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## Effect of Testosterone Undecanoate Hormone on Sperm and its Level in the Hemolymph of Male Mud Spiny Lobster, *Panulirus polyphagus*

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**Abstract:** The present study aimed to determine the effect of testosterone undecanoate hormone on sperm quality (sperm viability) and sperm quantity (sperm counts) and its levels in the hemolymph of male mud spiny lobster, *Panulirus polyphagus*. Male *P. polyphagus* was injected laterally in fifth abdominal segment of pure hormone, Testosterone Undecanoate (TU) and ethanol at days 1, 8, 15, 22 and 29. Hemolymph of *P. polyphagus* was taken every two weeks and checked with Enzyme-linked immunosorbent assay (ELISA) to measure hormone levels. The mean sperm quality and quantity were increased due to increase the TU dose and TU levels also increase. The sperm quality, quantity and hormone levels were relevance each others. These findings indicate that TU injection should be evaluated as a practical way of improving sperm quality and quantity in commercial operations.

**Key words:** Testosterone undecanoate, mud spiny lobster, *Panulirus polyphagus*, enzyme-linked immunosorbent assay

### INTRODUCTION

Spiny lobster, *Panulirus* spp., is one of the most important seafood products with high economic value and high prices in various international markets. In Malaysia, the culture of spiny lobsters is still in its early years with activities based in the State of Sabah mostly concentrated in Lahad Datu, Semporna, Kudat and Kota Kinabalu. Spiny lobster culture occupied both grow-out of juveniles in marine cages and hold of adults in cement tanks and marine cages (Tuan and Mao, 2004). The problems in spiny lobster including mud spiny lobster, *P. polyphagus* breeding technology are low survival rate in the larvae rearing of pueruli stage, difficult to maintain from pueruli until juvenile stages and very difficult to obtained the mature broodstock especially the male *P. polyphagus*. The availability of the mature males is important for induced mating trials in captivity or further trials on artificial insemination using ready sperm of *P. polyphagus*.

A number of steroids such as progesterone, 17 $\beta$ -hydroxyprogesterone, 6 $\alpha$ -hydroxyprogesterone, 20 $\beta$ -hydroxyprogesterone, pregnenolone, 17 $\beta$ -hydroxypregnenolone, 17 $\alpha$ -estradiol, estrone,

testosterone and others have been well-known in diverse crustaceans (Fingerman *et al.*, 1993; Subramoniam, 2000; Tsukimura, 2001; Wilder *et al.*, 2002; Oetken *et al.*, 2004). In crustaceans, steroid hormones levels have been studied and reported for many species such as the American lobster, *Homarus americanus* (Burns *et al.*, 1984; Couch *et al.*, 1987), European Green crab *Cacinus mianus* (Hazel, 1986) and the black tiger shrimp *Penaeus monodon* (Fairs *et al.*, 1990; Yashiro and Chaisriha, 1991). The existence of vertebrate-type steroids has been recognized in almost all invertebrate groups including crustaceans (Lehoux and Sandor, 1970; Lafont, 1991; Cardoso *et al.*, 1997).

Testosterone which a single sex differentiating hormones of vertebrates is commonly initiates in the hemolymph and tissues of decapods crustaceans (Ollevier *et al.*, 1986; Fairs *et al.*, 1989; Mackeviciene and Chibisova, 1995). Even though testosterone is not the major factor dependable for sex differentiation in decapods but, it was reported to stimulate growth of male reproductive organs (Nagabhushanam and Kulkarni, 1981), to alter sex ratios towards males (Baghel *et al.*, 2004) and still to transform ovaries into testes (Sarojini, 1963). Therefore, there is a chance for use Testosterone

Undecanoate (TU) for enhancing sperm in mature male *P. polyphagus*. The objectives of the present study were to determine the effect of TU on the sperm quality and quantity and TU levels in the hemolymph of *P. polyphagus*.

**MATERIALS AND METHODS**

Fifteen healthy and sexually matured *P. polyphagus* males were used in the experiment with 10 males for TU and 5 males for control treatments. One week acclimatization was adopted in order to reduce stress associated with handling and laboratory environment. The brood stocks were fed daily at 10% biomass with fresh squid, *Loligo* sp.

Two fiberglass tanks (5 tonnes capacity) were used for the TU treatments with dose of 0.01 µg g<sup>-1</sup> body weight (BW) (5 males) and 0.1 µg g<sup>-1</sup> body weight (BW) (five males). One fiberglass tank (5 tonnes capacity) was also used for control treatment (5 males). The hormone dose at 0.01 and 0.1 µg g<sup>-1</sup> BW used was modified from Alfaro (1996). Male *P. polyphagus* was injected laterally in fifth abdominal segment with 0.5 mL dilution of pure hormone TU and ethanol (control) at days 1, 8, 15, 22, 29 and 39. Day 42, the male *P. polyphagus* was dissected to check sperm quality and quantity and before dissect, hemolymph from male *P. polyphagus* was taken. The sperm quality and quantity of *P. polyphagus* at day 42 were determined using the percentage of viable sperm (sperm viability) and sperm count (sperm number), respectively. Percentages of viable sperm (sperm viability) was known when a live and death of sperm were counted. Percentages of viable sperm were calculated by following equation:

$$\text{Sperm viability (\%)} = \frac{\text{Observed No. of live sperm}}{\text{Total No. of sperm observed}} \times 100$$

Hemolymph of *P. polyphagus* was taken every 2 weeks and checked with Enzyme-linked immunosorbent assay (ELISA) (Cusabio Biotech Co. Ltd.,) to measure hormone level. For the ELISA assays, the microtiter plate provided in the kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples were added to the appropriate microtitre plate wells with a HRP-conjugated testosterone and antibody preparation specific for testosterone and incubated. The substrate solutions were then added to each well. The enzyme-substrate reaction was accomplished by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of testosterone in the samples was

determined by comparing the Optical Density (OD) of the samples to the standard curve (Cusabio Biotech Co. Ltd.).

One way ANOVA (Bonferroni) was used for the statistical evaluation of mean values. Significance was accepted at p<0.05.

**RESULTS**

The result for mean sperm viability percentage were 72.11±5.38% in control, 91.84±1.95% in dose 0.01 µg g<sup>-1</sup> BW and 92.14±3.56% in dose 0.1 µg g<sup>-1</sup> BW (Fig. 1). The mean sperm counts were 38.84×10<sup>6</sup> cells mL<sup>-1</sup> (control), 62.64×10<sup>6</sup> cells mL<sup>-1</sup> (dose 0.01 µg g<sup>-1</sup> BW) and 102.83×10<sup>6</sup> cells mL<sup>-1</sup> (dose 0.1 µg g<sup>-1</sup> BW) (Fig. 2). There was significant difference between each dose (p<0.05) for

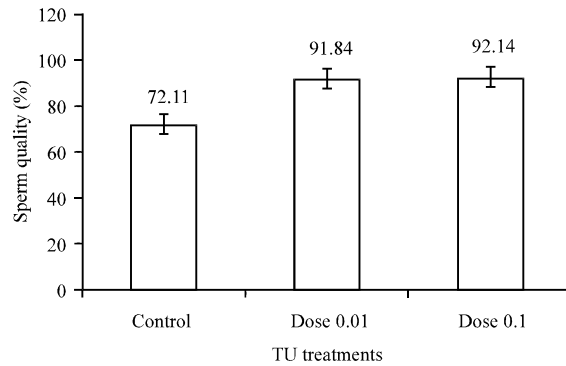


Fig. 1: Mean percentage of sperm viability (sperm quality) of *P. polyphagus* treated with difference dose of testosterone undecanoate (TU) for 42 days study period

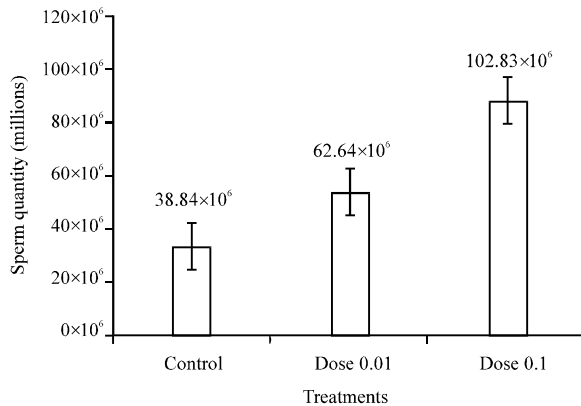


Fig. 2: Mean percentage of sperm counts (sperm quantity) of *P. polyphagus* treated with different dose of testosterone undecanoate (TU) for 42 days study period

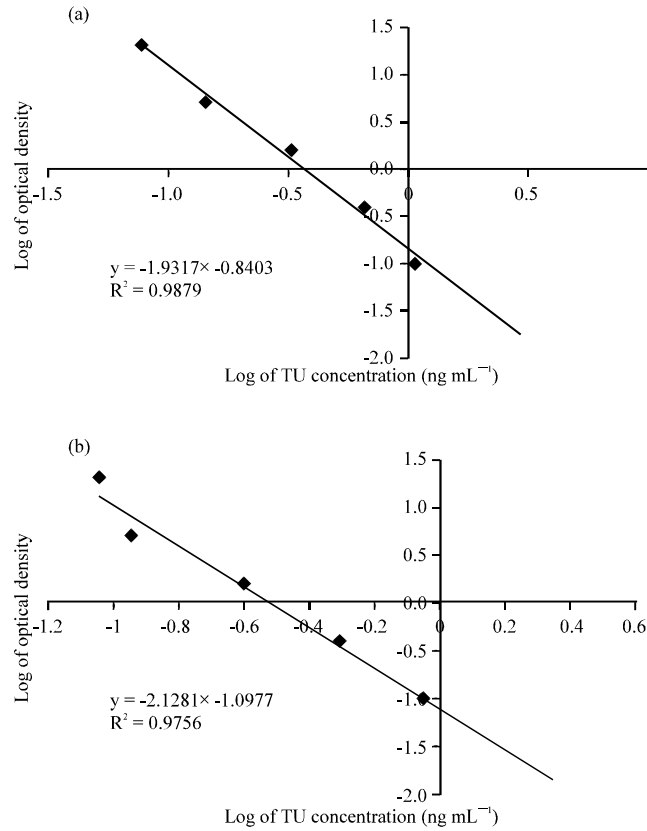


Fig. 3(a-b): ELISA standard curve of TU concentration (ng mL<sup>-1</sup>)

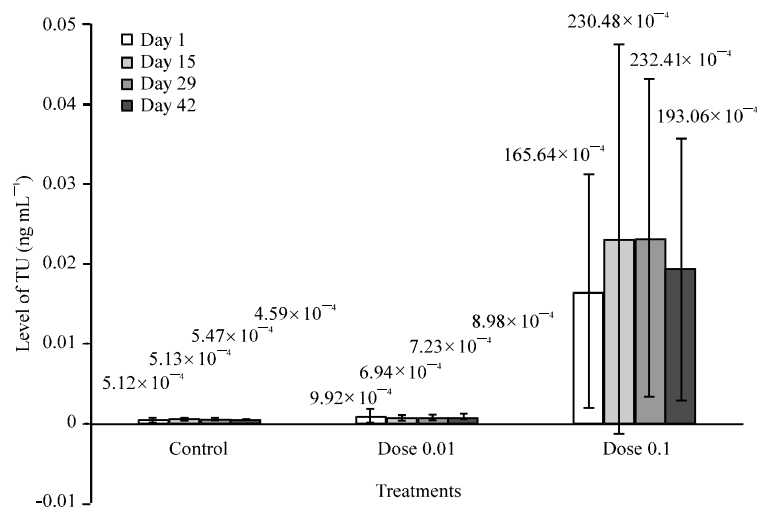


Fig. 4: Mean testosterone undecanoate (TU) levels in control, dose 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW in days 1, 15, 29 and 42 of *P. polyphagus*

the mean sperm quantity (sperm count). The mean sperm counts in dose 0.1  $\mu\text{g g}^{-1}$  BW was very higher than the other two treatments of control and dose 0.01  $\mu\text{g g}^{-1}$  BW.

The standard curves of TU were obtained (Fig. 3) before the TU levels in the hemolymph were determined. In the hemolymph, TU levels in control were low compared to

the treatment that injected with 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW. The mean TU levels of control were  $5.12 \times 10^{-4}$ ,  $5.13 \times 10^{-4}$ ,  $5.47 \times 10^{-4}$  and  $4.59 \times 10^{-4}$   $\text{ng mL}^{-1}$  for days 1, 15, 29 and 42. The means of TU levels of treatments for days 1, 15, 29 and 42 were  $9.92 \times 10^{-4}$ ,  $6.94 \times 10^{-4}$ ,  $7.23 \times 10^{-4}$  and  $8.98 \times 10^{-4}$   $\text{ng mL}^{-1}$ , respectively for 0.01  $\mu\text{g g}^{-1}$  BW dose and  $165.64 \times 10^{-4}$ ,  $230.48 \times 10^{-4}$ ,  $232.41 \times 10^{-4}$  and  $193.06 \times 10^{-4}$   $\text{ng mL}^{-1}$  respectively for 0.1  $\mu\text{g g}^{-1}$  BW dose (Fig. 4). The mean TU concentration for control and dose 0.1  $\mu\text{g g}^{-1}$  BW was increased in day 15 and 29 and decreased in day 42 (Fig. 4). But, in dose 0.01  $\mu\text{g g}^{-1}$  BW, the mean TU concentration was decreased in day 15 and increased in day 29 and 42 (Fig. 4).

### DISCUSSION

The mean sperm quality (sperm viability) had increase after been injected with 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW compared to the control. The sperm quantity (sperm counts) in control treatment was lower than the dose 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW. Therefore, the sperm quantity was an improvement by injecting of TU. There was significant difference ( $p < 0.05$ ) in sperm quantity in dose 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW from control treatment with significant improvement in injection of TU in dose 0.1 than dose 0.01  $\mu\text{g g}^{-1}$  BW.

In the present study, TU levels of male *P. polyphagus* measured using ELISA. There are a number of different techniques have been well-known to establish vitellogenin (VTG) levels in crustaceans including immunodiffusion (Quinitio *et al.*, 1990; Tom *et al.*, 1987), rocket immunoelectrophoresis (Yano, 1987) and ELISA (Sagi *et al.*, 1999; Tsukimura *et al.*, 2000; Vincent *et al.*, 2001). In ELISA, there were numerous advantages in terms of wellbeing, simplicity and speed and thus, is a method of choice for testing at hatcheries or on site (Mendoza *et al.*, 1993). One more advantage of the ELISA is that hemolymph samples can be assayed directly, not including extraction or hormone enrichment procedures (Chang *et al.*, 1998).

TU has significant effect on hormone level in *P. polyphagus*. There was no significant effect on hemolymph vitellogenin (Vg) levels of ridgeback shrimp, *Sicyonia ingentis* when injection of progesterone,  $17\beta$ -HP and  $17\alpha$ -estradiol (Tsukimura *et al.*, 2000) were given or used. There were difference between TU level in control and dose 0.01 with dose 0.1  $\mu\text{g g}^{-1}$  BW. For the control treatment, TU level lower than dose 0.01 and 0.1  $\mu\text{g g}^{-1}$  BW because TU level was naturally present in the *P. polyphagus* body with no other source of artificial injection. For dose 0.1  $\mu\text{g g}^{-1}$  BW, TU levels increase

dramatically because dose 0.1  $\mu\text{g g}^{-1}$  BW was enough to stimulate TU level in the body *P. polyphagus* and thus, the sperm quantity was increased. The TU levels and sperm quantity was an ascendancy each others.

### CONCLUSION

The present study shows that TU has affected the sperm quality (sperm viability) and sperm quantity (sperm counts) and hormone levels in mud spiny lobster, *P. polyphagus*. The injection of dose 0.1  $\mu\text{g g}^{-1}$  BW was higher and produced significant different ( $p < 0.05$ ) as compared to control and dose 0.01  $\mu\text{g g}^{-1}$  BW in mean TU levels.

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