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Effect of Androgen Hormone, Testosterone Undecanoate on the Banana Shrimp, Fenneropenaeus Merguiensis (De Man, 1888) Postlarvae

[1]M. Ikhwanuddin, [2]A.R. Khairul Hafiz, [1]M.B. Hafiz,
[1]M. Hidayah, [1]A.B. Noor-Hidayati and [2]A.B. Abol-Munafi

[1]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu, Malaysia
[2]Faculty of Fisheries and Aqua-Industry, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu, Malaysia

Abstract: This study was done to determine the effect of different concentrations of androgen hormone, Testosterone Undecanaote (TU) on the size increments, specific growth rate, survival rate, sex differentiation and sex ratio of banana shrimp, *Fenneropenaeus merguiensis* postlarvae. The experiment was carried out with different concentrations of TU (0, 200, 400, 600, 800 and 1000 mg/kg TU) which incorporated in the diet that was fed to PL 1 of *F. merguiensis* for 50 days study period. ANOVA analyzed showed that the mean size increments of body weight and total length, mean specific growth rate (SGR) of body weight and total length was significant different ($p<0.05$) among the different treatments. The highest mean percentage of survival rate was recorded for control (0 mg/kg TU) at 43.33 ± 4.41 % while the lowest mean percentages was recorded for 1000 mg/kg TU at 18.34+2.89. External sex differentiation of male *F. merguiensis* PL50 could be identified with the appearance of appendix masculine (AM) development at the second pair of pleopods and development of male gonophores complex (MG) at the fifth pereiopods and early male petasma development (endopodite without setae) at the first pair pleopods. In this experiment, 100% male was produced by every treatment from 200 mg/kg TU to 1000 mg/kg TU. The usage of androgen hormone TU can be an effective method for the production of 100% male *F. merguiensis* PL at least at the concentration of 200 mg/kg TU.

Key words: Fenneropenaeus merguiensis · Testosterone Undecanoate · Masculinization · Monosex

INTRODUCTION

Shrimp market demands cater for more uniform sizes, thus make the possibility of monosex culture may come into focus [1]. The world aquaculture in monosex culture technology had been done on crustacean culture [2, 3]. Monosex production would eliminate unwanted reproduction to fry during the critical period of post-hatching gonad in difference and permit farmers to achieve high yields of desirable size [1].

Banana shrimp, *Fenneropenaeus merguiensis* is one of the important penaeid species in Malaysia due to its good price and faster growth [4,5]. Occurrences of disease outbreaks such as White Spot Syndrome Virus (WSSV) and Early Mortality Syndrome (EMS) had cause a great loss to the shrimp industry. Since then, attentions also have been given to the culture *F. merguiensis*.

Monosex male production is preferred for shrimp culture because the absence of females prevents precocious spawning and eliminates behaviours that limit shrimp growth in mixed sex populations and early reproduction. Early maturation shunts energy to gonad growth rather than somatic growth and reproduction in ponds may lead to the harvest of many unmarketable fry. Individuals in mono-sex populations have increased somatic growth rate due to the avoidance of energy losses associated with gonad development and reproduction.

Corresponding Author: Ikhwanuddin, Deputy Director of Institute Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
Tel: + (6)09-6683638, Fax: + (6)09-6683390.

According to FDA Development and Approval Process in Designation List 2010, the usage of 17α-methyltestosterone hormone (MT) used in feed for the masculinization of female early life stage still under questionable. This hormone is illegal to be marketed or promoted until they are conditionally approved. Previous study by [5] state that MT treatment for the production all male postlarvae (PL) *P. monodon* gave bad implication to the aquaculture worker especially human handling on feed and methyltesterone 3 to 5 times daily more than 35 days which make them exposed to tumorigenic and terogenic cancer.

The use of new androgen hormone has sought to replace the harmful hormone, MT to ensure that all male PL production via monosex can still run. The alternative androgen hormone must be safe to human and effective in low dosage to minimize cost, sterility and environmental pollution. In this study, new androgen hormone, testosterone undecanoate (TU) in aquaculture were used to solve these problems. This hormone is safe for human usage [6, 7] and just required low dose to obtain good results. Thus this study aims to determine the effect of different concentration of androgen hormone, TU on the size increaments, specific growth rate, survival rate, sex differentiation and sex ratio of *F. merguiensis* PL.

MATERIALS AND METHODS

Broodstock and Larvae Rearing: This study was done at the Institute of Aquaculture Tropical, Universiti Malaysia Terengganu (UMT), Terengganu, Malaysia. Matured *F. merguiensis* broodstocks from Sungai Petani, Kedah coastal water, Malaysia (5°39'N; 100°19'E) were used in this experiment. Broodstocks were placed in a HDPE black tank (1 tonne capacity) where each tank has six female broodstocks and were waited until spawned.

After hatching, the high quality, disinfected and wash nauplii were counted by averaging the number from at least three small samples from the nauplius holding tanks before stocking. The larvae were acclimatized by flow through of water from the larval rearing tank until the temperature and salinity levels were equalized. The nauplii were stocked at a density between 100 and 150 nauplii per liter. The larval rearing tanks were filled to 50-75% of capacity with clean, disinfected, filter seawater at 30-35 ppt and 28-30°C. Clean water was slowly added during the delicate 4-6 day zoea stages.

Water was added daily until the tank was filled by early mysis larvae stage. Also the water was exchanged at 10-30% per day through the 4-6 day mysis larvae stage period until harvest at PL 1. If any disease or the water quality problems occur, water exchange rates were increased. Uneaten food and feces were siphoned out from the bottom of the tanks periodically. This was done by turning off the air and allowing the larvae to come to the surface of the tank. Debris from the bottom of the tank was siphoned into a net and the contents were put into a bucket to separate and return any larvae siphon from the tank. The larvae were cultured until they reach 1st day postlarvae stage (PL1).

Experimental Design: Stocking density of these PL1 is 10 PL/L^{-1} which every tank were stocked with 6L seawater and 60 PL. Total tank used in this experiment was 24 tanks for six treatments which were placed in the water batch culture tank of 500L capacity. The initial body weight (BW) and total length (TL) of a shrimp PL were recorded. The treatments were fed with commercial diets (Higashimaru CO; Ltd; Japan; 49% protein) of five concentrations of androgen hormone, TU at concentrations of 200, 400, 600, 800 and 1000 mg/kg feed. Each of the five experimental treatments had three replicates. The control was fed with the same commercial diet without supplement of TU (0 mg/kg) with also three replicate tanks. These PL shrimp was fed for 50 days study period at 4 times daily (0800, 1200, 1600 and 2000 hours).

Two submersible heaters have been used in the water batch culture tank to maintain a constant temperature of 30 ± 2°C. For aeration system, a blower was used and distributed to each aquarium tank by transparent long rubber tube which provide with ceramic air stones. The mild aeration was used to avoid disturbing the PL. The tanks were covered with black netting to give dark environment, about 90% of light blocking.

Preparation Hormonal Feed: This hormone was added by following a procedure by [5]. The appropriate quantity of the hormone was dissolved in 8 ml of 95% ethanol equivalent to 80% of the weight of commercial diet feed and was prepared for every week at 10g for each feed. Then, the hormonal feed diets were placed under a vented laboratory hood where it was covered from light. After three hours, most of the ethanol in the trays was evaporated. Then the diets were transferred onto aluminum foil trays and spread out into a thin layer to complete the evaporation of the remaining ethanol. The process was completed when there was no smell of ethanol. Then, the hormonal feed diets were packed into plastic containers and stored in a freezer until feeding.

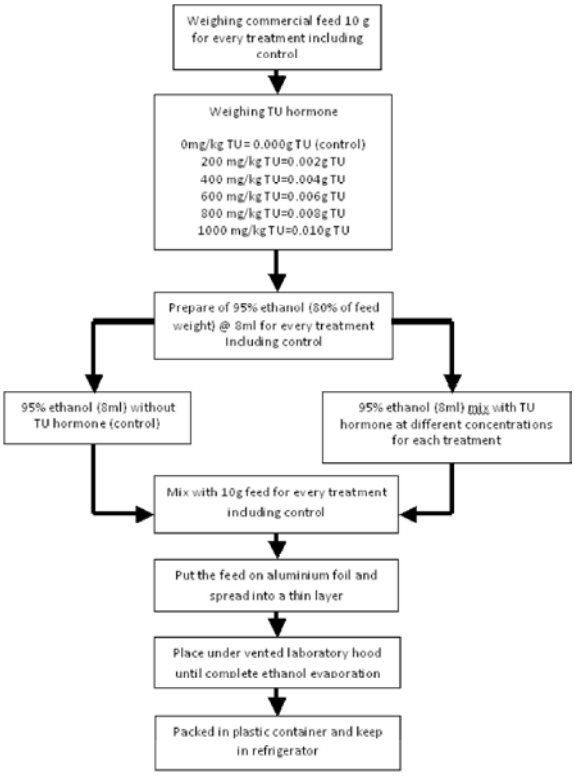

Fig. 1: Summary work flow for preparation of hormonal feed diets during the present study.

The control diet was prepared similarly without TU. The summary work flow for preparation of hormonal feed is as in Figure 1.

Data Collection

Size Increaments and Specific Growth Rate: The biological measurement of body weight (BW) and total length (TL) of random 30 PL *F. merguiensis* from every experimental aquarium were measured after 50 days at the end of the study period to determine the size increaments. The BW and CW measurements were also used to calculate the Specific Growth Rate (SGR). The SGR of PL was calculated following method by [8].

$$SGR = \frac{100\ (\ln \text{average final BW/TL} - \ln \text{initial BW/TL})}{\text{Number of culture days}}$$

Survival Rate: The survivals of *F. merguiensis* PL were also counted at the end of the study period using subsample technique. Survival rate (%) is equal to number of PL harvested divided by number of PL stocked multiply by 100% [5].

External Sex Differentiation and Sex Ratio: These external sex differentiation structures of *F. merguiensis* in the present study were described similar to the methods used by [5] for *Penaeus monodon* and by [9] for *Litopenaeus vannamei*. The sex of each shrimp was identified by (i) the development of the male petasma by cutting off the 1st pair of pleopods, (ii) the development of male Appendix Masculine (AM) by cutting off the 2nd pair of pleopods, (iii) the development of Gonophores Complex (GC) at the 5th pereiopods and (iv) the pair of female oblique Sharp Ridges Thelycum (SRT) on the anterior sternite XIV at the 5th pereiopods. The present of petasma, AM, GC and SRT were examined under the dissecting microscope [4]. Then, the sex ratio was determined.

Data Analysis: One way Analysis of variance (ANOVA) was used to determine the differences among treatments. Post host test and Tukey's test were used to determine the significantly different among treatment. The level of significance of the results was set at ($P < 0.05$). All statistics were performed using SPSS (version 16).

RESULTS

Size Increaments of Body Weight and Total Length: Mean initial BW for shrimp PL1 was measured at 0.0009 ± 0.001g before stocking. The results showed that the highest mean final BW were for treatment of 1000 mg/kg at 0.1314 ± 0.003g, followed by the other treatments of 800 mg/kg, 600 mg/kg, 400 mg/kg, 200 mg/kg and control at 0.1227 ± 0.004 g, 0.1144 ± 0.003 g, 0.1067 ± 0.003 g, 0.0916 ± 0.002 g and 0.0743 ± 0.004 g respectively (Figure 2).

Mean initial TL for random shrimp PL1 was measured at 5.13 ± 0.566 mm before stocking. The results showed that the highest mean final TL were for treatment of 1000 mg/kg at 31.22 ± 0.256 mm, followed by the other treatments of 800 mg/kg, 600 mg/kg, 400 mg/kg, 200 mg/kg and control at 30.54 ± 0.270 mm, 29.66 ± 0.273 mm, 28.83 ± 0.298 mm, 26.80 ± 0.360 mm and 24.26 ± 0.446 mm respectively (Figure 3).

Specific Growth Rate: The study showed that mean Specific Growth Rates (SGR) value for BW and TL increasing greatly from control to 1000 mg/kg TU. The results show that the highest mean SGR of BW were for treatment of 1000 mg/kg at 9.967 ± 0.048 %, followed by the other treatments of 800 mg/kg, 600 mg/kg, 400 mg/kg, 200 mg/kg and control at 9.830 ± 0.057 %, 9.690 ± 0.050 %, 9.550 ± 0.048 %, 9.245 ± 0.050 % and 8.826 ± 0.113 %

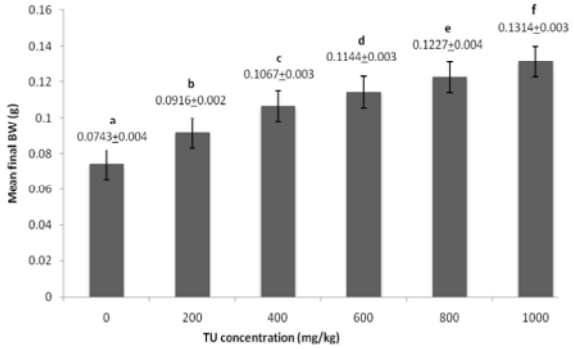

Fig. 2: Mean final body weight (BW) of F. merguiensis PL fed with different concentrations (0, 200, 400,600, 800 and 1000 mg/kg of Testosterone Undercanoate (TU) after 50 days study period. Analysis of variance showed that the mean final BW was significant different ($p<0.05$) among the different treatments with bars show different letters.

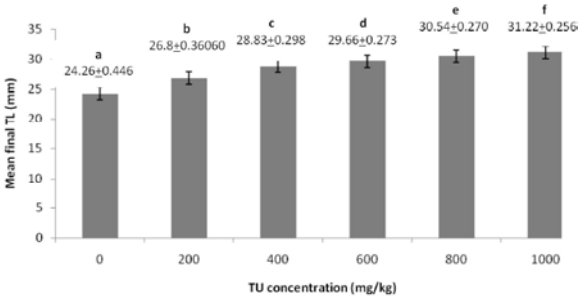

Fig. 3: Mean final body total length (TL) of F. merguiensis PL fed with different concentrations (0, 200, 400,600, 800 and 1000 mg/kg) of Testosterone Undercanoate (TU) after 50 days study period. Analysis of variance showed that the mean final TL was significant different ($p<0.05$) among the different treatments with bars shows different letters.

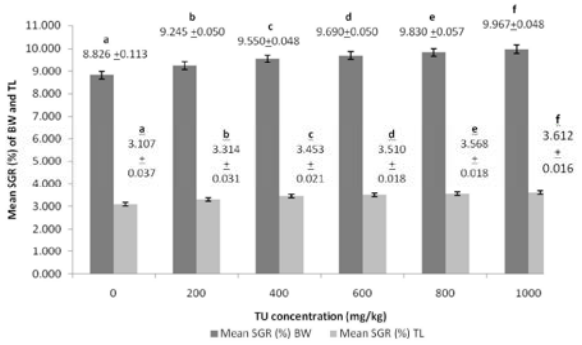

Fig. 4: Mean Specific Growth Rate (SGR) of body weight (BW) and total length (TL) of F. merguiensis PL fed with different concentrations (0, 200, 400,600, 800 and 1000 mg/kg) of Testosterone Undercanoate (TU) after 50 days study period. Analysis of variance showed that the mean SGR (%) of BW and TL was significant different with ($P < 0.05$) among the different treatments with bars shows different letters.

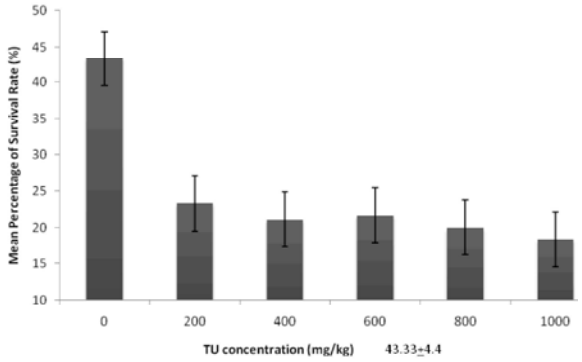

Fig. 5: Mean Survival Rate (%) of F. merguiensis PL fed with different concentrations (0, 200, 400, 600, 800 and 1000 mg/kg) of Testosterone Undercanoate (TU) after 50 days study period.

respectively (Figure 4). The highest SGR of TL were also for treatment of 1000 mg/kg at 3.612+0.016%, followed by the other treatments of 800 mg/kg, 600 mg/kg, 400 mg/kg, 200 mg/kg and control at 3.568+0.018%, 3.150+0.018%, 3.453+0.021%, 3.314+0.031% and 3.107+0.037% respectively (Figure 4).

Survival Rate: The highest mean percentage of survival rate was recorded for control (0 mg/kg TU) at 43.33 ± 4.41 % while the lowest mean percentages was recorded for 1000 mg/kg TU at 18.34+2.89%. The survival rate was decrease started from 200 mg/kg TU at 23.33 ± 1.67 % to 400 mg/kg TU at 21.11 ± 0.96 % and slightly increased from 600 mg/kg TU at 21.67 ± 1.67 %. It continued decreased again from 800 mg/kg TU at 20.00 ± 3.33 to 1000 mg/kg TU at 18.34 ± 2.89 (Figure 5).

Sex Differentiation: The study showed that the external sex differentiation of male *F. merguiensis* of PL50 could be identified with the appearance of AM development at the second pair of pleopods (Figure 6), the development of male GC at the fifth pereiopods (Figure 7) and the early male petasma development (endopodite without setae) at the first pair pleopods (Figure 8). While the female can be identified from anterior part of sternite XIV with SRT at the fifth pereiopods (Figure 9).

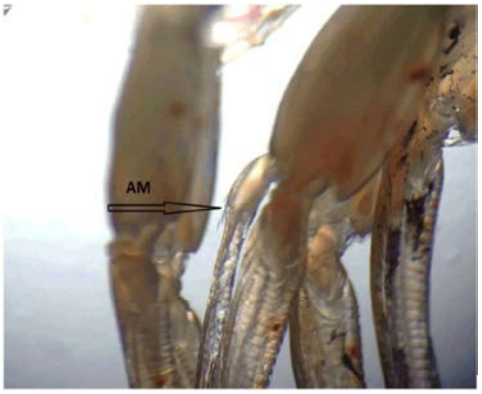

Fig. 6: Male Appendix Masculine (AM) at the second pair of pleopods of F. merguiensis at 100X magnification.

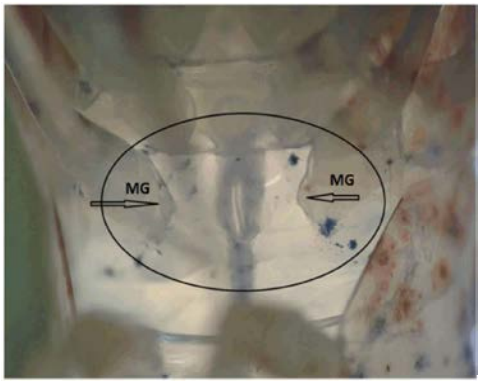

Fig. 7: Male Gonophores Complex (MG) at the fifth pereiopods F. merguiensis at 100X magnification.

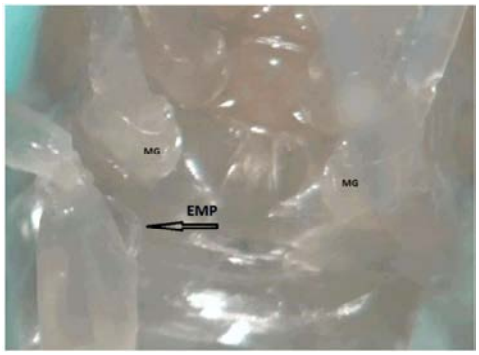

Fig. 8: Early male petasma (EMP), endopodite without setae at the first pair of pleopods and male Gonophores Complex (MG) of F. merguiensis at 100X magnification.

Sex Ratio: In this experiment, 100% male was produced by using TU concentration of 200 mg/kg to 1000 mg/kg (Figure 10) meanwhile, the control (0 mg/kg) shows highest females percentage of sex ratio with mean 57.72% females and 42.28% males (Figure 10).

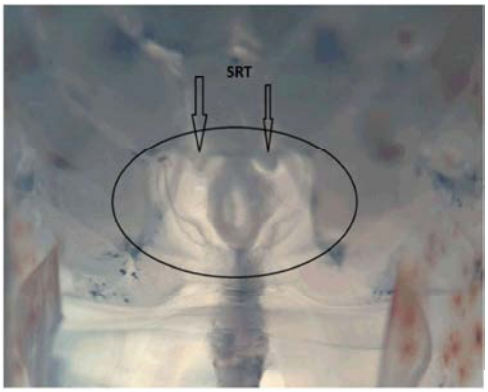

Fig. 9: Anterior part of sternite XIV with Sharp Ridges Thelycum (SRT) of female F. merguiensis at 100X magnification.

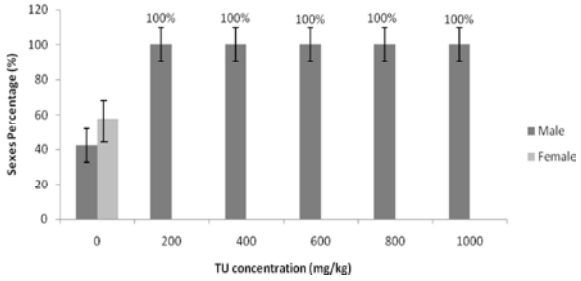

Fig. 10: Mean sexes percentage (%) of F. merguiensis PL fed with different concentrations (0, 200, 400,600, 800 and 1000 mg/kg) of Testosterone Undercanoate (TU) after 50 days study period.

DISCUSSION

Nowadays, hormones treatments are used to influence fish growth rates, sexual development and osmoregulation in aquaculture production. Sex reversal is also commonly been done using hormones for species such as tilapia where one sex grows faster than the other [10]. A previous study by [11] found that the injections of testosterone into the penaied prawn, *Parapenaeopsis hardwickii* can cause an increase in the weight, size and activity of the androgenic gland.

Based on the results of the present study, mean final TL and BW were increased started from 200 mg/kg TU until 1000 mg/kg TU as compared to control. Analysis of variance for both results also showed that the mean final TL and BW was significant different ($P < 0.05$) among the treatments and control. Therefore, it was concluded that the androgen hormone, TU had effectively become a growth promoter by improving the value of weight and length as compared to control, without TU hormone. This statement had been proven by the result of mean

SGR with the improvement of the SGR values started from 200 mg/kg TU to 1000 mg/kg TU and analysis of variance showed that the mean SGR values was significant different ($P < 0.05$) among the different treatments. The increased TU doses had also increased the growth and size of *F. merguiensis* PL due to the increasing somatic growth rate for each individual of the monosex population. In monosex population, the shrimp body will concentrate the energy from feed more on somatic growth than gonad development [5; 12].

TU hormone shows better growth promoter as compares to MT hormone where TU hormone was effective in growth rate (length and weight) started at early 200 mg/kg TU from the present study compare to MT at 600 mg/kg MT from study by [5]. On the other hand, analysis showed that SGR of TU hormone from the present study were significant differences among the treatments as compared to MT hormone where SGR were not significant differences among treatments from study by [5].

Based on the result of the study, it was found that the survival rate of *F. merguiensis* PL decreased with the increasing of TU concentrations. It was found that 200 mg/kg TU could produce all male with survival rate at 23.33%. The survival rate start decreased at 200 mg/kg TU with the survival rate of 400 mg/kg, 600 mg/kg and 800 mg/kg TU have no significant difference with survival rate between 20-22% and decreased to 18.34% at 1000 mg/kg TU. It was suggested that the *F. merguiensis* PL are in the stress with the increasing of TU concentrations in their body that will over react their body metabolism thus produced lower survival rate.

In the present study, the sex determination of *F. merguiensis* PL can be distinguished at PL50 with BW between 0.0743 to 0.2073g and TL between 24.26 to 37.13mm. It was found that individuals *F. merguiensis* PL having the three setae, developed a pair of oblique sharp ridges on the anterior part of sternite XIV that characterizes the female thelycum while for male, the individuals having a tubular appendage, developed the male gonophores located medially at the coxopod of the fifth pereiopods and the appendix masculine at the second pair of pleopods. It is found that an early male petasma development which was a pair of endopodite without setae at the first pair of pleopods with the pair of male gonophores appeared at the fifth pereiopods. Sex ratio with androgen hormone treatment showed that the application of TU can replace the former testosterone, MT used in aquaculture to produce male population of penaeid shrimps.

The results showed that TU hormone was proven can change sex of *F. merguiensis* into male 100% of the lower concentration at 200mg/kg TU and better than MT which only produced 76.62% male at 1000mg/kg MT [5]. Thus, it will be necessary in the future to identify the optimal dose of TU for better survival rate and at the same time could produce 100% male with doses lower than 200 mg/kg TU. TU treatment of 50 days could produce all male population of *F. merguiensis* postlarvae but it is still unknown what the implication if the treatment was below than 50 days or more than 50 days, may produce sterility of penaied shrimp PL.

The results from the present study show that the TU hormone can replace the MT hormone which is also non hazard to human. Study by [13] shows that MT hormone is carcinogenic which are not suitable for human consumption and can damage liver and trigger to cancer for human case. It is different situation to TU hormone which proven safe to human consumption without bad implication [13].

CONCLUSION

In conclusion, the present study demonstrates that the usage of androgen hormone, TU can be an effective method for the production of 100% male *F. merguiensis* PL at lowest concentration of 200 mg/kg TU. Thus, TU hormone can replace the MT hormone in aquaculture because of its effectiveness in producing male and also not harmful to human especially aquaculture workers and for human consumption. The results also showed that TU hormone can act as a growth promoter in penaeid shrimp PL either the survival rate decrease when the hormone concentration increasing.

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