Noradrenaline modulates the immunity of white shrimp \textit{Litopenaeus vannamei}

Winton Cheng\textsuperscript{a}, Hung-Tien Chieu\textsuperscript{a}, Mei-Ching Ho\textsuperscript{b}, Jiann-Chu Chen\textsuperscript{b,}\*

\textsuperscript{a} Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung, Taiwan 912, ROC
\textsuperscript{b} Department of Aquaculture, College of Life Sciences, National Taiwan Ocean University, Keelung, Taiwan 202, ROC

Received 15 February 2005; revised 15 July 2005; accepted 5 September 2005
Available online 27 December 2005

Abstract

The total haemocyte count (THC), phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency in response to pathogen \textit{Vibrio alginolyticus} were measured when the white shrimp \textit{Litopenaeus vannamei} (18.4 ± 1.2 g) were injected individually with noradrenaline at 10\textsuperscript{-8}, 10\textsuperscript{-7} and 10\textsuperscript{-6} mol shrimp\textsuperscript{-1}. For the shrimp that received noradrenaline at 10\textsuperscript{-8}, 10\textsuperscript{-7} and 10\textsuperscript{-6} mol shrimp\textsuperscript{-1}, the THC decreased by 15%, 21% and 32%, phenoloxidase activity decreased by 15%, 31% and 31%, respiratory burst decreased by 13%, 21% and 32%, and SOD activity decreased by 46%, 56% and 55%, respectively, after 2 h. The phagocytic activity and clearance efficiency of shrimp that received noradrenaline at either dose decreased significantly after 2 h. The THC, phenoloxidase activity, respiratory burst, SOD activity, phagocytic activity and clearance efficiency returned to normal values after 4, 4, 8, 24, 16 and 8 h, respectively, in the shrimp that received noradrenaline at either dose. In another experiment, \textit{L. vannamei} which had received noradrenaline at 10\textsuperscript{-8}, 10\textsuperscript{-7} and 10\textsuperscript{-6} mol shrimp\textsuperscript{-1} were challenged after 1 h by injection with \textit{V. alginolyticus} at 1.0 × 10\textsuperscript{5} colony-forming units (cfu) shrimp\textsuperscript{-1} and then placed in seawater of 20\textdegree C. The cumulative mortality of shrimp that received noradrenaline at either dose was significantly higher than that of shrimp that received saline after 4 h, and at the termination of the experiment (48 h after the challenge). It is therefore concluded that noradrenaline administration at 10\textsuperscript{-6} mol shrimp\textsuperscript{-1} or less causes immune modulation of \textit{L. vannamei}.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: \textit{Litopenaeus vannamei}; Noradrenaline; Total haemocyte count; Phenoloxidase activity; Respiratory burst; Superoxide dismutase activity; Phagocytic activity; Clearance efficiency

I. Introduction

White shrimp \textit{Litopenaeus vannamei}, which was introduced to the Eastern hemisphere in 1985, has since become the primary species currently being cultured in Thailand, Taiwan, and China [1]. Best survival of juveniles is between temperatures of 20 and 30 °C and salinity levels above 20\textdegree, [2]. Farmers are likely to add freshwater to adjust salinity...
levels lower than these levels, because they believe that the growth of shrimp in brackish water is better than that in seawater. Since 2001, shrimp farmers have experienced disease problems causing production declines in farmed *L. vannamei*. The symptoms differ from those of Taura syndrome virus (TSV) in Taiwan [3]. A bacterium *Vibrio alginolyticus* isolated from the diseased *L. vannamei* with whitish musculature and inactivity is considered to be a secondary and opportunistic pathogen, and causes mortality of shrimp under salinity change or ammonia stress [4–6].

In teleosts, the primary response to physiological stress involves the release of corticosteroids and catecholamines. These then induce hyperglycaemia as a secondary response [7]. Several biogenic amines which function mainly as neuropeptides (neurotransmitter and neuromodulators), including serotonin, dopamine, octopamine, histamine, noradrenaline (norepinephrine), adrenaline (epinephrine), tryptamine and tyramine, have been identified and quantitatively measured in crayfish *Pacifastacus leniusculus* and other decapod crustaceans [8–11]. It is known that shrimp *Upogebia littoralis* can synthesise noradrenaline from dopamine [12], and grass shrimp *Palaemonetes varians* and American lobster *Homarus americanus* can synthesise noradrenaline from tyrosine [13,14].

It is known that when Pacific oyster *Crassostrea gigas* are subjected to mechanical disturbance, noradrenaline and dopamine are released into the circulatory system, subsequently decreasing immune functions [15], and increasing susceptibility to *Vibrio splendidus* infection [16]. It is also known that, when the abalone *Haliotis tuberculata* are subjected to a 15-min mechanical disturbance, noradrenaline and dopamine are released, and immune parameters such as haemocyte counts decrease, as do migratory activity, phagocytic and respiratory burst capacity of the haemocytes [17]. Swamp crayfish *Procambarus clarkii* when subjected to constant exposure of illumination for 3 days, increases its noradrenaline level [18]. However, there is no knowledge on the release of biogenic amines and their potential roles in immunosuppression of penaeids shrimps under stress.

Environmental stressors like salinity, ammonia, nitrite and Cu$^{2+}$ have been reported to cause reduction in immune ability of white shrimp *L. vannamei* [4,6,19,20]. Blue shrimp *Litopenaeus stylirostris* in hypoxic condition also decreases its release of superoxide anion [21]. Dopamine has been found to mimic the action of crustacean hyperglycaemic hormone (CHH) in increasing the level of glucose in tiger shrimp *Penaeus monodon* [11]. It is assumed that penaeid shrimp under such stress may increase their levels of biogenic amines including noradrenaline and dopamine, which subsequently leads to immunosuppressive effects and increases susceptibility to pathogen infection. Accordingly, the purpose of the present study is to examine (1) the effect of noradrenaline on the susceptibility of *L. vannamei* to *V. alginolyticus*, and (2) the immune response of *L. vannamei* injected with noradrenaline. For the latter purpose, we examined total haemocyte count (THC), phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency of shrimp to *V. alginolyticus*.

2. Materials and methods

2.1. *L. vannamei*

*L. vannamei* juveniles (16–20 g) were obtained from a commercial farm in Pingtung, Taiwan, and acclimated in the laboratory for 2 weeks before experimentation. Only shrimp in the intermoult stage were used for the study. The moult stage was determined by the examination of uropods in which partial retraction of the epidermis could be distinguished [22]. For the susceptibility experiment, test and control groups were comprised of 10 shrimp each in triplicate. For the determination of immune parameters, tests were carried out in eight replicate test groups consisting of one shrimp each in 20 l PVC tanks containing 10 l aerated test solution. In all tests, the shrimp were fed twice daily with a formulated shrimp diet (Shinta Feed Company, Pingtung, Taiwan). No significant difference in weight was observed among the treatments. During the experiments, water temperature was maintained at 27 ± 1 °C, pH 7.8–8.2 while salinity was maintained at 20‰.

2.2. *V. alginolyticus*

A known pathogenic strain of *V. alginolyticus* (CH003), which had been isolated from diseased *L. vannamei* in Pingtung, Taiwan, was used for the study [5]. Stocks were cultured on tryptic soy agar (TSA supplemented with 2% NaCl, Difco) for 24 h at 28 °C and transferred to 10 ml tryptic soy broth (TSB supplemented with 2% NaCl, Difco) for 24 h at 28 °C as the culture for the test. The broth culture was centrifuged at 7155 × g for 20 min.
at 4 °C. The supernatant fluid was removed and the bacterial pellet was re-suspended in saline solution (0.85% NaCl) at 5 × 10⁶ and 1 × 10⁹ colony-forming units (cfu) ml⁻¹ for the susceptibility test, as well as for the tests of phagocytic activity and clearance efficiency, respectively.

2.3. Test solution

Noradrenaline (Sigma product no. A-7256) was dissolved in sterile saline (0.85% NaCl) to concentrations of 5 × 10⁻⁴, 5 × 10⁻³ and 5 × 10⁻² mol l⁻¹, before injection.

2.4. Effect of noradrenaline on the susceptibility of L. vannamei to V. alginolyticus

*L. vannamei* was injected individually with 5 × 10⁻⁴, 5 × 10⁻³ and 5 × 10⁻² mol l⁻¹ noradrenaline solution (around 20 μl) into the ventral sinus of the cephalothorax to reach doses of 10⁻⁸, 10⁻⁷ and 10⁻⁶ mol shrimp⁻¹, respectively. Challenge tests were conducted after 1 h with the injection of 20 μl of bacterial suspension (5 × 10⁵ cfu ml⁻¹) resulting in 1 × 10⁵ cfu shrimp⁻¹ into the ventral sinus of the cephalothorax. The shrimp that received no noradrenaline, and then received *V. alginolyticus* at 1 × 10⁵ cfu shrimp⁻¹ served as the challenged controls. The shrimp that received noradrenaline at 10⁻⁵ mol shrimp⁻¹, and then received saline (20 μl), however, served as the unchallenged controls (Table 1). Experimental and control shrimp (10 aquarium⁻¹) were kept in 60 l glass aquaria containing 40 l of seawater at 20°C. Therefore, there were a total of five treatments. Each treatment was conducted with 30 shrimp. The experiment lasted 48 h.

2.5. Effect of noradrenaline on the immune parameters of L. vannamei

*L. vannamei* was injected individually with 5 × 10⁻⁴, 5 × 10⁻³ and 5 × 10⁻² mol l⁻¹ noradrenaline solution into the ventral sinus of the cephalothorax to reach doses of 10⁻⁸, 10⁻⁷ and 10⁻⁶ mol shrimp⁻¹, respectively. Shrimp that received saline (20 μl) served as controls. There were four treatments (saline, 10⁻⁸, 10⁻⁷ and 10⁻⁶ mol noradrenaline shrimp⁻¹) with five sampling times (2, 4, 8, 16, and 24 h). Eight shrimps for each treatment and time were used for the studies. In addition, eight shrimps without any treatment were used as the initial control group.

After 0, 2, 4, 8, 16, and 24 h of injection, haemolymph (100 μl) was withdrawn from the ventral sinus of each shrimp into a 1 ml sterile syringe (25 gauge) containing 0.9 ml anticoagulant solution (trisodium citrate 30 mM, sodium chloride 0.34 M, EDTA 10 mM, pH 7.55, osmolality adjusted with 0.115 M glucose to 780 mOsm kg⁻¹). A drop of the anticoagulant—haemolymph mixture (100 μl) was placed on a haemocytometer to measure THC (Leica DMLM, Leica Microsystems, Wetzlar GmbH, Germany). The remainder of the haemolymph mixture was used for subsequent tests.

Following the procedures of Hernández-López et al. [23], phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA). The details of measurements were described previously [4]. The optical density of the shrimp’s phenoloxidase activity was expressed as dopachrome formation per 50 μl haemolymph.

As described previously [4], the respiratory burst of haemocytes was quantified using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of superoxide anion (O₂⁻). The optical density at 630 nm was measured.

Table 1

<table>
<thead>
<tr>
<th>Noradrenaline (mol shrimp⁻¹)</th>
<th>Bacterial dose (cfu shrimp⁻¹)</th>
<th>Cumulative mortality (%), time after challenge (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>Saline</td>
<td>0⁰</td>
</tr>
<tr>
<td>Saline</td>
<td>1 × 10⁵</td>
<td>0⁰</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>1 × 10⁵</td>
<td>10.0 ± 0³</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>1 × 10⁵</td>
<td>23.3 ± 3.3³</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>1 × 10⁵</td>
<td>56.7 ± 3.3³</td>
</tr>
</tbody>
</table>

Data in the challenge groups in the same column with different superscripts are significantly different (*p < 0.05*) among treatments. Values are mean ± S.E. (n = 30 shrimp in each case).
using a microplate reader (Model VERSAmax, Molecular Devices, Sunnyvale, CA, USA). Respiratory burst was expressed as NBT-reduction per 10 µl haemolymph.

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical dependent reactions using the Ransod kit (Randox, Crumlin, UK). The details of measurements were described previously [6]. A reference standard for SOD was supplied with the Ransod kit. One unit of SOD was defined as the amount required to inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units ml\(^{-1}\) [24].

2.6. Phagocytic activity and clearance efficiency of L. vannamei to V. alginolyticus

*L. vannamei* that received saline or noradrenaline was the same as those described above. Tests were carried out on eight shrimp. Twenty microlitres of bacterial suspension (1 \( \times \) 10\(^8\) cfu ml\(^{-1}\)) resulting in 2 \( \times \) 10\(^6\) cfu shrimp\(^{-1}\) was injected into the ventral sinus of each shrimp. After injection, the shrimp were kept for 1 h in a separate tank containing 40 l of water at 27.0 ± 1.0 °C. Then, 200 µl of haemolymph was collected from the ventral sinus and mixed with 200 µl of sterile anticoagulant. The methods for the measurements of phagocytic activity were described previously [4]. Two hundred haemocytes were counted. Phagocytic activity, defined as percentage phagocytosis (PR) was expressed as:

\[
PR = \frac{\text{number of phagocytic haemocytes}}{\text{total haemocytes}} \times 100
\]

Clearance efficiency was measured following the method of Adams [25]. The numbers of colonies from shrimp that received saline were the control values, and the numbers of colonies from shrimp that received noradrenaline at 10\(^{-6}\), 10\(^{-7}\) and 10\(^{-6}\) mol shrimp\(^{-1}\) after 2, 4, 8, 16 and 24 h were the test values. Clearance efficiency to *V. alginolyticus*, defined as percentage inhibition (PI), was calculated as:

\[
PI = 100 - \frac{\text{number of colonies in test group}}{\text{number of colonies in control group}} \times 100
\]

2.7. Statistical analysis

A multiple comparison (Tukey) test was conducted to compare the significant differences among treatments using the SAS computer software (SAS Institute Inc., Cary, NC, USA). Percent data (susceptibility study) were normalised using an arcsin transformation before analysis. For statistically significant differences, it was required that \( p < 0.05 \).

3. Results

3.1. Effect of noradrenaline on the susceptibility of L. vannamei to V. alginolyticus

All the unchallenged control shrimp that received noradrenaline at 10\(^{-6}\) mol shrimp\(^{-1}\) and then injected with saline survived. By contrast, death occurred after 4 h in the challenged shrimp that received noradrenaline at 10\(^{-6}\), 10\(^{-7}\) and 10\(^{-6}\) mol shrimp\(^{-1}\) after 2, 4, 8, 16 and 24 h were the test values. After 4–48 h, the mortality of shrimp that received noradrenaline at either dose was significantly higher than that of shrimp that received saline (Table 1).

3.2. Effect of noradrenaline on the immune parameters of L. vannamei

For the shrimp that received noradrenaline at 10\(^{-8}\), 10\(^{-7}\) and 10\(^{-6}\) mol shrimp\(^{-1}\) after 2 h, the THC decreased by 15%, 21% and 32% (Fig. 1A), and the phenoloxidase activity decreased by 15%, 31% and 31%, respectively (Fig. 1B). No significant differences in THC and phenoloxidase activity, however, were observed among the four treatments after 4, 8, 16 and 24 h.

For the shrimp that received noradrenaline at 10\(^{-8}\), 10\(^{-7}\) and 10\(^{-6}\) mol shrimp\(^{-1}\), the respiratory burst decreased by 13%, 28% and 34% after 2 h, but increased by 16%, 20% and 36% after 4 h, respectively. No significant difference in respiratory burst, however, was observed among the four treatments after 8, 16 and 24 h (Fig. 2A).
For the shrimp that received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}, the SOD activity decreased significantly by 47%, 56% and 55%, and by 28%, 34% and 41% after 2 and 4 h, respectively. For the shrimp that received noradrenaline at 10^{-7} and 10^{-6} mol shrimp^{-1}, the SOD activity decreased by 18% and 25% after 8 h, but increased by 32% and 33% after 16 h, respectively. No significant difference in SOD, however, was observed among the four treatments after 24 h (Fig. 2B).

3.3. Phagocytic activity and clearance efficiency of L. vannamei to V. alginolyticus

At time 0 h, phagocytic activity was 5%. For the shrimp that received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}, phagocytic activity decreased significantly to 3.8%, 3.5% and 2.8%, and decreased to 4.0%, 3.2% and 2.4% after 2 and 4 h, respectively. For the shrimp that received noradrenaline at 10^{-7} and 10^{-6} mol shrimp^{-1} after 8 h, phagocytic activity decreased significantly to 3.6% and 3.5%, respectively. No significant difference in phagocytic activity, however, was observed among the four treatments after 24 h (Fig. 2B).

For the shrimp that received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}, the SOD activity decreased significantly by 47%, 56% and 55%, and by 28%, 34% and 41% after 2 and 4 h, respectively. For the shrimp that received noradrenaline at 10^{-7} and 10^{-6} mol shrimp^{-1}, the SOD activity decreased by 18% and 25% after 8 h, but increased by 32% and 33% after 16 h, respectively. No significant difference in SOD, however, was observed among the four treatments after 24 h (Fig. 2B).

A similar trend was observed for clearance efficiency against V. alginolyticus. For the shrimp that received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}, clearance efficiency decreased by 162%, 486% and 681%, and decreased by 166%, 291% and 365% after 2 and 4 h, respectively. However, no significant difference in clearance efficiency was observed among the four treatments after 8, 16 and 24 h (Fig. 3B).
4. Discussion

In the present study, the white shrimp *L. vannamei* that received noradrenaline increased its susceptibility to *V. alginolyticus* infection. Previous research indicated that *L. vannamei* increased its susceptibility to *V. alginolyticus* infection by decrease in salinity, as well as the presence of ammonia, nitrite and copper sulphate in the rearing water [4,6,19,20]. For the Pacific oyster *C. gigas* that had been challenged with pathogen *V. splendidus* and subjected to a mechanical stress, the mortality increased [16]. Injection of noradrenaline, a key component of the neuroendocrine stress response system, also caused higher mortality in *C. gigas* [26]. Therefore, it is suggested that the physiological changes imposed by the stressors like salinity change, increased concentrations of ammonia and nitrite caused increases in the susceptibility of *L. vannamei* to *V. alginolyticus*.

Circulating haemocytes of *L. vannamei* displayed higher THC and phenoloxidase activities at the C stage and the lowest at the A stage [27]. In the present study, *L. vannamei* used were at the C stage and were therefore considered to be similar with regard to defence. Circulating haemocyte count was also affected by extrinsic factors like temperature and salinity variations, as well as nitrite and Cu$^{2+}$ in *L. vannamei* and *L. stylirostris* [19–21,28].

Both THC and phenoloxidase activity of freshwater prawn *Macrobrachium rosenbergii* were significantly higher at pH 7.5–7.7 and 30–31 °C than they were at pH 4.6–5.0 or pH 9.0–9.5 and 33–34 °C [29]. Exposure of common shrimp *Crangon crangon* to polychlorinated biphenyl 15 (PCB 15) resulted in significantly decreased THC and
phenoloxidase activity [30]. The phenoloxidase activity was significantly decreased in both L. vannamei and M. rosenbergii by exposure to ammonia-N at 0.55 mg l\(^{-1}\) or more [4,31]. Both THC and phenoloxidase activity decreased in L. vannamei following the exposure to 9.87 mg l\(^{-1}\) nitrite-N, as well as the exposure to 10 mg l\(^{-1}\) Cu\(^{2+}\) [19,20]. In the present study, L. vannamei that received noradrenaline at 10\(^{-6}\) mol shrimp\(^{-1}\) or less experienced decreased THC and phenoloxidase activity in 2 h, indicating that noradrenaline decreased the immune ability of shrimp.

Using Pacific oyster C. gigas, Lacoste et al. [32] reported that noradrenaline had a dose-dependent inhibitory effect on chemiluminescence (CL)-response at the physiological concentrations of 0.1 \(\mu\)M and above, and indicated that noradrenaline decreases the release of reactive oxygen species. They also reported that \(\beta\)-adrenergic receptors are present at the surface of oyster haemocytes, which allow noradrenaline to down-regulate the CL-response. The releases of superoxide anion (O\(_2^-\)) and hydrogen peroxide (H\(_2\)O\(_2\)) were considered to play a more important role in shrimp microbicidal activity than hypochlorites (OCl\(^-\)) and myeloperoxidase (MPO) [33]. The injection of fungicide propiconazole into the white shrimp L. vannamei induced an increase of superoxide anion at day 6, but caused a dose-dependent decrease in superoxide anion at day 13 [28]. It was proposed that the decreased production of superoxide anion in hypoxic P. stylirostris was due to the decrease of THC, and that the activity of NADPH oxidase responsible for the production of superoxide anion was not affected under hypoxia [21]. Exposure of L. vannamei to 11.10 mg l\(^{-1}\) ammonia-N for 48 h, to 9.87 mg l\(^{-1}\) nitrite for 96 h, or to 20 mg l\(^{-1}\) Cu\(^{2+}\) decreased its release of superoxide anion [4,19]. In the present study, we found that treatment with noradrenaline at 10\(^{-6}\) mol shrimp\(^{-1}\) or lower in L. vannamei for 2 h decreased their THC, superoxide anion and SOD activity. This fact suggests that the activity of NADPH oxidase responsible for the release of superoxide anion decreased with decrease in the activity of SOD responsible

![Fig. 3. Mean (± S.E.) phagocytic activity (A) and clearance efficiency (B) of L. vannamei that received noradrenaline at 10\(^{-8}\), 10\(^{-7}\) and 10\(^{-6}\) mol shrimp\(^{-1}\), and that received saline. See Fig. 1 for statistical information.](image-url)
for scavenging superoxide anion. The fact that the SOD activity recovered later than that of the respiratory burst suggests that the shrimp received noradrenaline causes immunomodulation to scavenge superoxide anion to other reactive oxygen intermediates (ROIs) including H$_2$O$_2$.

Phagocytosis is an important cellular defence mechanism, whereas clearance efficiency is an important humoral defence mechanism in molluscs and crustaceans [34,35]. A significant reduction of phagocytic activity and clearance efficiency against V. alginolyticus was observed in the white shrimp _L. vannamei_ following exposure to 1 mg l$^{-1}$ Cu$^{2+}$ [20], to 11.21 mg l$^{-1}$ ammonia-N [4], and when transferred to 5 and 15%$_{\text{o}}$ from 25%$_{\text{o}}$ seawater [6]. A significant reduction in phagocytosis of _Bacillus cereus_ was also observed in the shore crab _Carcinus maenas_ following 14 days exposure to 500 μg l$^{-1}$ Cd$^{2+}$ and 10 days exposure to 100 μg l$^{-1}$ Cu$^{2+}$ [36]. The phagocytic activity and clearance efficiency for _Vibrio harveyi_ decreased in tiger shrimp _P. monodon_ following exposure to 1.8–2.0 mg l$^{-1}$ O$_2$ for 6 h [37]. Noradrenaline had a dose-dependent inhibitory effect on phagocytosis at physiological concentration of 0.1 μM and above in the Pacific oyster _C. gigas_ [26]. Similarly, we found that noradrenaline had a dose-dependent inhibitory effect on phagocytic activity and clearance efficiency to _V. alginolyticus_ in _L. vannamei_. This correlated with the increased susceptibility of _L. vannamei_ to _V. alginolyticus_ when the shrimp received noradrenaline. Therefore, noradrenaline is considered to serve as an immune modulator in _L. vannamei_.

In conclusion, the present study documented that the white shrimp _L. vannamei_ experienced an increase in susceptibility to _V. alginolyticus_ after injections of noradrenaline at 10$^{-6}$ mol shrimp$^{-1}$ or less. Noradrenaline plays an important role in immune modulation by decreasing THC, phenoloxidase activity, and phagocytic activity and clearance efficiency of _L. vannamei_ against pathogen.

**Acknowledgements**

This research was supported by a grant from the National Science Council (NSC 92-2313-B-020-013), Republic of China. We appreciate Miss C. S. Wang for her technical assistance in the experiment.

**References**


Tseng IT, Chen JC. The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under nitrite stress. *Fish & Shellfish Immunology* 2001;17:325–33.


Adams A. Response of penaeid shrimp to exposure to *Vibrio* species. Fish & Shellfish Immunology 1991;1:59–70.


Liu CH, Yeh ST, Cheng SY, Chen JC. The immune response of the white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio* infection in relation with the molt cycle. Fish & Shellfish Immunology 2004;16:151–61.


