International Journal of Agricultural Technology 2022Vol. 18(1):311-318
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Study on different concentrations and timings of 17α-methyltestosterone to accumulate in water flea (Moina spp.) from lab-scale

Pilapang, K.[1], Dandrit, D.[2] and Yomla, R.[1*]

[1]Department of Animal Production and Fishery, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, THAILAND; [2]Amber Farm Co., Ltd., Bangkok, THAILAND.

Pilapang, K., Dangrit, D. and Yomla, R. (2022). Study on different concentrations and timings of 17α-methyltestosterone to accumulate in water flea (*Moina* spp.) in lab-scale. International Journal of Agricultural Technology 18(1):311-318.

Abstract The different concentrations and immersion times of 17α-methyltestosterone (17MT) on the accumulation in water flea were investigated. Results were found that amount of 17MT of both groups in each period displayed significantly different ($P < 0.05$) when compared to the control. The highest accumulation of 17MT was found at the concentration of 100 mg / L for 420 minutes equal to 0.46 ± 0.10 mg/L. In contrast, it was found that the amount of 17MT in both concentrations of water at three times was not statistically significant ($P > 0.05$)in each treatment. Therefore, results indicated that the accumulation of 17MT in water fleas can be applied for using accumulated water flea to produce male tilapia.

Keywords: 17 α-methyltestosterone, Water flea, Concentration, Immersion time

Introduction

Synthetic hormones 17 α-methyltestosterone (17MT) are generally used to mix in larval feed for producing all-male tilapia. The concentration at 60 mg per one kilogram of feed has been reported to fed tilapia larvae for 21 days (Beardmore *et al.*, 2001). However, the 17MT has been reported to detect in the sediment after using to mix in fish feed (Fitzpatrick and Contreras-Sánchez, 2000; Green and Teichert-Coddington, 2000; Mlalila *et al.*, 2015). Zooplanktons such as rotifers, copepods, or cladocerans (water fleas) as live food for fish larvae, are containing protein, carbohydrates, vitamins, minerals, and amino acids, including fatty acids, which support a high rate of survival, increase the growth of small aquatic animals (New, 1998). An environmentally friendly way may be to use zooplanktons enrich with the 17MT to gain sex reversal. Therefore, the objective of the preliminary study was to study on accumulation of 17MT in water flea immersing at different concentrations and duration times. Future benefits of

* **Corresponding Author:** Yomla, R.; **Email:** rungtawan.yo@kmitl.ac.th

being used water flea as a natural feed may support sex reversal and fast-growing of the nursing stage of tilapia.

Materials and methods

Stock hormone preparation

The concentration of 17MT hormone solution was modified from the method of Jensi *et al.* (2016) by using 200 mg of 17MT powder mixed with 20 ml ethanol. And then, this solution was kept at 4˚C (Barbosa *et al.,* 2013).

Sample preparation

Water flea sample in this experiment was randomly collected from snakehead fishpond near King Mongkut's Institute of Technology Ladkrabang, Bangkok. These were quickly disinfected with 10% formalin and then washed twice with tap water. These were stocked in clean water a day.

A total of 0.26 g of stock water flea was immersed into 17MT concentrations at 50 and 100 mg/L. Each concentration was designed to use water flea immersing 30, 60, and 420 minutes, respectively. The 17 MT extraction from water flea was used High Performance Liquid Chromatography (HPLC; HITACHI, Chromaste) at the Industrial University Collaborative Research Center, Faculty of Agricultural Technology for analysis.

17 MT evaluation using High Performance Liquid Chromatography (HPLC) technique

All immersed water fleas of each experiment were separately collected by washing in 5 ml of distilled water three times. Then, these water fleas were put into 1.5 ml test tubes and euthanized by freezing for 30 minutes. These samples were crushed and added with 0.6 ml of 95% ethanol. These samples were centrifuged at 10,000 rpm for 10 min at 4˚C, and the supernatant was used to pass in a 0.45-micrometer syringe filter. Later, approximately 1 ml of the extracted MT and immersed water was transferred into the vial and kept at 10℃ before detection. We also preserved immersed water to evaluate the residue 17MT.

To quantity all extracted 17 MT of each group, the mobile phase was prepared by mixing acetonitrile (HPLC grade) and ultrapure water (HPLC grade) at a ratio of 60:40 v/v and preparing 70% methanol for column washing. And it filtered with a mobile phase filter before using for analysis. The standard solution was diluted to a concentration at 0.01-0.25 mg/mL

with 50% ethanol and filtered by a syringe filter into a 1 ml of vial bottom. This method is modified from Barbosa *et al*. (2013)

The HPLC condition was based on a method adapted from Barbosa *et al*. (2013) and Luvizotto-Santos *et al*. (2009) with UV detection at a wavelength of 245 nm. The used column was the Inspire™ C18 normal phase column (150x4. 6 mm) in combination with the Phenomenex Security Guard column and the mobile phase is Acetonitrile: Ultrapure water (60:40 v/v) injection volume 10 µl at a flow rate of 1 ml/min at 25˚C.

Data analysis

Hormone dosage was calculated from the relationship curve between the standard 17MT concentration level and the area under the peak using the Microsoft Excel program. Then analyze the difference in the amount of accumulation of 17MT in water flea and periods by using SYSTAT® 13.

Results

The standard solution of the peak (retention time) of 17MT was found in the range of 4.44 to 4.56 minutes. We found all mean peak times of different concentrations (50 and 100 mg/L) and different periods (30, 60, and 420 minutes) displaying in the range of the peak of the standard (Figure. 1 and Figure 2). The standard curve was linear in the investigated range. The equation of the standard curve was obtained by regression analysis was: $y = 3E+07x + 41952$ ($R^2 = 0.9981$).

The results were shown statistically different significant ($P<0.05$) of the 17MT accumulation of both groups at different times, (Figure 3). The amount of 17MT in extracted water flea at a concentration of 50 mg/L within 30, 60, and 420 minutes was found 0.02 ± 0.02, 0.05 ± 0.02, and 0.12 ± 0.06 mg/10µl, respectively. At 100 mg/L was found 0.16 ± 0.02, 0.23 ± 0.06, and 0.46 ± 0.10 mg/10µl, respectively, In addition, it determined to random water sample after finishing to immerse water flea which 17MT, which shown not different significance ($P>0.05$) (Figure 4). The amount of 17MT at a concentration of 50 mg/L was displayed 0.04 ± 0.00, 0.04 ± 0.00, and 0.03 ± 0.00 mg/10µl within 30, 60, and 420 minutes, respectively. At 100 mg/L was found 0.05 ± 0.00, 0.04 ± 0.00, and 0.04 ± 0.00 mg/10µl within 30, 60, and 420 minutes, respectively. The peaking time at concentrations of 50 and 100 mg/L at 30, 60 and 420 minutes had the meantime of 4.44 minutes, all the same (Figure 5).

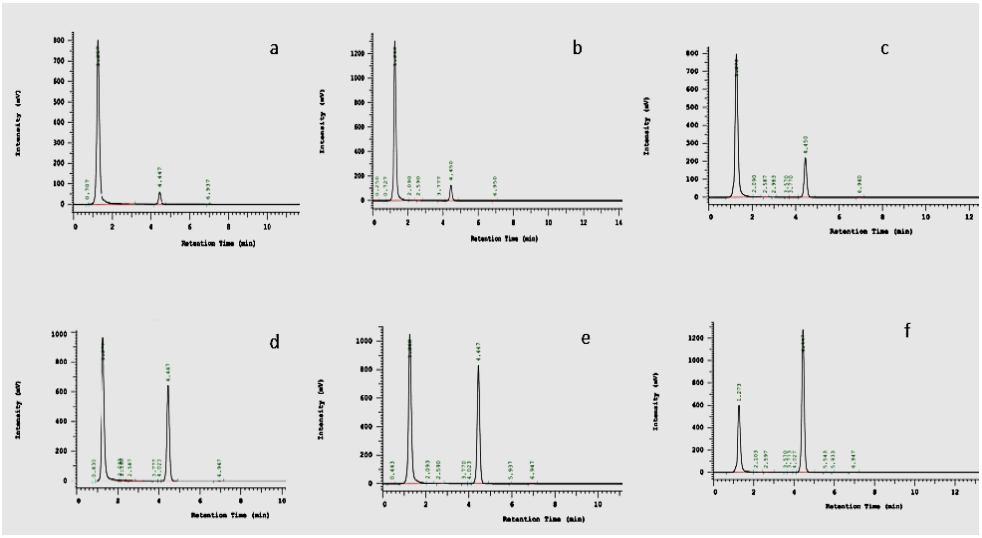

Figure 1. 17MT accumulation in water fleas at concentrations of 50 and 100 mg/L and times at 30, 60 and 420 mins. (50 mg/L; 30 (a) 60 (b) and 420 (c) and 100 mg/L; 30 (d) 60 (e), and 420 (f)

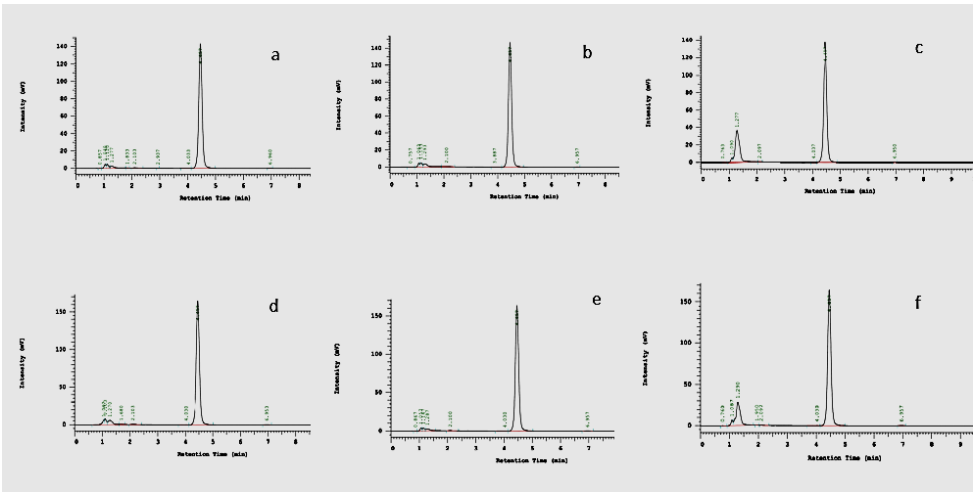

Figure 2. 17MT from immersed water at concentrations of 50 and 100 mg/L and times at 30, 60 and 420 mins. (50 mg/L; 30 (a) 60 (b) and 420 (c) and 100 mg/L; 30 (d) 60 (e), and 420 (f)

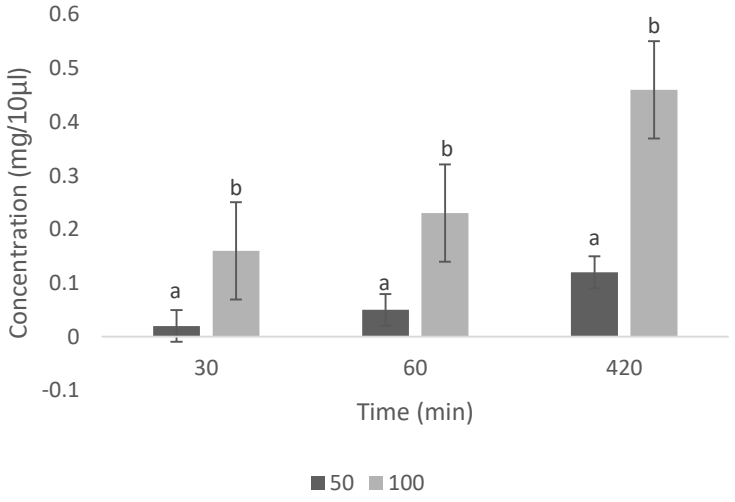

Figure 3. 17MT accumulation from water fleas extract at concentrations of 50 and 100 mg/L and immersion times at 30, 60, and 420 mins

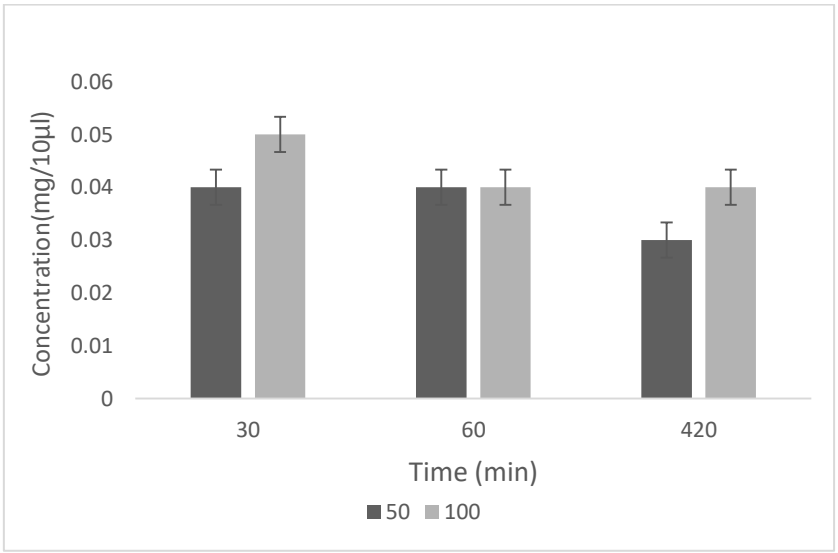

Figure 4. 17MT from immersed water at concentrations of 50 and 100 mg/L and times at 30, 60, and 420 mins

Discussion

The retention time of the 17MT in extracted water fleas to compare with the standard curve, which supported that the peak causing of the 17MT accumulation. It used a high concentration of 17MT for a long time to immerse water fleas, these were shown to have the highest accumulation in

extracted water flea. On the other hand, the lower concentration for a few times of immersion was detected the lower 17MT in extracted water flea. However, water fleas were immersed for a long time having low alive water flea. Moreover, all groups of immersed water after removing water flea were still to detect the residue 17MT after 420 mins. At the same of Kolodziej *et al.* (2004) is reported that the residue steroid hormones in dairy wastewater.

It should be noted that the 17MT at 50 mg/L be suitable used to immerse zooplanktons to reverse male production. Although, several articles have been reported that a high percentage of male tilapia requiring the 17MT in the range of 40-65 mg/kg (Marjani *et al.,* 2009; Celik *et al*., 2011; Dergal *et al*. (2016) and Jensi *et al*., 2016). Because of the 17MT at 50 mg/L was remained no difference from 100 mg/L at the end of immerse period (420 mins). In addition, the standard graph between the concentration and the area under the peak with a high positive correlation ($R^2 = 0.9981$). High concentration was displayed an increasing peak and decreased concentration displayed a decreased peak. Green and Teichert-Coddington (2000) reported that the 17MT was decreased at <100 pg./g at 8-40 days post-transsexual with 17MT and withdrawal periods were up to 120 days after sex reversal. However, the residue 17MT at the marketing size (4 to 5 months) was not harmful to consumers (White *et al*., 2006). We found the difference of residue 17MT in immersed water within 420 mins, which caused the effect of plankton, microbes, or photo-reaction. In addition, the 17MT was still detected in immersed water after 3 weeks. The reduction of hormones in an environment may react by photosynthesis or microbial degradation (White *et al*., 2006).

In conclusion, extracted water flea was found the highest 17MT accumulation at a concentration of 100 mg/L and immersion time at 420 mins. Future and research perspectives will focus on the potential of immersed water flea in the 17MT to reverse male production.

Acknowledgments

The author would like to thank Miss Nahatai Vijitrothai, the Industrial University Collaborative Research Center, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, who support working with the HPLC.

References

Beardmore, A., Mair, G. C. and Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects. Aquaculture, 197:283-301.

Barbosa, I. R., Lopes, S., Oliveira, R., Domingues, I., Soares, A. M. and Nogueira, A. J. (2013). Determination of 17α-methyl testosterone in freshwater samples of tilapia farming by high performance liquid chromatography. American Journal of Analytical Chemistry, 4:207.

Celik, I., Guner, Y. and Celik, P. (2011). Effect of orally-administered 17α-Methyl testosterone at different doses on the sex reversal of the Nile tilapia (*Oreochromis niloticus*, Linnaeus. 1758). Journal of Animal and Veterinary Advances, 10:853-857.

Dergal, N. B., Scippo, M-L., Degand, G., Gennotte, V., Mélard, C., Sidi-Mohammed Abi-Ayad, El-A. (2016). Monitoring of 17α-methyltestosterone residues in tilapia's (*Oreochromis niloticus*) flesh and experimental water after its sex reversal. International Journal of Biosciences, 9 (6):101-113.

Fitzpatrick, M. S. and Contreras-Sánchez, W. C. (2000). Fate of methyltestosterone in the pond environment: Detection of MT in soil after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, X. Cummings, and H. Egna (Editors), Seventeenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon,109-112.

Green, B. W. and Teichert-Coddington, D. R. (2000). Human Food Safety and Environmental Assessment of the use of 17α - methyl testosterone to produce male tilapia in the United States. Journal of the World Aquaculture Society, 31:337-357.

Jensi, A., Marx., K. K., Rajkumar, M., Shakila, R. J. and Chidambaram, P. (2016). Effect of 17α-methyl testosterone on sex reversal and growth of Nile tilapia (*Oreochromis niloticus* L., 1758). Ecology, Environment and Conservation, 22:1493-1498.

Kolodziej, E. P, Harter, T. and Sedlak, D. L. (2004). Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. environmental science and technology, 38:6377-6384

Luvizotto-Santos, R., Cordeiro, P. J. M. and Vieira, E. M. (2009). Analysis of diflubenzuron in tilapia filet by HPLC-DAD. Journal of chromatographic science, 47:785-788.

Marjani, M., Jamili, S., Mostafavi, P.G., Ramin, M. and Mashinchian, A. (2009). Influence of 17-Alpha Methyl Testosterone on Masculinization and Growth in Tilapia (*Oreochromis mossambicus*). Journal of Fisheries and Aquatic Science, 4 (1):71-74.

Mlalila, N., Mahika, C., Kalombo, L., Swai, H. and Hilonga, A. (2015). Human food safety and environmental hazards associated with the use of methyl testosterone and other steroids in production of all-male tilapia. Environmental Science and Pollution Research, 22:4922-4931.

New, M. B. (1998). Global aquaculture: Current trends and challenges for the 21st century. In: Anans do Aquacultura Brasil 98, Vol. I. Nov.2-6, Recife

White, J. R., Belmont, M. A. and Metcalfe, C. D. (2006). Pharmaceutical compounds in wastewater: wetland treatment as a potential solution. The scientific world journal, 6:1731-1736.

(Received: 8 November 2021, accepted: 30 December 2021)