EFFECT OF OESTROGEN HORMONE, 17β-ESTRADIOL ON FEMINIZATION, SURVIVAL RATE AND GROWTH RATE OF TIGER SHRIMP, *PENAEUS MONODON* (FABRICIUS, 1798) POSTLARVAE

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ABSTRACT. *This study was conducted to determine the effect of different concentrations of oestrogen hormone, 17β-estradiol (E_2) on feminization for the production of all female P. monodon postlarvae, as well as survival rate and growth rate. The treatments consisted of three replicates of each diet containing six concentrations of oestrogen hormone, E_2 at 0, 200, 400, 600, 800 and 1,000mg/kg feed. The experiment was carried out continuously for 50 days. At termination of experiment, the specimens in each treatment group were individually weighed and measured for their wet body weight (BW) and total length (TL) to estimate the mean final BW and TL, and the specific growth rate (SGR). The mean sex ratio values of male to female with the concentration of 0, 200, 400, 600, 800 and 1000 mg/kg were 1:1, 1:1, 1:2, 1:2, 1:4 and 1:5 respectively. The mean survival rate for 0, 200, 400, 600, 800 and 1000 mg/kg were 42.33% ± 2.52, 37.67% ± 2.52, 38.67% ± 2.08, 47.33% ± 2.52, 41.00% ± 3.00 and 40.67% ± 2.08 respectively. The mean final TL and BW were 24.57 mm ± 2.84 and 0.11 g ± 0.05 for control, 26.97 mm ± 2.99 and 0.16 g ± 0.07 for 200 mg/kg concentration, 28.95 mm ± 2.30 and 0.20 g ± 0.06 for 400 mg/kg concentration, 30.77 mm ± 4.33 and 0.24 g ± 0.09 for 600 mg/kg concentration, 32.97 mm ± 5.59 and 0.27 g ± 0.06 for 800 mg/kg concentration and, 34.54 mm ± 5.32 and 0.32 g ± 0.08 for 1000 mg/kg concentration. The mean SGR for control treatment of P. monodon PL after 50 days was 2.67 g/day ± 0.18 while for E_2 hormone concentration treatments of 200, 400, 600, 800 and 1000 mg/kg were 3.31 g/day ± 0.003, 3.86 g/day ± 0.13, 4.15 g/day ± 0.50, 4.41 g/day ± 0.02 and 4.74 g/day ± 0.30 respectively. The study shows that as the concentration of E_2 hormone increased, the female sex ratio and the growth rate of P. monodon PL also increased. The number of PL surviving in all hormone treated diets was similar to the control treatment. But, treatment of 600 mg/kg shows the highest survival rate compared to the other treatments. Treatment 600 mg/kg can be considered the most effective concentration for survival in the study.*

KEYWORDS. 17β-estradiol, feminization, *Penaeus monodon*, postlarvae, growth performance, survival rate.

INTRODUCTION

Aquaculture plays a major role in increasing seafood production in many countries. One of the fastest growing aquaculture production sectors is that of the Penaeid shrimp (Jiang *et al.*, 2009). The culture of more than 30 species of Penaeid shrimp has been attempted throughout the world in countries where they are endemic (Beard *et al.*, 1977) and they are among the most important extensively cultured crustaceans in the world (Shiau, 1998). In 1999, Penaeid shrimp culture accounted for 14% of the total aquaculture production value and the world shrimp production is dominated by tiger shrimp, *Penaeus monodon* which accounted for more than 50% of the production in 1999 (FAO, 2001).

According to Kungvankij and Chua (1986), Penaeid shrimps have attracted considerable attention from aquaculturists due to the technological breakthroughs in life cycle control, the

development of a variety of commercial feeds along with the establishment of more efficient farming practices, firm and high market prices, generation of employment, increase in foreign exchange earnings and unlimited market demand.

Thus, due to the perceptions of a market demand and high export price, many countries in the region rich in aquatic resources are encouraged to place high emphasis on the development of the shrimp culture industry (Kungvankij & Chua, 1986) and pressure shrimp producers to intensify their culture techniques to increase production (LeaMaster, 1998).

Kuperan (1988) stated that shrimp farming in Malaysia has become the primary aquacultural activity due to the fast growth and high price. Thus, it has become obvious that efficient biotechnology for producing all female Penaeid shrimp populations is required especially in countries in which economically valuable crustaceans constitute an important source of income (Sagi & Aflalo, 2005).

Due to the economic importance of Penaeid shrimp worldwide particularly in aquaculture base production, a great effort has been made to develop suitable and useful techniques in order to increase the total production (Franco *et al.*, 2006).

Besides, because of the market demand which caters for more uniform sizes, the possibility of monosex culture of female Penaeid shrimps could be a strategy to produce shrimp with larger tails (Gomelsky, 2003). The monosex culture of female Penaeid shrimps has become the main focus as this Penaeid species differs in growth rate, behaviour patterns and husbandry needs between the male and female shrimps (Aflalo *et al.*, 2006).

Basically, for Penaeid shrimp species, as they are observed in wild populations, females generally achieve a larger size than males (Garcia, 1985; Somers *et al.*, 1987). Some studies on shrimp aquaculture emphasize sexual size dimorphism and its possible advantages in improving production. Possibly, the larger size of females is influenced by female's growing faster than the males (Campos-Ramos *et al.*, 2006). Therefore, there is a significantly higher growth rate for females that would provide an incentive to investigate the potential for monosex culture of Penaeid shrimps as a technique for decreasing grow out period and/or increasing pond yield (Hansford & Hewitt, 1994).

The monosex production needs to be adapted to the species of interest. Thus, the rationale in production of both female and male monosex is not specific to the sex chosen for monosex production but it is specific to the particular situation. This is because, female and male monosex have their own benefits in aquaculture production depending on the species used. In the majority of species in which monosex culture is practiced at the present time, the female is more economically attractive than the male because of faster growth rate (Beardmore *et al.*, 2001).

The technique based on monosex sub-population culture strategy has become a common practice in fish-based aquaculture (Beardmore *et al.*, 2001) and thus, attempts have been made to apply this technique to crustacean culture (Siddiqui *et al.*, 1997) especially marine and freshwater shrimps (Devlin & Nagahama, 2002). This monosex culture technique was more recently successfully applied to production of all male giant freshwater prawn, *Macrobrachium rosenbergii* using 17α-methyltestosterone (Antiporda, 1986). The study of monosex female culture of Penaeid shrimps may come into focus due to the success in the production of *M. rosenbergiii*. In contrast with *M. rosenbergii*, the Penaeid shrimps have larger females than the males (Rungsin *et al.*, 2006).

In order to achieve the culture of monosex females, oestrogenic steroids were used to change the sex of fish and other animals from males into females (Jensen & Shelton, 1979). The administration of oestrogen hormone through prepared feed has proved successful not only in reversing sex but also as a growth promoter (Fagerlund *et al.*, 1979). In addition, hormone-treated fish grew faster and utilized food more efficiently than controls (Antiporda, 1986). Thus the main objectives of this study are to determine the effect of different concentrations of oestrogen hormone, 17β-estradiol (E_2) on feminization for the production of all female *P. monodon* PL, as well as survival rate and growth rate of.

METHODOLOGY

Sea water supply

This experiment was conducted at the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Terengganu, Malaysia. The sea water was pumped from nearby coastal water to the sedimentation tank and left overnight. The water was disinfected with 30 ppm active chlorine (calcium hypochlorite) for 16 h and de-chlorinated with 30 ppm sodium thiosulfate before use in the experiment. A cotton bag filter (50 µm) was used to remove detritus, small organisms, dirt and turbid water.

Experimental culture system

The culture system consisted of 18 aquaria containing 10 L of seawater each. All the aquaria were put in a big tank containing freshwater which acted as the water bath. Submersible heaters were placed in the freshwater tank to maintain a constant temperature of 28-30^{o}C in the experimental test. Aeration was distributed from a control air blower to each aquarium through an air stone. The top of each aquarium was covered with black hollow plastic net to maintain a stable temperature throughout the study period. *P. monodon* PL3 were randomly stocked at a density of 10 ind./L in each experimental aquarium. So, the number of *P. monodon* PL stocked in each experimental aquarium was 180 individuals. Thirty PL shrimp samples were measured for total length (mm) and wet body weight (g). The treatment consisted of diets containing five concentrations of oestrogen hormone, E_2 at 200, 400, 600, 800 and 1000 mg/kg feed mixed with the artificial diet of HIGASHIMARU brand with three replicates for each treatment. Three replicates of PL shrimps for control were fed with a diet without supplemental E_2 for comparison with the treatments.

Preparation of diets

The PL shrimps were fed with shrimp commercial diet of HIGASHIMARU brand (49% crude protein) mixed with different concentrations of oestrogen hormone, E_2. One feed served as control and the five others were supplemented with 200, 400, 600, 800 and 1000 mg E_2/kg. The feed was prepared by the alcohol preparation method described by Guerrero (1975). The amount of E_2 was calculated to achieve the desired dietary concentration and then weighed and dissolved in ethanol (95% denatured) equivalent to 80% of the weight of feed (20 mL ethanol in 25 g feed). The weight of E_2 hormone added to 25g of diet was 0.005, 0.010, 0.015, 0.020 and 0.025g for the concentration of 200, 400, 600, 800 and 1000mg/kg respectively. The mixture of dissolved E_2 and ethanol (20 mL) for each treatment was added into the diet (25g) and mixed well using a spatula. The diet containing E_2 was transferred onto aluminium foil and spread in a thin layer for the ethanol to evaporate and leave the E_2 hormone in the diet. After 45 min, the diet (E_2 and feed) was transferred to a bottle covered with aluminium foil for protection from UV light that can affect the hormone and then stored in a refrigerator at 0^{o}C until feeding time. The control diet was prepared without addition of E_2 hormone.

Larval rearing

The daily amounts of feed (which included quantity and number of feeding times) were adjusted to compensate for growth and survival depending on the age of PL shrimps. The fed was given four times a day at 0800, 1200, 1600 and 2000h at the rate of 10% wet body weight/day as recommended by Kian *et al.* (2004). The experiment was continued for 50 days. Water was renewed daily by siphoning out 30-40% of the water. The removed water was then replaced with new clean water and aerated. During the study the seawater parameters were maintained at salinity 25-30ppt, DO more than 6 mg/L , temperature 28-30^{o}C pH 6.0-8.7 for as suggested by Kungvankij *et al*. (1985). After 50 days, the PL shrimps in each aquarium were harvested by first siphoning out the water level to about 1/3 of the depth and finally collecting the shrimp using a bag net at the end of a siphoning pipe. The PL shrimps also were harvested using the scoop net. The number of harvested PL in each aquarium was counted to determine their survival rate and weight and length measured for the growth rate analysis. The PL were preserved in 70% alcohol and put in a sample bottle according to their concentration of treatment before the determination of their sex.

Sex determination

Following the protocol of Campos and Ramos (2006) the differentiation between male and female *P. monodon* PL was determined by examining the morphological sex structure. The female PL has a thelycum which is described as a small elevation between the fourth and fifth pereiopods on each side. While the male PL have a masculine appendix at the second pair of pleopods and gonophores complex at the fifth pereiopods.

Data collection

At termination of the experiment, the specimens in each treatment group were individually weighed and measured for their wet body weight (BW) and total length (TL) to estimate the mean final wet body weight and total length, and the specific growth rate (SGR). Measurement of the SGR value used the formula by Bautista-Teruel *et al*., (2003). The SGR formula follows:

$$\text{Mean SGR (g/day)} = \frac{\text{Mean final BW (g)} - \text{Mean initial BW (g)}}{\text{Culture period (days)}}$$

The BW and TL of each PL shrimp were measured using a digital weighing machine with an accuracy of 0.0001g and vernier calliper with an accuracy of 0.1mm respectively. While the survival rate of PL shrimps in each treatment group was calculated using the formula below;

$$\text{Survival rate (\%)} = \frac{\text{Final number of PL}}{\text{Initial number of PL}} \times 100$$

The survival rate and growth rate of all specimens in each treatment group were analyzed using variance analysis (ANOVA) as available in SSPS 16.0 software. The results were expressed as mean of triplicate treatment ± standard deviation (SD). Mean percentage of sex ratios in hormone treatments were transformed prior to analysis. One-way ANOVA and Duncan's multiple range tests were used to detect significant differences in the transformed data between treatments. $P<0.05$ was defined as a statistically significant difference.

RESULTS

Sex ratio

The sex ratio values gained from the treatments were based on the mean of 30 individuals of *P. monodon* PL for the three replicates of each treatment. The mean sex ratio values of male to female gained from control to the highest E_2 hormone concentration of 0, 200, 400, 600, 800 and 1000 mg/kg were 1:1, 1:1, 1:2, 1:2, 1:4 and 1:5 respectively (Figure 1). The sex ratio values gained from the treatments were based on the mean of 30 individuals of *P. monodon* PL for each treatment. The mean sex ratio values of male to female increased as the concentration of E_2 hormone increased from control treatment (0mg/kg) until the highest treatment concentration (1000 mg/kg) (Figure 1). The results gained were significantly different ($P=0.00$, $P<0.05$) among the treatments of 0, 200, 400, 600, 800 and 1000 mg/kg. There was no significant difference between the treatments of 800 and 1000 mg/kg ($P=1.00$, $P>0.05$) nor between the treatments of 400 and 600 mg/kg ($P=1.00$, $P>0.05$) and nor between the treatment of 200 mg/kg and control ($P=1.00$, $P>0.05$).

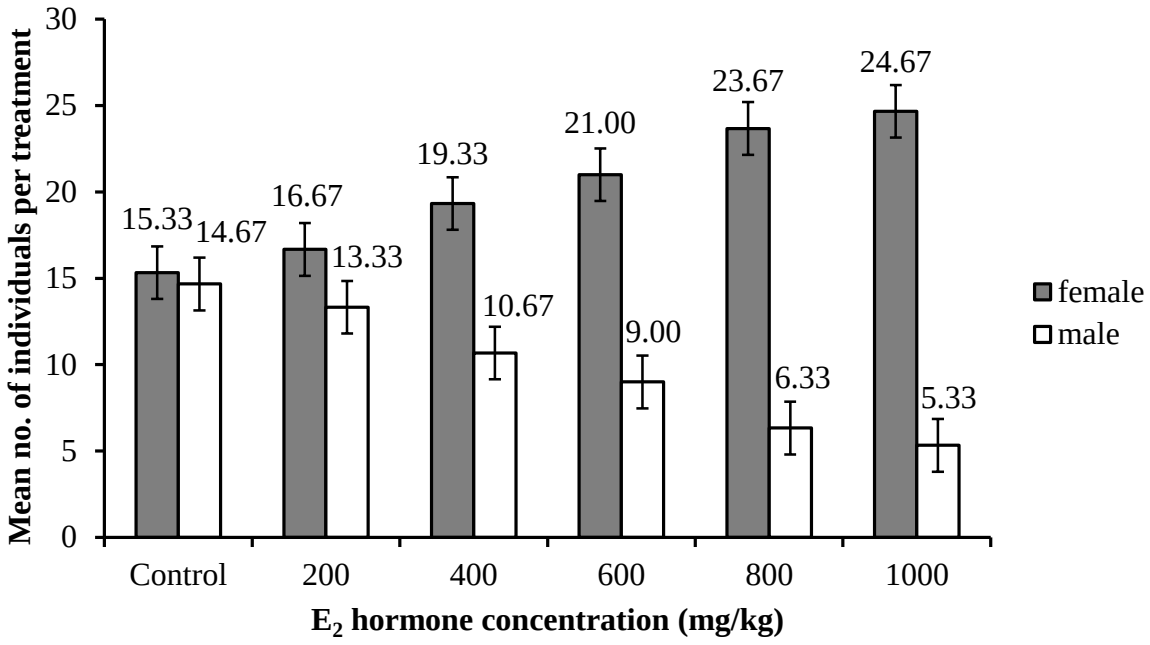

Figure 1. Mean number of individuals per treatment (male and female) of *P. monodon* PL produced from different E_2 hormone concentrations.

Survival rate

In all treatments, the mean percentages for survival rate of PL shrimps fed with different concentrations of E_2 at the end of experiment ranged from 37.67% ± 2.52 to 47.33% ± 2.52. For the normal feed with no E_2 hormone added to the diet (0mg/kg), the mean survival rate was 42.33% ± 2.52 (Figure 2). The treatment with E_2 hormone concentration of 600mg/kg of diet showed the highest mean survival rate of 47.33% ± 2.52 (Figure 2). The treatments of 200, 400, 800 and 1000mg/kg showed lower mean survival rates than the control treatment (Figure 2). The introduction of E_2 hormone concentration of 200mg/kg to PL shrimps showed the lowest mean survival rate which was 37.67% ± 2.52 (Figure 2). The treatment of 400, 800 and 1000mg of E_2 hormone per 1kg of feeding diets gave the mean survival rate 38.67% ± 2.08, 41.00% ± 3.00 and 40.67% ± 2.08 respectively (Figure 2). No significant difference ($P=0.17$, $P>0.05$) was detected among all the treatments except for 600mg/kg.

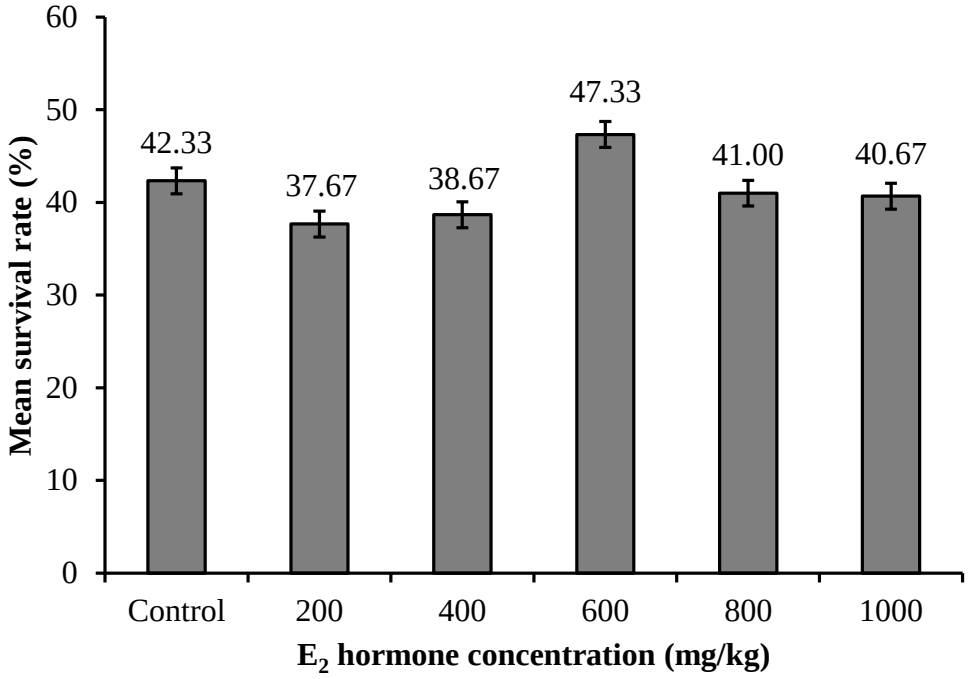

Figure 2. Mean survival rate of *P. monodon* PL fed with different E_2 hormone concentration.

Growth rate

The initial means of TL and BW were 3.61 ± 0.38mm and 0.03 ± 0.003g for all the treatments respectively. The final mean TL and BW of *P. monodon* PL after 50 days of treatment increased as the concentration of E_2 hormone increased. The final mean TL and BW were 24.57 ± 2.84mm and 0.11 ± 0.05g for control, 26.97 ± 2.99mm and 0.16 ± 0.07g for 200mg/kg concentration, 28.95 ± 2.30mm and 0.20 ± 0.06g for 400mg/kg concentration, 30.77 ± 4.33mm and 0.24 ± 0.09g for 600mg/kg concentration, 32.97 ± 5.59mm and 0.27 ± 0.06g for 800mg/kg concentration and, 34.54 ± 5.32mm and 0.32 ± 0.08g for 1,000mg/kg concentration (Figure 3 and Figure 4).

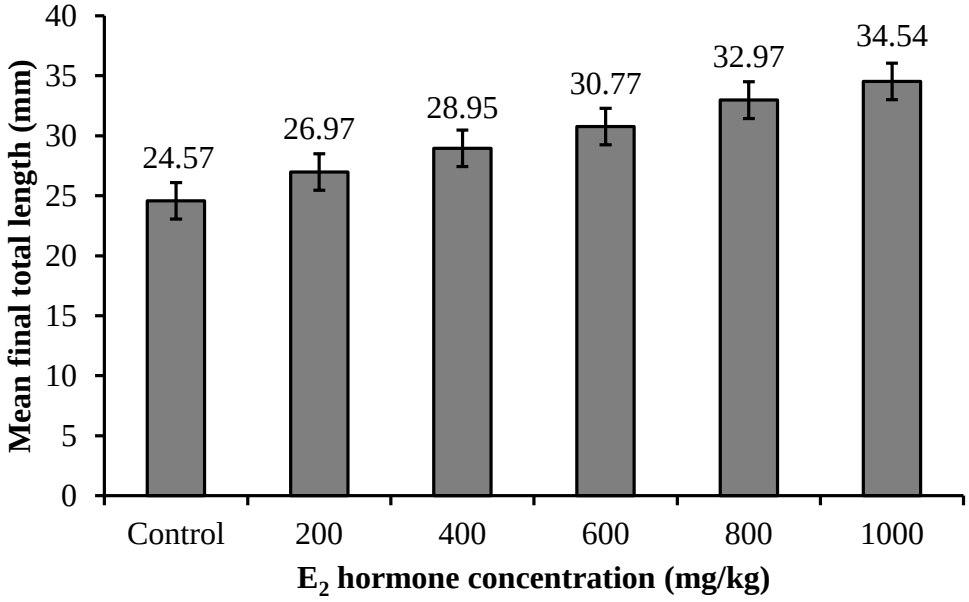

Figure 3. Mean final total length (TL) of *P. monodon* PL fed with different E_2 hormone concentrations.

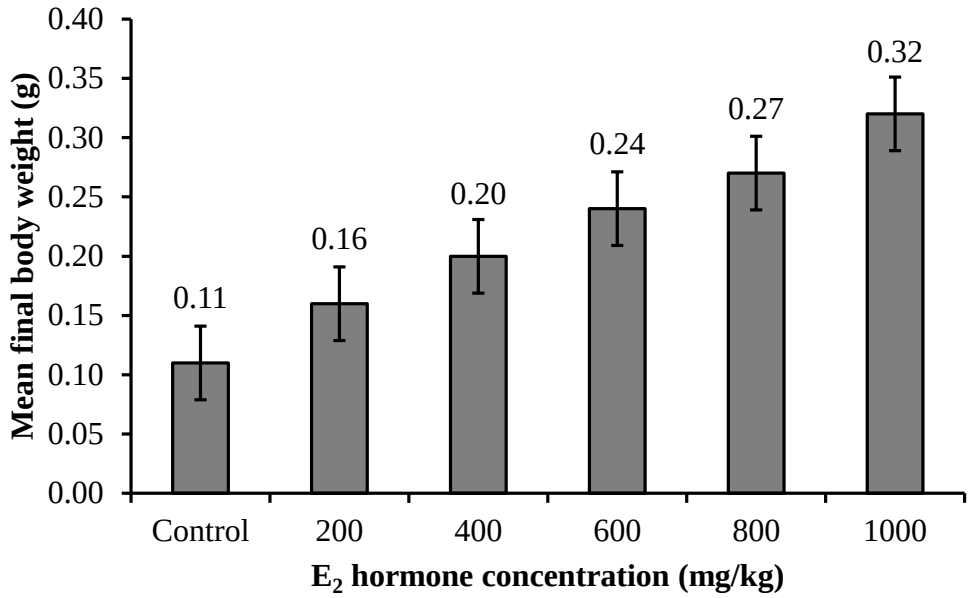

Figure 4. Mean final body weight (BW) of *P. monodon* PL fed with different E_2 hormone concentrations for each treatment.

The mean specific growth rates (SGR) for PL shrimp fed with different treatments of E_2 hormone concentration ranged between 2.67 ± 0.18 g/day and4.74 ± 0.281 g/day. The study shows that the mean SGR increased as the concentration of E_2 hormone increased (Figure 5). The mean SGR for control treatment of *P. monodon* PL after 50 days was 2.67 ± 0.18 g/day (Figure 5). The mean SGRs for E_2 hormone concentration treatments of 200, 400, 600, 800 and 1000mg/kg were 3.31 ± 0.003 g/day, 3.86 ± 0.13 g/day, 4.15 ± 0.50 g/day, 4.41 ± 0.02 g/day and 4.74 ± 0.30 g/day respectively (Figure 5).

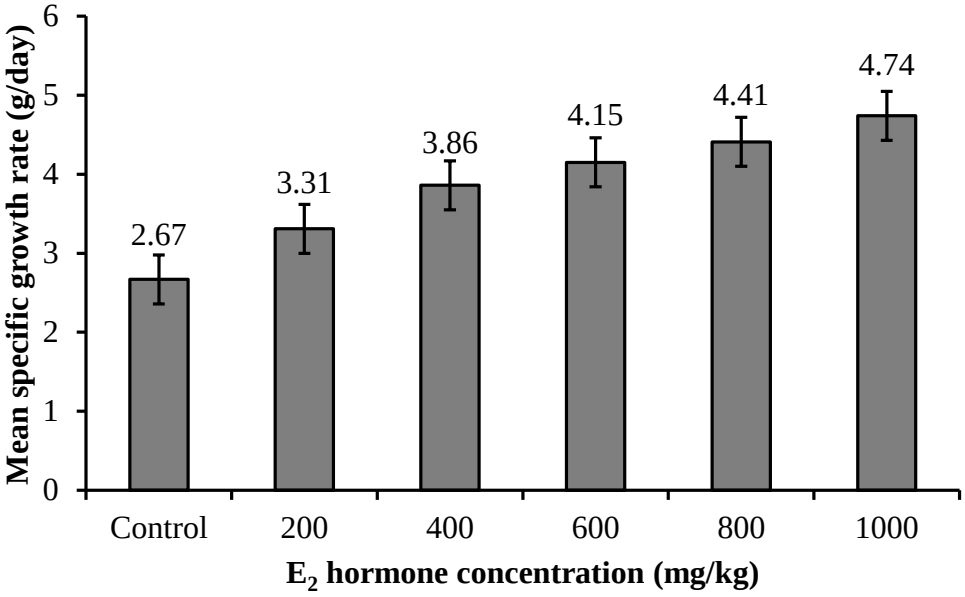

Figure 5. Mean specific growth rate (SGR) of *P. monodon* PL fed with different concentrationsof E_2 hormone.

DISCUSSION

Sex Ratio

The administration of E_2 hormone can improve the sex reversal of *P. monodon* PL into the females. A similar result was also obtained by Wang *et al.* (2008) in blue gill sunfish, *Lepomis macrochirus* where it was stated that E_2 hormonal induction can produce sex reversal not only for fish species but also for Penaeid shrimps. However, 100% feminization was not achieved during the treatment. This result is not similar to that reported by Wang *et al.* (2008) where, the sex ratios after exposure to various doses of E_2 hormone produced almost 100% females except for the control treatment. One of the possible reasons for the failure to produce 100% feminization in the present study may be the result of E_2 loss from the diets during storage and when the feed particles containing E_2 were not immediately consumed by the PL shrimp. This loss of E_2 from the diets was also reported by Beardmore *et al.* (2001) and Ohs *et al.* (2006). The second possible reason for the failure to induce 100% feminization of *P. monodon* PL in this study might be that the treatment was given outside the labile period which covers PL3 to PL53, while E_2 hormone should be introduced at an earlier during protozoea or mysis stage. This was proved in bluegill sunfish where sex reversal was almost 100% successful when E_2 hormone was administered during the early stages of their lives (Wang *et al.*, 2008). The same result was also obtained by Baker *et al.* (1988) to masculinate the Chinook salmon, *Oncorhynchus tshawytscha* using 17α-methyltestosterone at the time of hatching. Another possible reason may be that the treatment period of 50 days in this experiment was not long enough to activate the effectiveness of E_2 hormone for 100% feminization. The same results were also obtained by Macintosh *et al.* (1985) who also suggested that a longer period of treatment may be necessary as the effect of the hormone is time-dependent.

Survival rate

At the end of the experiment, the mean percentage of survival rates in all treatments was below 50% but ranged between 37.67 and 47.33%. The treatment of 600mg of E_2/kg of feed showed the highest mean survival rate compared to the other treatments. While the lowest mean survival rate was in 200mg/kg treatment. Variance Analysis showed that there was no significant difference among all the treatments. Thus, no signs of toxicity of behavioural differences between treatment groups and control PL shrimps were observed during and after the treatment. The results obtained in this study are almost similar to the information reported by Wang *et al.* (2008) on *L. macrochirus*.

Growth rate

The study showed that the SGR of PL shrimp increased as the concentration of E_2 increased in each treatment. Thus, these five different concentrations of E_2 hormone treatment all supported faster growth of PL. These results were similar to Cowey *et al.* (1973) where the oestrogenic synthetic hormone increases the weight gain of marine flatfish, *Pleuronectes platessa*. Study by Wang *et al.* (2008) *L. macrochirus* also showed considerable success in inducement of E_2 hormone not only on sex reversal but as a growth promoter. Personal observation showed that the food intake was higher in the steroid supplemented diet groups. Apparently, these compounds stimulated greater appetite where the PL appeared to be more active and always hungry for food. Thus, they ate more and grew faster as explained by Yu *et al.* (1979) on coho salmon, *Oncorhynchus kisutch*. This result may not be similar if the same hormone is applied to other aquatic species. This was also been pointed out by Woo *et al.* (1993) who suggested that the different growth enhancement is produced by different species and type of steroid hormone used. The estradiol may accelerate the growth of coho salmon, however, it may retard the growth of brown trout (Ashby, 1957). Presumably, different species of organisms respond differently to the type of steroid compound.

CONCLUSION

This study offers a new feasible way to improve *P. monodon* yields by introducing the oestrogenic hormone, 17β-estradiol (E_2) for the production of a higher female PL ratio. In addition, by considering all the effects of E_2 on sex ratio, survival and growth it can be concluded that the application of E_2 at

various doses was an effective way to regulate feminization and growth of *P. monodon* PL. The concentration of E_2 hormone increased the female sex ratio and growth rate of *P. monodon* PL. However, considering the time-dependent effect of E_2 hormone, it cannot be determined exactly which dose has the best effect on the PL shrimp growth and leads to 100% feminization. It is suggested that the treatment period should be increased to more than 50 days. In contrast to the result of feminization and growth, the application of this E_2 hormone does not provide a significant survival rate of PL produced. The number of PL surviving in all hormone treated diets was almost similar with the control treatment except for treatment of 600mg/kg. The E_2 concentration of 600mg/kg can be considered the most effective concentration for survival rate in the study.

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