control)	2	3	4	5
Crude protein	40.55 ± 0.06	40.97 ± 0.19	39.95 ± 0.25	40.18 ± 0.15	40.51 ± 0.16
Crude lipid	7.75 ± 0.70	7.65 ± 0.20	7.25 ± 0.15	7.57 ± 0.32	7.64 ± 0.52
Crude ash	9.45 ± 0.06	9.62 ± 0.06	9.47 ± 0.04	9.36 ± 0.07	9.52 ± 0.00
Moisture	6.48 ± 0.13	4.93 ± 0.15	6.28 ± 0.17	7.16 ± 0.19	5.39 ± 0.23

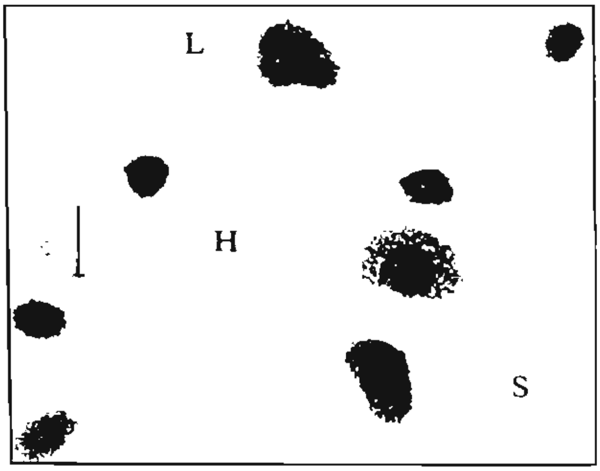
4. Effect of ALA on the immunity of shrimp

ALA is biodegradable herbicide and insecticide (Sasaki *et al.*, 2002). A common measure of initial acute toxicity is the lethal dose (LD_{50}) that causes death from the single exposure in 50 percent of treated animal. LD_{50} is generally expressed as the dose in milligram of chemical per kilogram of body weight. Office of Pesticide Programs (OPP) of the U. S. Environmental Protection Agency (EPA) uses qualitative descriptors to describe different levels of acute toxicity, the most likely kind of effect of modern pesticides. The LC50 or EC50 (immobility in aquatic invertebrates) would simply be expressed as >100 ppm. When no lethal or sub-lethal effects are observed at 100 ppm, OPP considers the pesticide will have "no effect" on the species (www.epa.gov/oppfead1/endanger/effects/acephate-analy.pdf). Toxicity of ALA on black tiger shrimp (average weight 15 ± 2 g) was determined in the ranges of 0-3,300 ppm for 7 days. LD_{50} of ALA was estimated to be 113.33 ppm for 7 days. Therefore ALA is practically non-toxic as LD_{50} was higher than 100 ppm (Zucker, 1985).

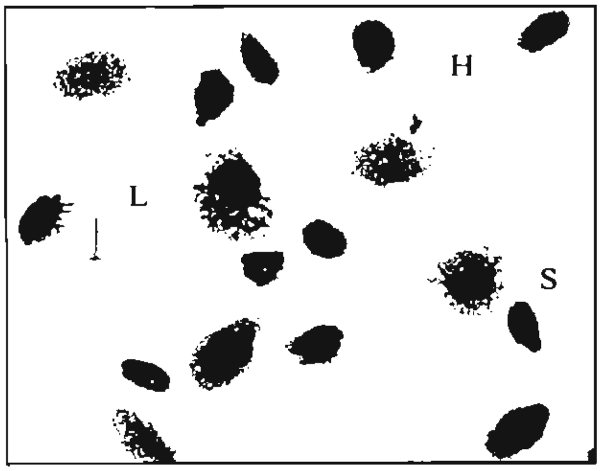
4.1 Immune response in haemolymph of black tiger shrimp (*in vitro*)

Here, phenoloxidase (PO) activity was examined as indicators for stimulatory activity of ALA in haemocytes (*in vitro*) of black tiger shrimp. PO is a redox enzyme which is capable of oxidizing phenols to quinines, which leads to spontaneous formation of melanin as an end product. Both melanin and quinines have also been found to act as inhibitors of bacterial enzyme and to be a fungistatic (Sung *et al.*, 1998). Although PO has long been considered to be the key enzyme in the biosynthesis of melanotic material in insects, there just has direct evidence verifying its role in parasite melanization of microfilaria by mosquitoes. Compared with controls, hemolymph PO activity in *Armigeres subalbatus* mosquitoes traduced with antisense RNA which targeted to highly conserved copper-binding region of *A. subalbatus* PO gene, was significantly reduced (Shiao *et al.*, 2001). Therefore, it was anticipated that proPO system plays an important role in shrimp defense mechanism. PO is the terminal enzyme in the prophenoloxidase (proPO) activating system. The proPO system protein released from semigranular haemocytes of *P. monodon* during degranulation. ALA (10-1,000 ppm) could stimulate the degranulation (Fig. 6) of semigranular cell higher than granular haemocytes (Table 5). The semigranular cells were most sensitive and they are the first respond to minuscule amount of microbial cell wall (pg/l), lipopolysaccharide and β-1,3-glucan, by degranulation and then the proPO system were released (Johansson and Söderhäll, 1985). Together with degranulation, other important components were released from haemocytes which directly involved functioning as cell adhesion and encapsulating factor.

The proPO activating system comprises an enzyme cascade leading to activation of proPO and other compounds with related activities. Upon activation by β-1,3-glucan, the zymogens proPO is activated by a serine protease, the prophenoloxidase activating enzyme (ppA) and other factor located in haemocytes, through cleavage to PO (Hölmblad and Söderhäll, 1999). HLS treated with 0.1-1,000 ppm ALA revealed higher PO activity compared with negative PO activity (Table 6). ALA was assessed to function as an activator of proPO activity by increment efficiency of ppA activity. However, exceed concentration of ALA was negative effect to ppA activity. ALA had affected to stimulate proPO activating system by increased degranulation and activated ppA efficiency so it was the new kind of shrimp immunostimulant.



a



b

Fig.6 Degranulation of haemocyte : control (a) , degranulation (b)
H = hyaline cell, S = semigranular cell, L = large granular cell

Table 5 Degranulation of haemocytes induced by ALA

Treatment	% degranulation	
	Large granular cell	Semigranular cell
Control	8 ± 4	12 ± 5
ALA 10 ppm	10 ± 5	29.5 ± 11[a]
ALA 100 ppm	10 ± 5	32.5 ± 13[a]
ALA 1000 ppm	11 ± 5	32 ± 11[a]

Table 6 Effect of ALA on specific PO activity (*in vitro*) of black tiger shrimp (*Penaeus monodon*)

Treatment	Specific PO activity (unit/min/mg protein)
Negative control[1)]	404.36 ± 71.71[a]
ALA 0.1 ppm	496.09 ± 22.68[a]
ALA 1 ppm	470.58 ± 79.83[a]
ALA 10 ppm	471.41 ± 95.38[a]
ALA 100 ppm	488.48 ± 95.26[a]
ALA 1,000 ppm	422.75 ± 70.57[a]
ALA 10,000 ppm	157.92 ± 88.51[b]
Positive control[2)]	1141.66 ± 229.96[c]

[1)] Na-CAC buffer was used instead of trypsin solution.

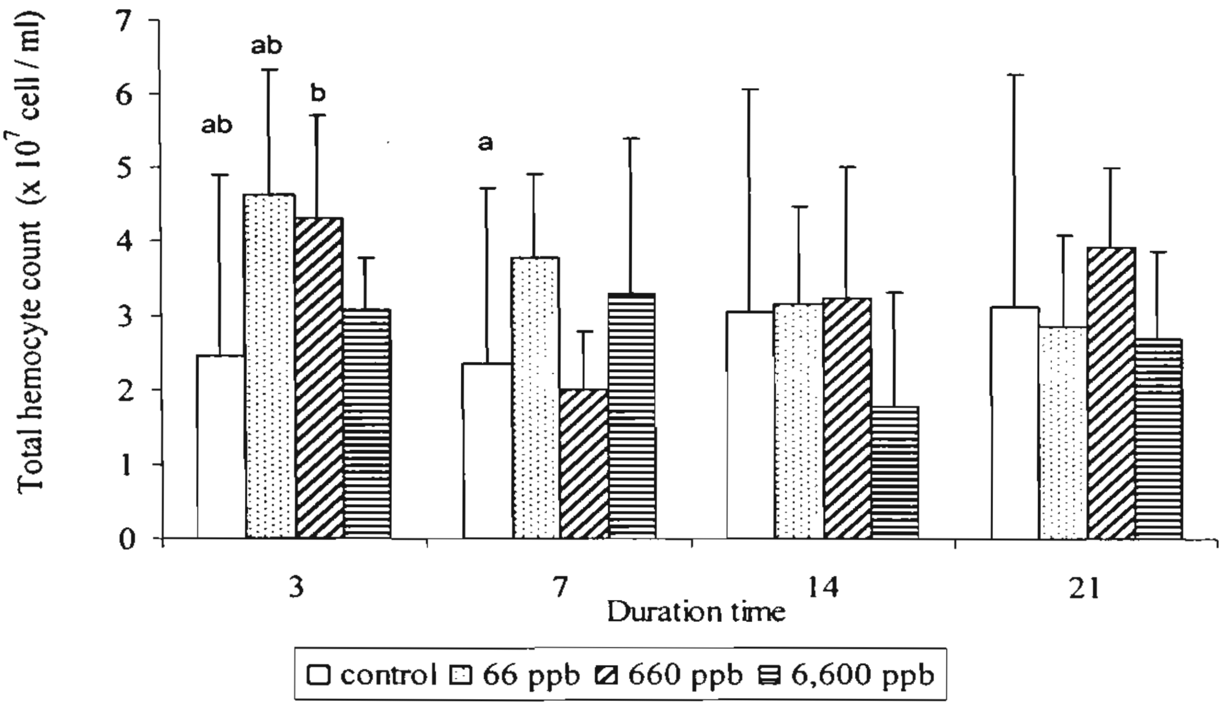
[2)] Trypsin solution was added to activate the proPO (zymogen) to PO (active form). Na-CAC buffer and L-DOPA were the blank

4.2 Immune responses in black tiger shrimp (*in vivo*)

Injection administration is the direct method to assess the potential of chemical property. ALA at 66 and 660 ppb had affected to increase THC and activated PO activity (Fig. 7). The increment of haemocytes in circulatory system was the good responses of shrimp immunity caused of hemocytes constitute an important line of defense involving processes such as phagocytosis, encapsulation, melanization (proPO activating system) and coagulation (Johansson *et al.*, 2000). In addition, haemocytes play an important role in the production and discharge of agglutinin (Kopacek *et al.*, 1993) of antibacterial peptides (Destoumieux *et al.*, 1997) and of cytotoxic molecules such as lysomal enzyme (lysozyme, esterases, phosphotases, phospholipases, peroxidase and protease) (Millar and Ratcliffe, 1994). Moreover, shrimp which had been received ALA after 3 days injection showed resistance to White Spot Syndrome Virus (WSSV) challenge (Fig. 8).

4.3 Effect of ALA on growth, feed performance, survival and immunological functions

There were no significant difference in growth, survival and feed conversion ratio (FCR) of shrimp among the diets after 8 weeks of feeding (Table 7). The final body weight of shrimp fed with the control, containing ALA and experimental diet (PSB containing ALA) were 8.935, 8.025, 8.582, 8.419 and 8.560, respectively. Survival and FCR of shrimp fed treatment diet between 95.2 – 97.6 % and 1.85 – 2.00 respectively. Weight gain was between 215.92 - 246.64 % and had significantly difference of weight gain lipopolysaccharide (LPS) which is cell wall component of *R. sphaeroides* SH5 biomass, containing in diet no 3-5 may reduce appetite and suppress growth of shrimp (Raa, 2000). After 4 weeks cultivation, temperature changes within a raining day (24-32°C) resulted in higher FCR. During cultivation, shrimp stress frequently causes by means of physical (temperature change) chemical (water quality) or biological (nutritional or infections) factors, which can result in a reduction of shrimp immunological resistance and thus facilitate infection (Chen *et al.*, 1995). Temperature has a direct effect on other environmental parameters such as salinity and oxygenation of the water. Temperature is a key determinant of growth and production in shrimp farming (Jackson and Wang, 1998).



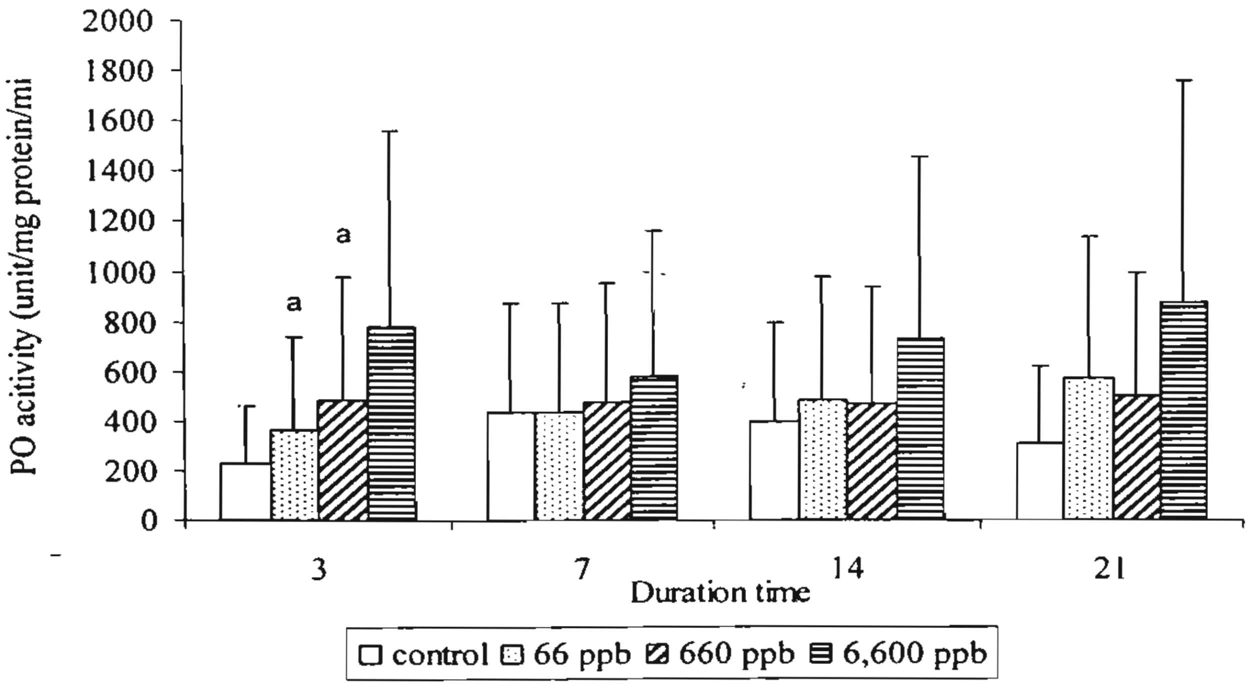


Fig.7 Effect of commercial ALA (*in vivo*) 66, 660, 6,600 ppb injection on THC and specific PO activity of *Penaeus monodon* during 3-21 days

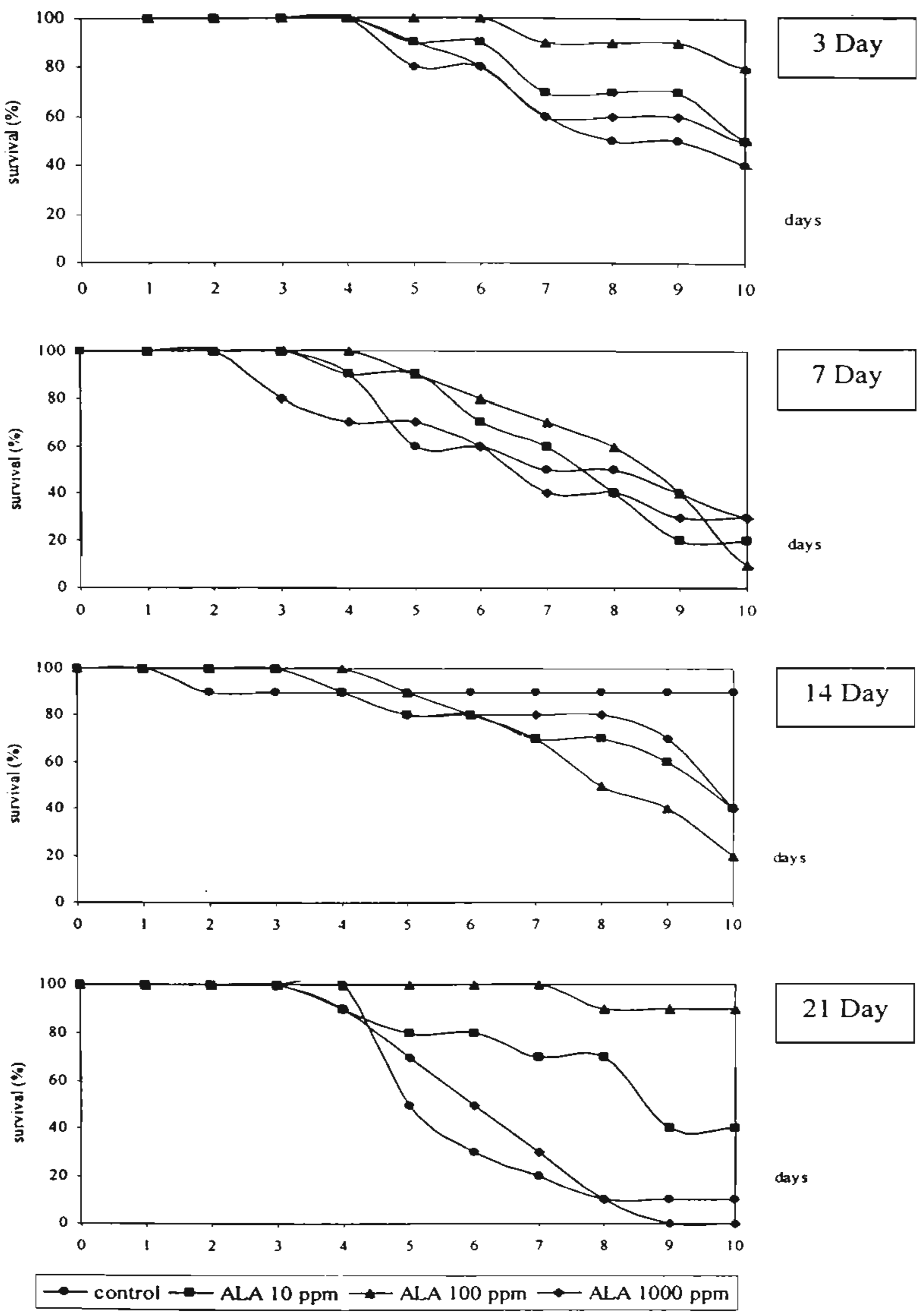


Fig.8 WSSV challenge of *Peneaus monodon* after injection ALA 66, 660, 6,600 ppb during 3-21 days

Table 7. Effect of biomass of *Rhodobacter sphaeroides* SH5 containing ALA on growth and feed performance of *Penaeus monodon* affect 8 weeks feeding period

Treatment	Average weight (g)		Weight gain	Survival	FCR
	Initial	Final	(%)	(%)	
Feeding 1 (control)	2.577 ± 0.021	8.935 ± 0.517^{b}	246.64 ± 18.07^{b}	97.6 ± 3.58	1.853 ± 0.118
Feed 2	2.549 ± 0.042	8.025 ± 0.228^{a}	215.92 ± 9.643^{a}	95.2 ± 5.21	2.007 ± 0.179
Feed 3	2.563 ± 0.022	8.582 ± 0.378^{a}	234.86 ± 14.75^{ab}	95.2 ± 1.79	1.951 ± 0.179
Feed	2.579 ± 0.028	8.419 ± 0.477^{ab}	226.43 ± 17.95^{ab}	96.0 ± 5.65	1.962 ± 0.191
Feed5	2.561 ± 0.019	8.560 ± 0.526^{ab}	234.14 ± 19.89^{ab}	97.6 ± 2.19	1.894 ± 0.101

Feed 1 was used as the control.

Feed 2 contained commercial ALA (2 mg/kg).

Feed 3 contained 3% broken-biomass with normal level of ALA (0.17 μg/g DCW).

Feed 4 contained 3% non-broken-biomass with high level of ALA (70.59 μg/g DCW).

Feed 5 contained 3% broken-biomass with high level of ALA (67.32 μg/g DCW)

4.3.1 Effect of ALA on growth, feed performance, survival and immunological functions

Total hemocyte count

Total haemocyte count (THC) of *P. monodon* fed different ALA containing diets were illustrated in Fig 9. Normally THC for *P. monodon* has been reported to be in the range of 20-40 x 10^{6} cells ml^{-1} (Chang *et al.*, 1999). Experimental feeds (no. 2-4) could increase agranular or hyaline cells whereas only feed no.4, contained biomass containing high level of ALA, could increase both of granular and agranular cells. Significantly higher THC were observed in shrimp fed diets supplemented with both broken-biomass containing normal level of ALA (feed no. 3) and biomass containing high level of ALA (feed no. 4) than shrimp fed with control feed (feed no.1). Feed no.3 represented the effect of lipopolysaccharide (LPS) as immunostimulant for comparison with the effective of ALA and LPS containing in feed no.4. Among the four ALA sources, shrimp fed with feed no.4 had the highest THC both in the term of granular and agranular cells than fed with the other diets. High THC after fed with feed no.3 and 4 in this present study suggests that many haemocytes were used in the reaction against LPS and ALA+LPS containing in biomass of *R. sphaeroides* SH5, respectively. This is in accordance with the knowledge that the circulating hemocytes play important roles in the invertebrate immune response (Söderhäll and Cerenius, 1992). THC was suggested to have the influence on the capability of the host to react against foreign material and varies in response to infection, environmental changes and ecdysis in most crustaceans (Persson *et al.*, 1987).

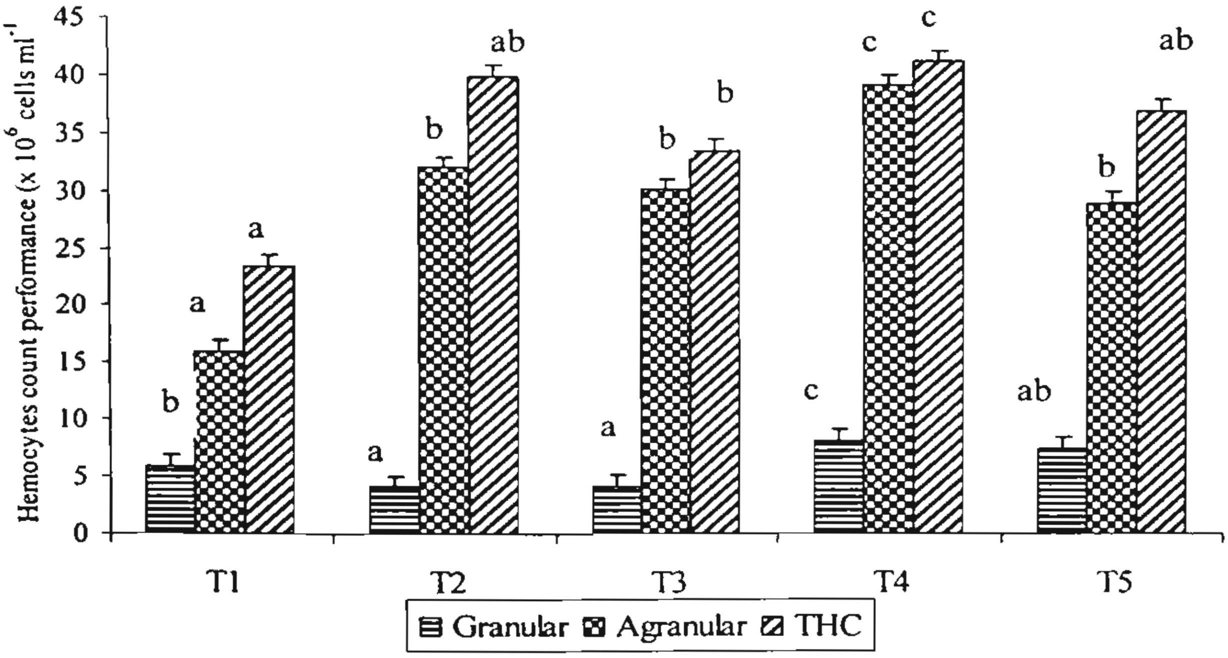


Fig.9 Effect of ALA on haemocytes count performance of *Penaeus monodon* after 8 weeks feeding period

In feed no.2 and no.5 containing broken-biomass containing high ALA, ALA may loss during the feed preparation and solubilized from diet into seawater in aquarium which due to alkaline pH of sea water in aquarium (8.0-8.5).

The stability of ALA is dependent on parameter such as pH, temperature and time (Didden *et al.*, 1994). ALA solution was stable at pH 2.35 where the amino group is protonated so it is important to use ALA at lower pH. The temperature dependence of condensation reaction of ALA and Arthenius plot was linear and hence prediction of stability at lower temperature was possible (Elfsson *et al.*, 1998).

Phenoloxidase activity

The prophenolosidase (proPO) system had been considered to play an important role in the defense system of crustaceans (Smith and Söderhäll, 1983). Activation of the proPO system which was measured in terms of the PO activity has been used by some investigation to measure immunostimulant in shrimp (Sung *et al.*, 1994, Devaraja *et al.*, 1998). In this study, the PO activity in haemocytes of *P. monodon* was used as one indicator for the immunostimulating properties of ALA containing in biomass of *R. sphaeroides* SH5. The specific of PO activity from the haemocytes of shrimp in control group was 523.57 ± 99.92 (unit/mg protein) (Table 8). Shrimp fed diets containing ALA showed no promising results on specific of PO activity from haemocytes after 8 weeks feeding period. Agranular (hyaline) cell was found to be negative for proPO, while the granular (semigranular and large granular cells) cells were positive. Although, shrimp fed with feed no.4 had higher granular than feed no.1 (control), specific PO activity showed no difference. The increment ratio of agranular (hyaline) cell was higher than granular cell, resulting from the enhancement of protein content in HLS so no different significance of specific PO activity. Oral administration of biomass containing ALA could not stimulate PO activity was contradicted to the previous study by injection of ALA. The discrepancy could be explained by the difference in exposure period (injection method) in which PO activity exposed at day 3 and 7 day whereas in the oral administration method, PO activity was not performed until after 8 weeks feeding.

Superoxide dismutase activity

When microorganism is engulfed by hemocytes, a series of microbicidal substances are generated. These include superoxide radicals (O^{2-}), hydrogenperoxide (H_2O_2), hydroxide (OH^-), singlet oxygen (O_2^1), myeloperoxide (MPO^-)-catalyzed hyprochlorite and digestive enzymes within cytoplasmic granules (Sagal, 1989). They can either inhibit microbial activities or completely digest the microorganisms. As it is nonspecific, O^-_2 is capable of attacking both microorganisms and host cells and probably can do harm to shrimp itself when O^-_2 is over produced. Due to the specificity of superoxide dismutase (SOD), in catalyzing the dismutation of O^-_2 into hydrogen peroxide, is involved in protective mechanism within tissue injury following oxidative process and phagocytosis. The high SOD value indicated that more O^-_2 need to be reacted (Chien *et al.*, 2003). The mean SOD activity value in treated shrimp was significantly higher than control shrimp (Table 8). In this present work, it would appear that ALA containing in feed no.2, 4, 5 induced the haemocytes to produce SOD. Hölmblad and Söderhäll (1999) first related SOD to immunity in crustacean. They speculated that peroxinectin and extracellular SOD cooperate during respiratory burst to destroy ingested or encapsulated parasites. Neves *et al.* (2000) attributed lower SOD activity in shrimp infected by a gill parasite to change in oxygen consumption when respiration of the infected animal was impaired by the parasite. However, SOD was seldom used as one of the health indices.

Table 8 Effect of biomass of *Rhodobacter sphaeroides* SH5 containing ALA on specific phenoloxidase (PO) and superoxide dismutase (SOD) activity of *Penaeus monodon* affect 8 weeks feeding period

Treatment	Specific PO activity (unit/min/mg protein)	Specific SOD activity (unit/min/mg protein)
Feeding 1 (control)	523.57 ± 99.92	8.622 ± 4.1713^a
Feed 2	450.46 ± 128.35	13.266 ± 2.856^b
Feed 3	510.47 ± 121.05	13.122 ± 1.492^b
Feed 4	525.55 ± 160.03	14.075 ± 3.612^b
Feed 5	508.52 ± 136.31	13.289 ± 1.946^b

Feed 1 was used as the control.

Feed 2 contained commercial ALA (2 mg/kg).

Feed 3 contained 3% broken-biomass with normal level of ALA (0.17 µg/g DCW).

Feed 4 contained 3% non-broken-biomass with high level of ALA (70.59 µg/g DCW).

Feed 5 contained 3% broken-biomass with high level of ALA (67.32 µg/g DCW)

Clearance ability

The ability of shrimp to remove foreign particles from blood circulation can be used as an indicator of immune function in shrimp. Results from this study showed that biomass of *R. sphaeroides* SH5 in feed no.4 could enhance the ability of shrimp to remove bacterial cells from the blood circulating. Shrimp fed with feed no.4 exhibited higher efficient removal of bacteria from blood circulating than the control shrimp (Table 9).

Table 9 Effect of PSB containing ALA on the ability to remove bacterial cells from the blood circulating system of *Penaeus monodon* after 8 weeks feeding period

Treatment	Total vibrio count ($\times 10^3$ CFU/ml of haemolymph)
	(initial bacterial count $= 3.7 \times 10^7$ CFU/ml)
Feeding 1 (control)	122.50 ± 41.58
Feed 2	135.00 ± 94.42
Feed 3	111.11 ± 37.73
Feed 4	97.22 ± 36.32
Feed 5	110.00 ± 67.90

Feed 1 was used as the control.
Feed 2 contained commercial ALA (2 mg/kg).
Feed 3 contained 3% broken-biomass with normal level of ALA (0.17 μg/g DCW).
Feed 4 contained 3% non-broken-biomass with high level of ALA (70.59 μg/g DCW).
Feed 5 contained 3% broken-biomass with high level of ALA (67.32 μg/g DCW).

4.3.2 Effect of ALA on disease resistance and stress test

Disease resistance

Disease problems have grown proportionally with the intensive culture of shrimp worldwide. Intensive stocking density, use of infected carriers and facilities, and water quality management are among the reasons for disease problems. However, the most important reason for the greater susceptibility of shrimp to disease may be that their mechanism of resistance is weakened under intensive culture conditions. PSB containing ALA, an immunostimulant, is being fed to shrimp to enhance disease resistance (WSSV). The results of this attempt for the enhancement of disease resistance had been mixed in feed. Results showed that shrimp fed with feed no.4 had the highest resistance to infection (50% survival post-infection). While shrimp fed with feed no. 2, 3 and 5 showed similar in term of disease resistance (35-30% survival post-infection) but had better results than the control group (30% survival post-infection) (Fig. 10). These results indicate that ALA containing in feed no.4 plays an important role on disease resistance. The mechanism of ALA which provokes the immune system in shrimp is still unknown. However, direct injection of ALA could increase total hemocytes count as well as other immune functions in term of PO activity and WSSV resistance.

Stress test

Environmental stress such as low oxygen tension hampers the metabolic performance in shrimp and can reduce growth and molting frequency (Allan and Maguire, 1991) and cause mortality (Madenjian *et al.*, 1987). The mortality of stressed shrimp fed with feed no.2 was 30% while the mortality of the control shrimp was 0% (Fig. 11). In *P. monodon*, the phagocytic activity of haemocytes was less efficient in oxygen-depleted shrimp (Direkbusarakom and Dannayadol, 1998). The average clearance efficiency of the oxygen-depleted shrimp was approximately 50% less than in control shrimp. The decrease of defence parameter, THC in *P. stylirostris*, phagocytosis in *P. monodon*, may explain why in penaeid shrimp, low oxygen level in pond water causes, even at short-term, an increased susceptibility to infectious disease (Le Moullas *et al.*, 1998).

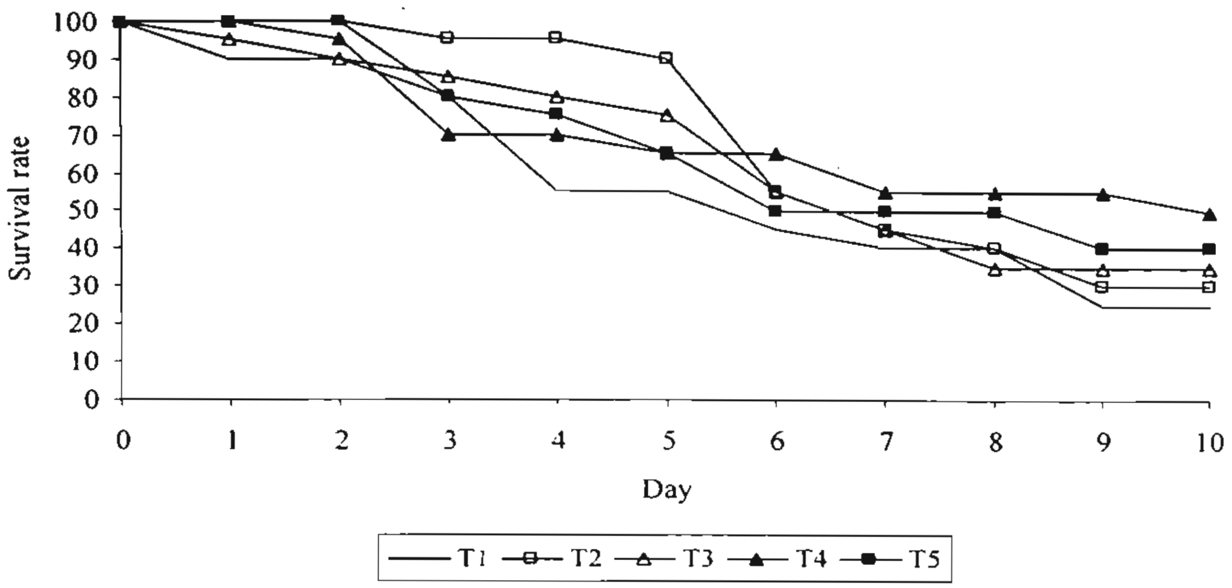


Fig.10 WSSV challenge of *Penaeus monodon* after fed PSB containing ALA for 8 weeks feeding period

Feed 1 was used as the control.

Feed 2 contained commercial ALA (2 mg/kg).

Feed 3 contained 3% broken-biomass with normal level of ALA (0.17 μg/g DCW).

Feed 4 contained 3% non-broken-biomass with high level of ALA (70.59 μg/g DCW).

Feed 5 contained 3% broken-biomass with high level of ALA (67.32 μg/g DCW)

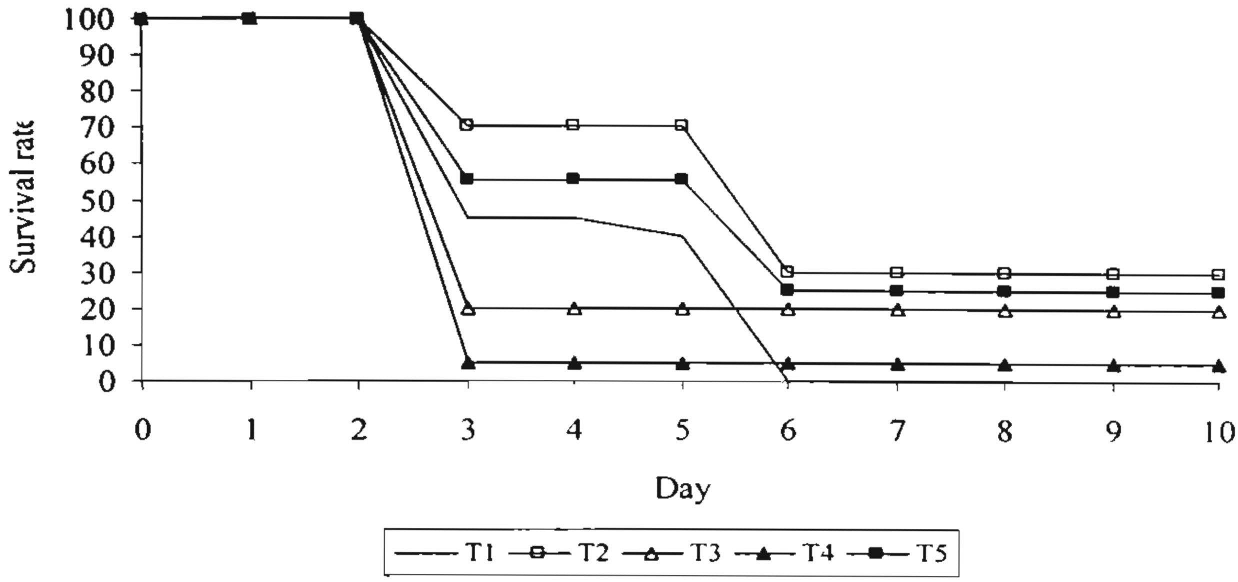


Fig.11 Effect of PSB containing ALA on stress test of *Penaeus monodon* after fed 8 weeks feeding period

Feed 1 was used as the control.

Feed 2 contained commercial ALA (2 mg/kg).

Feed 3 contained 3% broken-biomass with normal level of ALA (0.17 µg/g DCW).

Feed 4 contained 3% non-broken-biomass with high level of ALA (70.59 µg/g DCW).

Feed 5 contained 3% broken-biomass with high level of ALA (67.32 µg/g DCW).

4.4 Mechanism of ALA act as immunostimulant in black tiger shrimp

Due to the increment of PO activity of shrimp injected with ALA, the expression of proPO was used to determine the mechanism of ALA. Primers based on shrimp haemocyte (HE) cDNA library proPO (GenBank accession no.AF099741) was used to produce a proPO specific probe. By using these primers with haemocyte cDNA as a template, a 113 bp PCR product was amplified. The PCR product was subcloned into T-vector and sequenced. BLAST results showed sequence similarity of this clone to shrimp HE-Mn-SOD (accession no. AF099741). The experiment was carried on by injection of ALA (66 ppb) into shrimp and shrimp total RNA was collected on day 3 and 7. Control group was injected with saline solution. Samples of these 2 groups were collected on day 3 and 7. The results showed no significant difference between all treatments suggesting that no effect of ALA injection on Mn-SOD transcriptional level (Fig. 12).

Although PO activity of shrimp fed with ALA was no significant difference, SOD activity significantly increased. Therefore, the mechanism of ALA acted as immunostimulant not only study on expression of MnSOD, but also the expression of PO. Primers based on shrimp haematopoietic tissue (HPT) cDNA library Mn-SOD (GenBank accession no.) was used to produce a Mn-SOD specific probe. By using these primers with haemocyte cDNA as a template, a 428 bp PCR product was amplified. The PCR product was subcloned into T-vector and sequenced. BLAST results showed sequence similarity of this clone to shrimp HPT-Mn-SOD (accession no.). Specific primer was further used to study the expression profile of shrimp haemocyte Mn-SOD by effect of ALA treatment.

The effect of ALA on expression of Mn-SOD in shrimp fed with ALA. Shrimp were divided into 5 groups ;

Group 1 : fed with control feed

Group 2 : fed with purified ALA (2 mg/kg feed)

Group 3 : fed with feed pellet mixed with sonicated PSB cells containing normal level of ALA

Group 4 : fed with feed pellet mixed with intact PSB cells containing high level of ALA (2 mg/kg feed)

Group 5 : fed with feed pellet mixed with sonicated PSB cells containing high level of ALA

The trial was performed for 2 months with daily fed (5 meals/days). After 2 months, hemocytes were collected from 10 shrimp for determination of cytosol Mn-SOD expression by RT-PCR. The results showed that no significant difference was observed for all 5 treatments (Fig.12) suggesting that ALA had no effect at transcriptional level by Mn-SOD. Elongation factor (accession no. X94913) was used as constitutively expressed control gene. The increase of total SOD activity in circulating haemocytes in shrimp was observed.

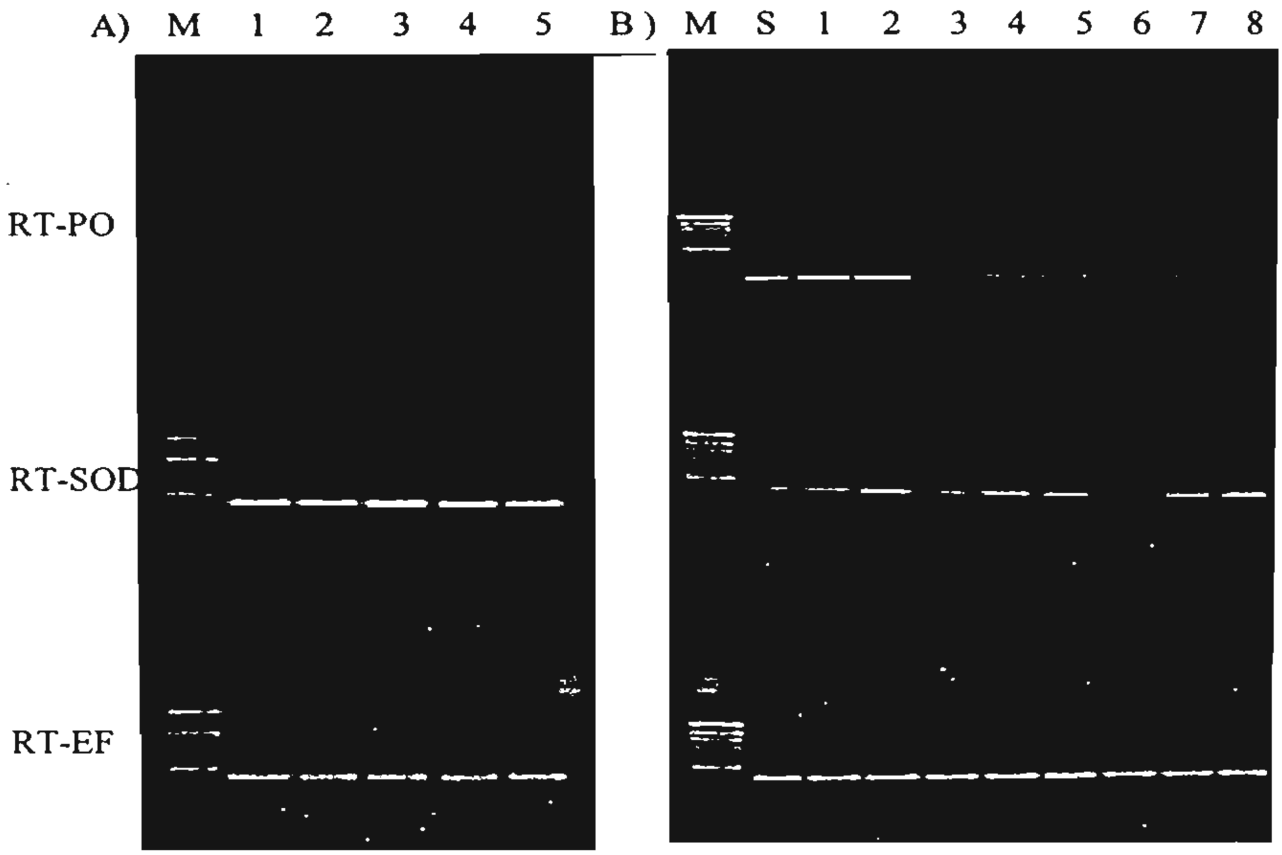


Fig.12 Semi-quantificated detection of PO and SOD mRNA by RT-PCR in an 1.5% agarose gel compared to the constitutive elongation factor gene. Panels A, Lane M : molecular standard 100-bb DNA ladder. Lanes 1, 2, 3, 4 and 5. Group 1 : fed with control feed, Group 2 : fed with purified ALA (2 mg/kg feed), Group 3 : fed with feed pellet mixed with breaked PSB cells containing normal level of ALA, Group 4 : fed with feed pellet mixed with intact PSB cells containing high level of ALA (2 mg/kg feed), Group 5 : fed with feed pellet mixed with sonicated PSB cells containing high level of ALA. Panels B :, Lane M : molecular standard 100-bb DNA ladder. Lane S : normal shrimp. Lane 1, 2 : injected with normal saline Day3. Lane 3, 4 : injected with 66 ppb ALA Day3. Lane 5, 6 : injected with normal saline Day 7. Lane 7, 8 : injected with 66 ppb ALA Day 7.

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Output จากโครงการวิจัยที่ได้รับทุนจากสกว. (คปก. และ วปก.)

1. **ผลงานตีพิมพ์ในวารสารนานาชาติ**

   Tungprasittipap, A., Sasaki, K., and Prasertsan, P. Screening of halotolerant photosynthetic bacteria and increment of its intracellular 5-aminolevulinic acid. (manuscript)

2. **การนำผลงานไปใช้ประโยชน์**

   - **เชิงพาณิชย์**

   - 2.1) งานวิจัยนี้กำลังอยู่ระหว่างการจดสิทธิบัตร เรื่อง "กรรมวิธีการผลิตกรด 5-อะมิโนลีวูลินิกและการใช้เป็นสารกระตุ้นภูมิคุ้มกันโรค" ซึ่ง สกว. ได้มอบหมายให้สถาบันทรัพย์สินทางปัญญาแห่งจุฬาลงกรณ์มหาวิทยาลัยเป็นผู้ดำเนินการในการขอจดสิทธิบัตร

   - 2.2) หากการดำเนินการในการจดสิทธิบัตรแล้วเสร็จ งานวิจัยนี้ก็มีโอกาสให้ประโยชน์เชิงพาณิชย์ได้

   - **เชิงสาธารณะ** (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)

   - งานวิจัยนี้สามารถสร้างความร่วมมือทั้งภายในและต่างประเทศ โดยภายในประเทศ เป็นการสร้างความร่วมมือและทำวิจัยร่วมกันระหว่างอาจารย์ต่างคณะใน
   มหาวิทยาลัยสงขลานครินทร์ (คณะอุตสาหกรรมเกษตรและคณะทรัพยากรธรรมชาติ) และอาจารย์ต่างมหาวิทยาลัย คือ มหาวิทยาลัยมหิดล (ศูนย์ความเป็นเลิศด้านกุ้ง คณะวิทยาศาสตร์) ส่วนความร่วมมือกับต่างประเทศ ได้ร่วมกับอาจารย์ของ Hiroshima Kokusai Gakuin University ประเทศญี่ปุ่น

   - **เชิงวิชาการ**

   - งานวิจัยเรื่องนี้ได้พัฒนานักศึกษาระดับปริญญาเอกภายใต้ทุน คปก. ให้มีความรู้ความสามารถด้านเทคโนโลยีการหมัก ด้านเทคนิคชีวโมเลกุล โดยเฉพาะอย่างยิ่งการศึกษา กลไกการเป็นสารภูมิคุ้มกันโรคให้กับกุ้งของกรด 5-อะมิโนลีวูลินิก

3.อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในต่างประเทศ การเสนอผลงานในที่ประชุม หนังสือ การจดสิทธิบัตร)

ผลงานวิจัยนี้อยู่ระหว่างการดำเนินการจดสิทธิบัตร (ดูข้อ 2.1)