CHAPTER VI

CONCLUSION

1. The optimal temperatures for the condition of non-lethal thermal shock on shrimps were ranged from 15 to 35°C with 6 h of exposure. Shrimps survived in this condition for more than 2 days. Exposing to the thermal shock with higher or lower temperature caused complete mortality of the shrimps within 10 and 15 min.

2. Protein levels of haemolymph from induce shrimps raised significantly in corresponding to the level of the increase temperature in heat shock and decreased when exposed to cold shock. The response occurred within an hour after exposure. There was no evidence on induction of *Vibrio* exposure on plasma protein level. The plasma protein level in *P. monodon* depended on the raise of surrounding temperature and returned to normal level after the thermal condition had passed.

3. Glucose levels of haemolymph from induce shrimps raised significantly in corresponding to the level of the increase temperature in heat shock and decreased when exposed to cold shock. The increase of glucose level was found in heat shock shrimps 3 hours after the thermal treatment. Low glucose level was found in cold shock shrimps and the level increased in corresponding to post exposure time.

4. Protein analysis of thermal-shock shrimps with and without *vibrio* infection using SDS-PAGE showed several different protein bands in the unstressed and stressed samples. However, the results were not conclusive due to the un-consistency of the protein profiles between replications and the interference of haemocyanin in the samples.

5. The Western blotting analysis of the haemocyte lysates showed a considerably clear signal of cross reaction of monoclonal anti-HSP70 antibody and the proteins at 76 kDa. The increasing intensity of the band correlated to the level of thermal induction and time, indicating that HSP70 was inducible by heat and cold condition.

The changes of HSP60 and HSP90 in thermal shock shrimps, however, were not detected by this method.

6. Fifty percent mortality (LC_{50}) of the shrimps exposed to *V. harveyi* at the concentrations of 10^6, 10^7, 10^8, and 10^9 CFU/ml were detected at 6, 5, 3, and 2 days, respectively.

7. Pre-heat treatment at 35°C for 6 h can enhance the *V. harveyi* tolerance in *P. monodon*.

8. The differential expressed genes in the haemocytes of the shrimps in response to heat shock were detected by RAP-PCR technique. From the result of amplification with 10 primer combinations, 17 DNA fragments were obtained. Three fragments showed significant similarity to some known genes in the GenBank.

9. The expression levels of RAP12, 16, 22, and 58, HSP70 and HSP90 from heat-induced shrimps were induced by heat shock but not by the exposure to *vibrio*. The response of HSP60 genes to heat shock and *vibrio* induction was un-detectable. *Vibrio* treatment did not affect the expression level of those genes. The expression of PO gene was up-regulated by the *vibrio* treatment but not by heat shock.