Reliability of Total Haemocyte Count as Stress Indicator in Giant Tiger Shrimp (*Penaeus monodon*)

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Abstract

Stress is the main factor causing losses to the aquaculture industry including shrimp farming. It depresses the immunity of shrimp, increasing susceptability to disease and thus causing high mortality and morbidity. Stress in shrimp basically come from the management and farming activity such as transportation, handling, ablation of eye stalk for reproduction, anesthesia for mild to painful procedure and other practice. Previous reports have shown the reduction of total hemocyte count (THC) when shrimp was challenged with stress. In the present study, THC of giant tiger shrimp *(Penaeus monodon)* were compared between control group and 3 treatments i.e., subjected to unilateral eyestalk ablation, post 10% ethanol anesthesia stress and transport stress respectively. Statistical analysis showed that there was significant ($P<0.01$) difference for THC between control group and those with unilateral eyestalk ablation. Significant mean difference ($P<0.01$) was also seen in control group and those with post-anesthesia stress using 10% ethanol. However, there was no significant mean difference between control group and those subjected to transport stress.

Keywords: Penaeus monodon Total hemocyte count, transport stress, eyestalk ablation, stress indicator.

Introduction

Penaeus monodon is a marine crustacean that is popular for its firm meat texture. With two-thirds of *P. monodon* production coming from farming, mainly in South-East Asia, with over 900,000 tons of tiger shrimp consumed annually. However, despite the efforts and attention given to shrimp farming, the production and performance of *P. monodon* is still not satisfactory. Therefore, there is a need to reevaluate current practices and production management.

Animal health status is a very vital factor in the production and profit of aquaculture industry. However, the use of practical and reliable methods for a rapid analysis of shrimp health status is still uncommon among shrimp farmers. In this industry, the shrimp health is normally evaluated through reduction in growth, abnormal behavior or even widespread mortalities. Apart from infection, people always overlook the effect of stress when considering the shrimp health status. Certain culture practices frequently cause stress to the animals by means of physical (transport and eye ablation) or chemical (post anesthesia stress) means, which can result in a reduction of the shrimp immunological resistance and, thus, facilitate infections.

Eye ablation is a culture practice normally used in ovarian development in brood stock female shrimp. A complete ovarian maturation often ensues 3 to 10 days after unilateral eyestalk ablation, provided that other stress factors such as transport stress, temperature factor does not interfere. The latency period between the eye ablation and ovarian maturation depends on the readiness of the population and also the other concurrent stress factors. The theory behind this practice is that the gonad inhibitory hormone (GIH) which is produced in the neurosecretory complexes in the eyestalk is reduced through removal of the eyestalk. However, in addition to ovarian hypertrophy, other physiological function such as molting, osmotic and ionic balance and pigmentation in crustaceans are also affected and may contribute to major mortalities follow improper eyestalk ablation technique. Increase of susceptibility to infection following these stressful practices is also of great concern. Thus, the stress caused by the eye ablation needs to be evaluated for its negative effects.

Transport stress factor raises more industry concern nowadays as it is always blamed to be the root of other potential problem such as handling stress, heat stress, mechanical shock through vibration, and deterioration of water chemistry. Indicator of stress due to transport would be an interesting area to study.

Chemical stress factor, such as post anesthesia stress has always been neglected. However, it is one of the problems that should not be ignored. Anesthesia for crustacean can be induced either through cooling or use of chemical. Shrimp anesthesia is used prior to transportation or minor invasive procedure such as sample taking or eyestalk ablation. This practice is intended to reduce the movement of animals and also to calm down the animals. However, it is important to understand the stress that the anesthesia procedure imposes on the shrimp. For the post-anesthesia stress physiological mechanism is assumed to be similar to those in small animal anesthesia and no research has been done in this area.

The objective of this study was to evaluate whether total hemocyte count is a reliable and rapid stress indicator for shrimp that has been subjected to eye ablation, transportation and anesthesia.

Materials and Methods

Adult male and female tiger shrimps were purchased from Prawn Planet at Equine Park, and transferred to the Hatchery Unit of Aquatic Animal Health Laboratory, UPM. The shrimp were kept in seawater with salinity of 23.5% at stocking rate of 10 shrimps/ 20 L. The animals were kept in the laboratory for at least 12 hours prior to their use. Only apparently healthy animals with body length around 17cm were used in these experiments.

The shrimps were divided into four groups of 20 shrimp in each group: the first group (G1) was subjected to unilateral right eye ablation. The eye ablation technique commonly practiced by farmer was used whereby slitting one eye with a razor blade was done. Then crushing eyestalk, with thumb and index fingernail, beginning one-half to two-thirds

down the eyestalk and moving distally until the content of eyes was removed. Animals were evaluated one hour post treatment.

The second group of animals (G2) underwent 3 hours transport stress from UPM to Klang and back to UPM again. Animals were packed at high stocking rate (10 shrimps /10 L). Portable aeration was supplied along the journey to meet the high demand of oxygen for these animals. Animals were evaluated immediately post treatment.

The third group (G3) was anesthetized using ethanol 10% and was evaluated after their recovery from lateral recumbency. Absolute 100% ethanol was added into the seawater to obtain a solution at 10% concentration of ethanol. This concentration was chosen based on the results from pretreatment trial.

Group four (G4) composed of animals which were not treated and showed no stress signs. They were kept as the control for this experiment. Animals for G4 were selected randomly from the same stock with the 3 group animals stated above.

Shrimp hemolymph was collected by inserting a 25-gauge needle attached to a 1 mL syringe into the ventral sinus of animals, transferred to hematocrit tubes which contain sodium heparin to prevent clotting. The hemolymph was later transferred into a Neubauer Hemocytometer for examination under light microscope. This procedure was done with extra care to prevent debris or clot from entering the Neubauer chamber.

Total hemocyte count was determined using a Neubauer Hemocytometer under light microscope. The depth of the Neubauer chamber was 0.1 mm with area of 0.0025 mm^2 per square. The THC from two partition of Neubauer chambers were counted as A and B respectively to obtain the average value. From each partition, five chambers were counted to represent the whole partition. This is similar to the procedure for sperm count. The unit used was cell/mL. The formula derived was 50000 × average values from microscopic count.

Results and Discussion

Statistical analysis showed that there was a significant difference ($P<0.01$) for THC between control group and those from unilateral eyestalk ablation. Between control group and the group that underwent post anesthesia stress with 10% ethanol, there was also significant mean difference ($P<0.01$). There was no significant difference in mean THC between the group exposed to transport stress and the control.

From the results of this experiment, there was a significant mean difference in both eyestalk ablation and post anesthesia group compared to the control group. For the eyestalk ablation, the procedure as described by Perazzolo et al. (2002) was adapted. The significant mean differences for unilateral eyestalk ablation and post anesthesia group shows that THC is very suitable to evaluate stress conditions resulting from the two treatments. However, different individual may respond differently to stress in terms of THC. This can be explained by the different stage of growth (especially molting stage),

stress tolerance, surrounding factors, and health status of the shrimp. So, to set a certain standard mean value for stress-free shrimp as a guideline is tedious. Probably the THC as an indicator of stress can only be used for the same stock of shrimp. Whether the THC value is representative for the degree of stress is questionable as the actual physiological pathway that causes the reduction in THC has not being studied.

The results of the present study showed that eyestalk ablation and post anesthesia effect caused significant stress to the animals. There is a need to improve the induce spawning technique and anaesthesing the shrimp so as to reduce the stress factor carried by current practices.

For the third treatment group, which was exposure to transport stress did not show significant difference probably due to the following reasons. Firstly, there are no clear procedure on how to induce transport stress. In the present study, a car was used instead of a lorry. Maybe the shrimp should have been exposed in conditions with a vibrator or shaker at temperature similar to what the animals undergo during transport. In the present study the animals were transported in the morning to avoid heat stress and prior to transport they were not fed 12 hours to avoid deterioration of the water quality as what is being practiced by the farmer. Maybe these steps help to minimize the stress during transportation. Therefore, it is important for farmers to control the temperature and fast the animals prior to transport.

In the present study, the total volume of hemolymph was withdrawn from the ventral sinus. The hemolymph volume collected was found to be higher in the treatment group compared to control. This was a consistent finding for all the treatment groups. From this observation, the assumptive theory will be that the THC reduction was more on hemolymph volume rather than to the actual reduction in cell numbers. According to study by Guadagnoli et al. (2005) on changes in cardiac output and hemolymph flow during hypoxic exposure in the gravid grass shrimp, there was a redistribution of hemolymph flow away from main vessels that results in an enhanced hemolymph flow to the sternal artery that supplies the ventral segmental system, the gills, the buccal apparatus and the ventral nerve cord. According to Guadagnoli et al. (2005), this mechanism is more like a survival method for crustacean to cope with stress and life threatening situations. This finding raises the thought and consideration of using hemolymph volume as stress indicator which is less labour consuming and cheap.

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