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THE EFFECTS OF 17α-METHYLTESTOSTERONE ON SEX REVERSAL OF RED TILAPIA (*OREOCHROMIS NILOTICUS* X *OREOCHROMIS MOSSAMBICUS*) IN THE FREE-SWIMMING STAGE

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ABSTRACT: The effects of 17α-Methyltestosterone (MT) on sex reversal of Red tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*) in the free-swimming stage by immersion and feeding techniques were studied at the Department of Fisheries, Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Pathumthani Province from April to July 2017. The experimental design was a factorial experiment in CRD which studied two factors: 1) sex reversal by immersion treatment of 17α-MT (0, 150 and 300 µg/l) for 3 hours (T_1, T_2, and T_3) and 2) sex reversal by feeding treatment of 17α-MT (40, 60 and 80 mg/kg) for 28 days (F_1, F_2 and F_3). Results from these studies indicated that during the free-swimming stage, the immersion treatment of 17α-MT in 300 µg/l for 3 hours (T_3) had the highest growth rate in terms of weight, total length (28 days), ADG, FCR and survival rate (0.731 g, 3.340 cm, 0.024 g/d, 0.627 and 95.443 % respectively). The immersion treatment of 17α-MT in 150 µg/l for 3 hours (T_2) had a medial growth rate among the other factors. The immersion treatment of 17α-MT in 0 µg/l for 3 hours (T_1) had a minimal growth rate but had the highest sex ratio of males (83.889 %). The effects on sex reversal by feeding treatment of 17α-MT in 60 mg/kg for 28 days (F_2) had the highest growth rate in terms of weight, ADG and FCR (0.644 g, 0.022 g/d and 0.714 respectively). The effects on sex reversal by the feeding treatment of 17α-MT in 80 mg/kg for 28 days (F_3) had a medial growth rate and the highest survival rate (95.037 %) and sex ratio of males (85.667 %). The effects on sex reversal by feeding treatment of 17α-MT in 40 mg/kg for 28 days (F_1) had a good growth rate but the other factors were minimal.

Keywords: 17α-Methyltestosterone, Sex reversal, Red tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*), *Free-swimming stage*

1. INTRODUCTION

Tilapia are freshwater fish belonging to the family Cichlidae. They are native to Africa but were introduced into many tropical, subtropical and temperate regions of the world during the second half of the 20th century [1]. Tilapia is a worldwide fish of great commercial importance and it is recognized as one of the most important aquaculture species of the 21st century. Tilapia is currently ranked second only to carps in global production [2]. The world's total tilapia production in 2012 was 4.2 million tons. Tilapia aquaculture is rapidly expanding with global production of about 2.8 million metric tons in 2008 [3] and estimated to increase to 8.89 million metric tons by the year 2020 [4].

This rapid global production of tilapia is due to the introduction of improved strains of Nile tilapia (*Oreochromis niloticus*) (Fig.1) which is the major farmed tilapia species. The important aquaculture species are the nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*), Java tilapia (*O. mossambicus*) and Zanzibar tilapia (*O. hornorum*). The red tilapia (*Oreochromis* hybrids) (Fig.2) developed from crosses of the main *Oreochromis* culture species also has become popular in recent years. Other tilapia species are cultured only by small-scale farmers in Africa [5]. Nile tilapia was then introduced in different countries during the 1970s but problems including inbreeding, insufficient fish seed supply, stagnant production and poor fish growth hindered small-scale aquaculture production. Large-scale genetic improvement programs have been established for *O. niloticus* in Asia [6] and genetic methodologies to control sex have now resulted in the reliable production of all male fry to help overcome the problem associated with excessive fry production in culture ponds [7].

The Genetic Improvement in Farmed Tilapia (GIFT) project in the Philippines created strains of *O. niloticus* that grew up to 60% faster than their relatives [8]. The benefits of the GIFT strain include significantly faster growth rates than other farmed strains, improved survival in polluted waters and they can be raised in extensive systems without the need for commercial feeds. The development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male monosex populations to be raised to uniform, marketable sizes. Although several species of tilapia are cultured commercially, research on nutrition and culture systems, along with market development and processing, including value addition in Nile tilapia, made the species the predominant cultured species worldwide. The species matures at a larger size and is less fecund and thus less prone to overpopulation. Today, tilapia are often farmed with multiple species in the same pond, such as shrimp and milkfish. This not only optimizes the financial return if space is limited but also helps to prevent the growth of harmful bacteria and serves to remove excess organic matter in the water [9].

Fig.1 Morphology of Nile tilapia (*Oreochromis niloticus*)

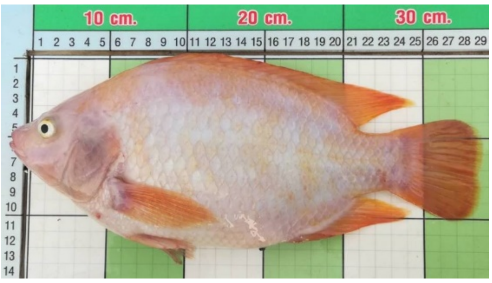

Fig.2 Morphology of red tilapia (*O. niloticus* x *O. mossambicus*)

Initially, GIFT trials were conducted in Bangladesh, China, Thailand and Vietnam, as well as in the Philippines. Today, 13 countries in Asia have received GIFT to develop national breeding and dissemination programmes and GIFT's fast growth and high yield have encouraged many rural families to take up environmentally friendly aquaculture across the region. As many new fish farmers are women, this empowers them as it improves local supplies of high-quality, affordable protein and benefits the nutrition of the household. The "GIFT Fish" was the result of this carefully conducted genetic selection and improvement programme based on broodfish collected from four African countries (Egypt, Ghana, Kenya and Senegal) and four commercial *O. niloticus* strains (from Israel, Singapore, Taiwan, Province of China and Thailand) used in the Philippines [10]. In the initial phase of the research, it was evident that the gain in growth and survival through crossbreeding were less than expected. This was followed by a pure breeding strategy among the best performing purebred and crossbred groups that led to the build-up of a genetically-mixed base population. This population formed the basis for the final selection program through a combined family and within family selection strategy [11]. Subsequent selection resulted in the emergence of the GIFT strain, which is purported to have an 85 percent cumulative genetic gain compared to the base population [6].

Hormonal sex reversal has been extensively used for sex determination and producing monosex fish for aquaculture purposes. Steroid hormones or hormone analogues, as well as non-steroid compounds, are commonly used for producing monosex tilapia. The hormones are generally incorporated into larval feeds and administered to undifferentiated larvae at very early larval stages (preferably at first feeding) for sufficient time to enable sex reversal. The use of hormones has been under increasing public criticism due to their possible health and environmental impacts. As a result, the use of hormones for sex reversal of tilapia is either licensed (in the USA) or banned (in Europe) [12]. Androgens are steroid hormones derived from cholesterol synthesized naturally by the adrenal cortex. Steroids are groups of lipids with several unique properties affecting growth and development. Steroids are called androgens if they are able to induce male characteristics and estrogens, if they induce female characteristics. The hormone testosterone is ineffective as an androgen when given orally and has a short duration of action when administrated parenterally because of rapid hepatic metabolism [13].

"Aquatic Animals" provide a healthy diet which is rich in protein, high-quality fat and many types of fatty acids. This is especially true of tilapia (*O. niloticus*), which is considered good protein and cheap. All people around the world can access this fish. Tilapia is a good source of fat and contains all kinds of essential fatty acids and lower cholesterol when compared to other meats or aquatic animals. In addition, the texture of tilapia meat is firm and it turns white when cooked to make a colorful, appetizing and delicious meal. It can be cooked in a variety of ways. For this reason, Tilapia is popular among consumers both in Thailand and abroad. Sex reversal by immersion and feeding may lessen the duration of treatment and lower the cost of hormones used. The present study aims to investigate the potential of different doses of 17α-MT with respect to the determination of the optimum dose.

2. METHODOLOGY

The experiment was conducted at the laboratory of Tilapia Hatchery, Division of Fisheries, Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi (RMUTT).

2.1 Experiment Design

This study was designed as a factorial experiment in CRD. The experiment was divided into 9 treatments with 3 replications per trail and carried out in a 50 liter glass cabinet filled with 30 liters of water (total of 27 experimental units) for 28 days. The experiments in this study were conducted to investigate two factors: immersion and feeding. There are 3 concentration levels of 17α-Methyltestosterone (MT): 0, 150 and 300 micrograms per liter (T_1, T_2 and T_3) in the first factor (immersion) and 3 concentrations levels of 17α-MT of 40, 60 and 80 milligrams per kilogram feed (F_1, F_2 and F_3) in the second factor (feeding).

2.2 Preparation of 17α-MT for immersion

Free-swimming stage (3 days old) (Fig.3) whose yolk sac have been absorbed were collected from the hatchery between 1,600-1,700 hours and were transferred to the laboratory. Four thousand and fifty free-swimming stages were immersed in different concentrations of 17α-MT hormone concentrations of 0, 150 and 300 µg l^{-1} for three hours. Immersion was done using plastic containers (1.5 liter capacity each) which were suspended in an aquarium measuring 24 cm x 50 cm x 30 cm. Aerators were provided in each container to facilitate the continuous movement of the free-swimming stage in the water column.

Fig.3 Morphology of the red tilapia free-swimming stage (scale = 1 mm)

2.3 Preparation of 17α-MT for Feeding

Hormone treated feed was prepared as described [14]. The 17α-MT was the hormone used. A stock solution was made by dissolving the hormone in 50 ml of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from the stock solution and preparing different doses of 60 mg and 80 mg 17α-MT kg^{-1}. This solution was evenly sprayed over 500 g of shrimp starter feed containing 40% protein and then mixed thoroughly. The feed-alcohol-hormone mixture was mixed again and again to ensure an equal distribution of the 17α-MT throughout the feed. Treated diets were dried at room temperature for 24 hours and then kept in airtight containers since androgens will breakdown when exposed to sunlight or high temperature. The prepared diets were stored at 4°C in a refrigerator before fed to fry of red tilapia. The diets containing 17α-MT were characterized as follows:

T_1: diet + 40 mg 17α-MT kg^{-1} of diet
T_2: diet + 60 mg 17α-MT kg^{-1} of diet
T_3: diet + 80 mg 17α-MT kg^{-1} of diet

2.4 Rearing

Sex reversal of tilapia in the free-swimming stage whose yolk sac have been absorbed was done by oral administration of 17α-MT through feed. After three days of hatching, the free-swimming stage were shifted to nine glass aquaria. There were three replicates for each treatment having 30 liters of water each and containing 300 nos of free-swimming stage for sex reversal treatment. During the free swimming stage, there was an oral administration of 17α-MT mixed feed containing 40% crude protein at three different dose rates viz. 40 (T1); 60 (T2) and 80 (T3) mg 17α-MT kg^{-1} of feed. The feeding occurred 6 times (7.00 9.00 11.00 13.00 15.00 and 17.00) daily during the day light hours for 28 days.

During the rearing period, water quality parameters (temperature, pH, dissolved oxygen (DO), Total Ammonia and Nitrite) in the net enclosures were measured weekly up to the end of the experiment. Temperature and dissolved oxygen concentrations were measured by a YSI DO meter Model 55 while pH was measured by a pen-type HANNA pH meter. Total Ammonia and Nitrite were measured by titration. The mean length, weight and survival rate of the fish in each treatment were recorded. After twenty eight days, the percentage of male and females were recorded.

2.5 Specifying Sex Rates

The sex was determined depending on the secondary sexual characteristics (Fig.4-5). All surviving fish were placed in an ice box and immediately dissected for sex differentiation. Sexes (male female, intersex) of fry (Fig.6) were identified by the gonad squash technique [15].

2.6 Statistical Analysis

In order to calculate the statistical significance between the growth of the groups treated with different doses of 17α-MT, a comparison of the different parameters was described according to [16]. The T-test was used to find out the statistical significance in terms of growth parameters, survival rates and sex ratio.

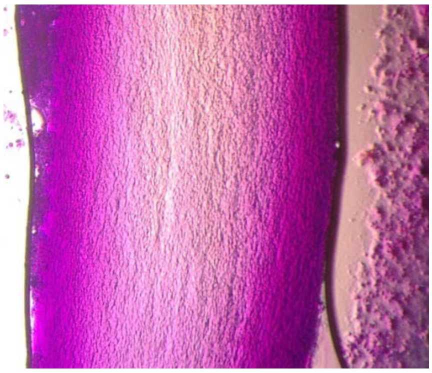

Fig.4 The secondary sexual characteristic of male

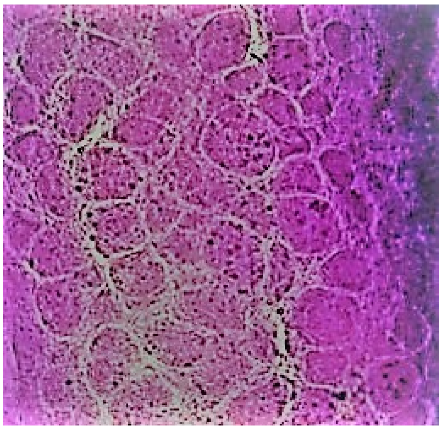

Fig.5 The secondary sexual characteristic of Female

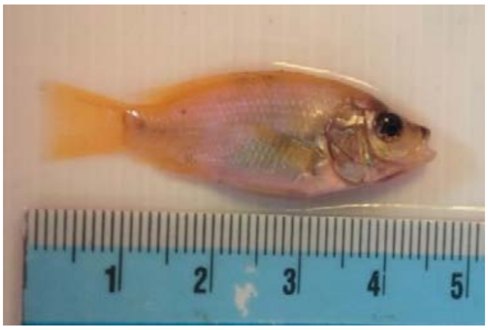

Fig.6 Morphology of red tilapia fry

3. RESULTS AND DISCUSSION

This study shows the effects of 17α-MT on sex reversal of red tilapia (*O. niloticus* x *O. mossambicus*) (Fig.2) in free-swimming stage by immersion and feeding techniques. The experimental design was a factorial experiment in CRD which studied two factors: 1) sex reversal by immersion treatment of 17α-MT (0, 150 and 300 µg/l) for 3 hours (T_1, T_2 and T_3) and 2) sex reversal by feeding treatment of 17α-MT (40, 60 and 80 mg kg^{-1}) for 28 days (F_1, F_2 and F_3). The two factors were not combined. The results are as follows. (Table 1)

3.1 Growth Parameters and Survival Rate

3.1.1 Average Weight and Length

The red tilapia underwent twenty eighty days of 17α-MT treatment with three concentrations of 0, 150, 300 mg l^{-1} in the free-swimming stage by the immersion technique. The experiment was carried out in a laboratory with a 30 fish per liter density with an average weight of 0.482, 0.698 and 0.731 grams respectively and with an average length of 3,156, 3,331 and 3,340 centimeters respectively. The results show that the immersion of 17α-MT with a concentration of 300 mg l^{-1} produced the heaviest weight and length followed by concentrations of 150 and 0 mg l^{-1}. The feeding diets of 40, 60 and 80 mg kg^{-1} produced an average weight of 0.624, 0.644 and 0.642 grams respectively and an average length of 3.292, 3.282 and 3.252 centimeters respectively. It was found that feeding the hormone 60 mg kg^{-1} produced the highest weight followed by 80 and 40 mg kg^{-1}. The length produced by feeding 40 mg kg^{-1} was the longest followed by 60 and 80 mg kg^{-1}. (Table 1) This was better than the results of [17] that was conducted to produce sex reversal in tilapia (*O. niloticus*) seed in fiberglass tanks in recirculating systems with different densities for 21 days. It was found that with 10 densities l^{-1}, the final weight was 0.066 ± 0.03 grams and the mean length was 1.00 ± 0.36 centimeters. The technique for administering these feedings was to introduce the feed little by little until the fish were full. Each feeding took about 30 minutes for the fish to be full then the weight and length were considered in good standing.

3.1.2 Average Daily Gain (ADG)

The ADG of the free-swimming stage for the 3 concentrations (0, 150 and 300 µg l^{-1}) by immersion were 0.018, 0.021 and 0.024 g d^{-1} respectively. (Table 1) It was found that 300 µg l^{-1} produced the best growth rate followed by 150 and 0 µg l^{-1}. The ADG by feeding at 3 concentrations (40, 60 and 80 mg kg^{-1}) were 0.022, 0.022 and 0.019 g d^{-1} respectively. (Table 1) It was found that feeding at 40 and 60 mg kg^{-1} was the best growth rate followed by 80 mg kg^{-1}. There was an experiment on the level of 17α-MT concentration on sex reversal of three strains of Nile Tilapia in Thailand. It found that the growth rate of Red Tilapia was 0.023 g d^{-1} which is close to the present experiment and shows that red tilapia have the best daily growth rate [18].

Table 1 The effects of 17α-MT on sex reversal of red tilapia (*O. niloticus* x *O. mossambicus*) in the free-swimming stage by immersion and feeding techniques.

Factor	Weight on 28 day (g)	Weight gain (g)	Length on 28 day (cm)	Increased length (cm)	ADG (g d^{-1})	FCR	Survival rate (%)	Male ratio (%)
Hormone Concentration (immersion)								
T_1	0.428^b	0.472^b	3.156^b	3.066^b	0.018^b	0.928^a	94.852^{ab}	83.889^a
T_2	0.698^a	0.688^a	3.331^a	3.241^a	0.021^{ab}	0.646^b	93.222^b	82.111^a
T_3	0.731^a	0.721^a	3.340^a	3.243^a	0.024^a	0.627^b	95.443^a	82.222^a
Hormone Concentration (feeding)								
F_1	0.624^a	0.614^a	3.292^a	$3.2.2^a$	0.022^a	0.742^a	94.110^a	80.667^a
F_2	0.644^a	0.634^a	3.282^a	3.315^a	0.022^a	0.714^a	94.370^a	81.889^a
F_3	0.642^a	0.632^a	3.252^a	3.192^a	0.019^a	0.743^a	95.037^a	85.667^a

Note: Different English letters in the vertical mean significantly different (P <0.05).
T_1 = Hormone concentration 0 µg l^{-1} used for immersion
T_2 = Hormone concentration 150 µg l^{-1} used for immersion
T_3 = Hormone concentration 300 µg l^{-1} used for immersion
F_1 = Hormone concentration 40 mg 17α-MT kg^{-1} used for feeding
F_2 = Hormone concentration 60 mg 17α-MT kg^{-1} used for feeding
F_3 = Hormone concentration 80 mg 17α-MT kg^{-1} used for feeding

Table 2 Water quality parameters observed in the glass tank of red tilapia

Water Quality Parameters	Min-Max	Standard value
pH	8.38 ± 0.00-9.31 ± 0.59	6.59-.0
DO (mg l^{-1})	3.38 ± 0.01-5.91 ± 0.02	≥ 3
Temperature (°c)	27.00 ± 0.00-29.00 ± 0.10	32.00-25.00
Total Ammonia (mg l^{-1})	0.01 ± 0.01-0.36 ± 0.04	≤ 0.5
Nitrite (mg l^{-1})	0.00 ± 0.00-0.02 ± 0.02	≤ 0.1

Note: *The standard of water quality for fish farming

3.1.3 Feed Conversion Ratio ((FCR)

The FCRs of the free-swimming stage for 3 concentrations (0, 150 and 300 µg l^{-1}) by immersion were 0.928, 0.646 and 0.627 respectively. (Table 1) It was found that 300 µg l^{-1} gave the best FCR followed by 150 and 0 µg l^{-1}. The FCR by feeding at 3 concentrations (40, 60 and 80 mg kg^{-1}) were 0.742, 0.714 and 0.743 respectively. (Table 1) It was found that feeding at 60 mg kg^{-1} gave the best FCR followed by 40 and 80 mg kg^{-1}. This was better than the results of [17] that was conducted to produce sex reversal tilapia (*O. niloticus*) seeds in fiberglass tanks in recirculating systems with different densities for 21 days. It was found that the FCR of Red Tilapia was 2.860. This may be due to the feeding technique which has less food loss than other methods resulting in lower FCR values.

3.1.4 Survival Rate

The survival rates of the free-swimming stage at 3 concentrations (0, 150 and 300 µg l^{-1}) by immersion were 94.852, 93.222 and 95.443 % respectively. (Table 1) It was found that 300 µg l^{-1} was the best survival rate followed by 0 and 150 µg l^{-1}. The survival rates by feeding at 3 concentrations (40, 60 and 80 mg kg^{-1}) were 94.110, 94.370 and 95.037 % respectively. (Table 1) It was found that feeding at 80 mg kg^{-1} was the best survival rate followed by 60 and 40 mg kg^{-1}. This was better than the results of the experiment on the effects of 17α-MT on sex reversal of Nile Tilapia (*O. nilotica*) by immersion at 300 µg l^{-1} and feeding of 60 mg kg^{-1}. The results of the survival rate were 83.8±7.1, 77.3±13.1, 78.0±5.7, 74.4±5.1, 79.9±12.5 and 66.1±10.9 % respectively. In this present study, the factors were controlled in all experiments, especially the water quality and the environment. The fish were fed regularly and were not under any stress, making for good health and good growth. This produced a higher survival rate [19].

3.2 Sex ratio

The sex ratios of the free-swimming stage at 3 concentrations (0, 150 and 300 µg l^{-1}) by immersion were 83.889, 82.111 and 82.222 % respectively. It was found that 0 µg l^{-1} was the best male ratio followed by 300 and 150 µg l^{-1}. The sex ratios by feeding at 3 concentrations (40, 60 and 80 mg kg^{-1}) were 80.6667, 81.889 and 85.667 % respectively. (Table 1) It was found that feeding at 80 mg kg^{-1} was the best male ratio followed by 60 and 40 mg kg^{-1}. There was an experiment of 17α-MT on sex reversal of Nile Tilapia (*O. nilotica*) by immersion of 300 µg l^{-1} and feeding of 60 mg kg^{-1} for 45 days. The result of this experiment showed a sex ratio of 100 % which is higher than this present study. This may be due to the longer duration of hormone exposure [19].

3.3 Water Quality

The red tilapia were fed hormones by different methods and with different concentrations for a period of 28 days. The water quality was checked every day throughout the trial period. It was found that the pH was 8.38 ± 0.00-9.31 ± 0.59, Dissolved Oxygen (DO) amounts were 3.38 ± 0.01-5.91 ± 0.02 mg l^{-1}, Water temperatures were 27.00 ± 0.00-29.00 ± 0.10 ° C, Total Ammonia amounts were 0.01 ± 0.01-0.36 ± 0.04 mg l^{-1} and Nitrites were 0.00 ± 0.00-0.02 ± 0.02 mg l^{-1}. (Table 2) It was found that the water quality in this present study was satisfactory [20] due to the quality control of the water throughout the duration of the experiment. The fish were not under stress and had a higher survival rate.

4. CONCLUSION

This study looked at the effects of 17α-MT on sex reversal in the free-swimming stage of Red Tilapia (*O. niloticus* x *O. mossambicus*) by immersion (0, 150 and 300 µg/l) for 3 hours and feeding (40, 60 and 80 mg/kg) for 28 days. It was found that during the free-swimming stage the immersion treatment of 17α- MT in 300 µg l^{-1} (T_3) produced the highest growth rate in terms of weight, total length (28 days), ADG, FCR and survival rate. The immersion treatment of 17α-MT in 150 µg l^{-1} (T_2) produced a medial growth rate among the other factors. The immersion treatment of 17α-MT in 0 µg l^{-1} (T_1) gave the minimal growth rate but had the highest sex ratios of males. Sex reversal by feeding treatment of 17α-MT in 60 mg kg^{-1} (F_2) produced the highest growth rate in terms of weight, ADG and FCR. Sex reversal by feeding treatment of 17α-MT in 80 mg kg^{-1} (F_3) gave a medial growth rate and the highest survival rate and sex ratios of males. Sex reversal by feeding treatment of 17α-MT in 40 mg kg^{-1} for 28 days (F_1) had a good growth rate but the other factors were minimal.

5. ACKNOWLEDGEMENTS

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