Pro-PO based assessment of eco-friendly immunostimulation in *Penaeus monodon* (H. Milne Edwards) 

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**ABSTRACT**  
A 30-day experiment with *Penaeus monodon* was conducted to evaluate the efficacy of seaweed *Ulva lactuca* as marine immunostimulant. The shrimps were fed with the experimental diets coated with different concentrations of *Ulva lactuca* viz., 100, 250, 750 and 1000 mg/kg. One group of animals were challenged with the marine pathogen *Vibrio parahaemolyticus* to assess the lethal dose in terms of their survival rate. The other group was used for drawing the haemolymph to assess the increase in the prophenoloxidase activity. The highest prophenoloxidase activity (0.72) and the highest survival (75%) were recorded on the 1st day of 750 mg/kg of the experimental diet. Hence the 750 mg/kg of *Ulva lactuca* addition in the diet would prove to be an eco-friendly and economically viable immunostimulant.

**Introduction**  
Considering the importance of shrimp aquaculture industry and the limitation of the production due to pathological problems, it is important to develop right immunostimulants by which the shrimp can develop greater immune response to fight back the microbial diseases. Shrimps posses only a very primitive specific defense system and therefore non-specific immune system plays a vital role (Anderson, 1992). Cellular defense mechanisms in crustaceans generally rely on haemocytes with several functions such as coagulation, phagocytosis, encapsulation and prophenoloxidase system. The pro-PO mechanism is one of the main defensive mechanisms as a non-self recognition system in crustaceans (Le Moullac *et al.*, 1997).

Studies on the immune system and phenoloxidase activity would certainly be valuable for providing a better understanding of its susceptibility to microorganisms and the defense reactions elicited during such infections (Perazzolo and Barracco, 1997). Immuno-recognition is thought to be mediated through the prophenoloxidase system present in the haemocytes which are activated by the presence of non-self molecules (Soderhall, 1992).

Immunostimulants are thought to be safer than chemotherapeutics and
their range of efficacy is wider than vaccination (Masahiro Sakai, 1998). Thus with a detailed understanding of the efficacy and limitations of immunostimulants, they may become powerful tools to control fish/shrimp diseases. Use of marine resources, which are abundantly available in the Gulf of Mannar region, as a source of immunostimulants, will be an added advantage to the aquaculture sector in general and shrimp culture in particular.

Materials and methods

The seaweed, Ulva lactuca collected from the Gulf of Mannar coast was dried under room temperature and prepared in fine powder form. The basal shrimp feed was prepared as per the standard formulation of Chin, 1998. The basal shrimp feed was given a coating with seaweed powder, using egg albumin as binder. The inclusion level of the seaweed was at four different concentrations viz. 100, 250, 750 and 1000 mg/kg. PCR negative tiger shrimp (Penaeus monodon) seeds (PL-20) were reared in nursery tanks (2 ton capacity) at the stocking density of 250/m² till they attained 4 gm size. They were distributed (6 each) to 90 numbers of experimental tanks of 50 L capacity, kept on the water recirculation system (WRS) and were acclimatized to the laboratory conditions. While four tanks were maintained as control, six more tanks were used for the estimation of LD₅₀ by injecting Vibrio parahaemolyticus, a bacterial pathogen isolated from infected shrimps. The other 80 tanks were fed with the experimental feed.

The seaweed (Ulva lactuca) coated feed was fed for 5 days to the treatment shrimps followed by control feed for the next 12 days. Haemolymph was drawn from the experimental shrimps (8-10 nos every time per concentration) at regular intervals viz. 1st, 3rd, 6th, 9th and 12th day after feeding with the treatment feed for quantitating the prophenol oxidase activity (pro-PO) level.

On the same day of the haemolymph collection, immune enhancement in treated shrimps (100, 250, 750 and 1000 mg/kg of seaweed) was detected by challenging them with Vibrio parahaemolyticus (LD₅₀ = 5.6 x 10⁷ cfu / shrimp). For each concentration, two tanks of 6 shrimps each were used in challenge experiment. Pro-PO activity in haemolymph was measured as detailed by Le Molluac et al. (1997) using ELISA reader (Lab Systems, Finland). Protein content of haemocyte suspension was measured (Lowry et al., 1951) to estimate the pro-PO activity for 0.001/ min/mg of protein.

The bacterial concentration of 5.6 x 10⁷ cfu/shrimp was estimated as LD₅₀ from the pathogenicity test and the dose was used to challenge the immunostimulant (Ulva lactuca) treated shrimps.

Results

The pro-PO activity estimated for 5 days treatment of Ulva lactuca coated on the basal shrimp feed in different concentrations viz. 100, 250, 750 and 1000 mg/kg revealed that the highest activity (0.72 units/min/mg of protein) was recorded on the 1st day of 750 mg/kg of experimental feed when control samples recorded only 0.42 units/min/mg of protein pro-PO activity. The pro PO level however recorded a continuous reduction from 3rd day to 12th (Table 1).

In challenge study, 750 mg/kg on the 1st day and 100 mg/kg on the 12th day recorded the highest survival (75% each) (Table 2).
Pro-PO based immunostimulation in shrimp

Table 1: Haemocyte based phenoloxidase (pro-PO) activity in Penaeus monodon

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment (mg/kg of feed)</th>
<th>Pro-PO activity (u/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1.</td>
<td>100</td>
<td>0.36</td>
</tr>
<tr>
<td>2.</td>
<td>250</td>
<td>0.17</td>
</tr>
<tr>
<td>3.</td>
<td>750</td>
<td>0.72</td>
</tr>
<tr>
<td>4.</td>
<td>1000</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 2: Challenge study using V. parahaemolyticus in P. monodon (Dose 5.6 x 10<sup>6</sup> cfu/shrimp)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Immunostimulant dose (ppm)</th>
<th>% of Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; day</td>
</tr>
<tr>
<td>1.</td>
<td>100</td>
<td>41.66</td>
</tr>
<tr>
<td>2.</td>
<td>250</td>
<td>33.33</td>
</tr>
<tr>
<td>3.</td>
<td>750</td>
<td>75.00</td>
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<tr>
<td>4.</td>
<td>1000</td>
<td>50.00</td>
</tr>
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</table>

Discussion

Use of immunostimulants for boosting the defense mechanism in crustaceans in general and shrimps in particular is a new and promising field (Sung et al., 1994; Newman, 1996). Itami and Takheshi (1989) demonstrated that the immunized shrimps (*Penaeus japonicus*) were better protected against challenge with pathogenic organisms. Induction of resistance of *P. monodon* against challenge with *Vibrio vulnificus* after treatment with b-Glucan was reported by Sung *et al.* (1994) and recorded that the preferred route of delivery in aquaculture system would be the oral route.

Seaweeds contain carbohydrates, protein, vitamin, minerals and micronutrients. They are the potential renewable source of food, fertilizer and renewable energy (Dave and Chawhan, 1989).

The pro-Phenol Oxidase (pro-PO) activity of haemolymph, an important enzyme linked mediator of crustacean immunity has been demonstrated to be an effective tool that can be used along with challenge studies for confirmation of immune enhancement pattern in penaeid shrimps (Devaraj *et al.*, 1998; Indrani *et al.*, 1999 and Felix and Sivakumar, 2003; Felix and Murugan, 2003).

It has been confirmed that the 750 mg/kg concentration of *Ulva lactuca* coated on the basal shrimp feed enhanced pro-PO activity better than the control and the other concentrations. Incorporation at this level was proved to be effective to shrimp against *V. parahaemolyticus*, the widely present shrimp pathogen of marine eco-system.

The reduction in pro-PO level observed as the duration increases revealed that incorporation of this immunostimulant in shrimp feed throughout the crop might be helpful to sustain better immunity in shrimps. It was further noticed that from palatability point of view this seaweed did not exhibit any inherent problems...
during the experimental period.

The effectiveness of the Ulva lactuca as immunostimulant in penaeid shrimps is comparable to those of other immunostimulants (LPS, Chitin and b-Glucan) attempted with pro PO effects. It would prove to be an eco-friendly and economically viable source of marine immunostimulant for penaeid shrimps due to their abundant availability as marine resources all along the Indian coast in general and the Gulf of Mannar in particular.

Acknowledgement

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References


Pro-PO based immunostimulation in shrimp

