International Journal of Agricultural Technology 2023 Vol. 19(4):1765-1776
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The effect of *Moina* sp. immersion with 17alpha-methyl testosterone (17MT) on growth performances and sex reversal of Nile tilapia

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Pilapang, K., Ssijad, S. and Yomla, R. (2023). The effect of *Moina* sp. immersion with 17alpha-methyl testosterone (17MT) on sex reversal of Nile tilapia. International Journal of Agricultural Technology 19(4):1765-1776.

Abstract The results found that *Moina* sp. immersion with 17alpha-methyl testosterone (17MT) has a non-effect on the growth performances and non-effect on the sex reversal of Nile tilapia. The higher length, weight, and specific growth rate were found in the PC (PC: 60 mg17MT/kg mixing commercial feed) and the NC (only commercial feed without 17MT) than in other groups (50 and 100 mg17MT/l immersing *Moina* sp.). The length and the weight of the PC and NC groups showed no significant difference. But these groups were different from the groups of 50 and 100 mg/l. The average length of the PC, NC, 50, and 100 mg/l were displayed at 2.67±0.08, 2.86±0.17, 1.45±0.07 and 1.46±0.02 cm, respectively. The average weight of the PC, NC, 50, and 100 mg/l was displayed as 0.40±0.07, 0.51±0.08, 0.08±0.10 and 0.07±0.01 g, respectively. The highest specific growth rate was found in the NC group (2.37±0.00%). The survival rate of the NC group was not significantly different from other groups. 17MT does not affect growth performances in the larval stage. However, dried *Moina* sp. immersion in 17MT could be caused an effect on growth performances and sex reversal.

Keywords:17 α-methyltestosterone, *Moina* sp., Nile tilapia

Introduction

Nile tilapia, *Oreochromis niloticus* (L.), is the most economic species of freshwater aquaculture in Thailand. Tilapias can be grown and reproduced in a wide range of environmental conditions and tolerate stress induced by handling (Tsadik and Bart, 2007). To achieve more productivity in tilapia growing, farmers produced all-male production (Mair and Little, 1991). Because females are a greater reallocation of metabolic energy toward reproduction. Despite males using metabolic energy to channel toward growth. Thus, this is their benefit from anabolism-enhancing androgens (Tran-Duy *et al*., 2008, Angienda *et al*., 2010). Then, synthetic hormones 17 α-methyltestosterone (17MT) are

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generally used to mix in feed for producing all-male tilapia, which began to feed in tilapia fries (El-Greisy and El-Gamal, 2012). The 17MT concentration has been reported using 30–60 mg/kg feeding larval post-hatching until the 25–60th days (Macintosh and Little, 1995). Beardmore *et al*. (2001) reported using 17MT at the concentration of 60 mg/kg feeding tilapia larvae for 21 days, which produce all sex-male. Histological observations, the first sign of gonadal development is the formation of the genital ridge and the appearance of primordial germ cells (PGC). They were located between the gut and kidney (El-Greisy and El-Gamal, 2012), which are similar observations of two species of salmonids (Nakamura,1984). In both the control and treated groups, the initial ovaries were easily distinguishable due to the presence of well-developed perinucleolus oocytes. However, there was no clear differentiation observed in the testes, which appeared similar to undifferentiated gonads (El-Greisy and El-Gamal, 2012). A study by Sacobie and Benfey (2005) reported that *T. mossambica*, when subjected to a methyltestosterone dose of 50 mg/g of diet, certain germ cells in the gonads of potentially female individuals underwent oogenesis despite the influence of androgens, but eventually degenerated (Nakamura *et al*., 1974).

Zooplankton plays a crucial role as a food source for young and some adult freshwater fish. Throughout the year, the dominant groups of zooplankton in freshwater ecosystems include rotifers, cladocerans, and copepods. These organisms provide essential nutrients such as protein, carbohydrates, vitamins, minerals, amino acids, and fatty acids. By consuming zooplankton, larval aquatic animals experience improved survival rates and increased growth (Kenneth, 1990; Hutchinson, 1967). One specific freshwater cladoceran, the water flea *Moina macrocopa*, shows great potential as a live food source for both finfish and crustacean larvae (Alam *et al*., 1993; Kang *et al*., 2006). According to Rottmann *et al*. (2003), *Moina* typically contains around 50% protein in terms of dry weight, with adult females containing 20-27% fat per dry weight and juveniles containing 4-6% fat. Cladocerans, particularly *Moina* species, are highly nutritious, easily consumed, and digested by fish larvae. They meet the dietary requirements of larvae and contribute to water quality improvement by reducing the need for artificial feeding (He *et al*., 2001). Nevertheless, not all types of zooplankton are appropriate for rearing fry. However, it has been reported that live rotifers, *Moina*, and *Daphnia* species are beneficial freshwater zooplankton that can significantly improve the protein and overall nutritional composition of farmed fish. A study conducted by Islam *et al*. (2017) demonstrated that the growth performance, as measured by feed conversion ratio, specific growth rate, and protein content, of tilapia fry (*O. niloticus*) was notably enhanced when cultured with *M. macrocopa*.

Moreover, Jul-a-dung and Komanpririn (2007) studied 17β-estradiol hormone on the feminization of climbing perch (*Anabas testudineus*) by using water fleas and rotifer in the first 2 weeks after hatching for juvenile feed. *M. macrocopa*, a species of water flea, has emerged as a new and innovative method for delivering medication to freshwater aquatic animals. When medicated water fleas are orally administered to fish, the concentration of the drug in the fish's body tissues increases with each subsequent dose, reaching its peak after three feedings. This primary food source shows great potential as a drug delivery system for aquatic animals. Additionally, this approach is environmentally friendly, as it utilizes zooplankton to enrich with 17MT, facilitating sex reversal (Wiwattanapatapee *et al*., 2002). In a prior investigation (Pilapang *et al*., 2020), the focus was on assessing the levels and timeframes required for 17α-methyltestosterone (17MT) to accumulate within water fleas. The aim was to examine the effects on growth performance and sex reversal by immersing *Moina* in various concentrations of 17MT. The results were compared to a positive control group treated with 60 mg of 17MT per kilogram, while the negative control group solely received a commercial feed.

Materials and methods

Fish preparation

In this trial, totaled 1,200 Nile tilapia larvae at the age of 3 days after hatching were taken from Aquaculture and Aquatic Resources Management, Asian Institute of Technology (AIT) to Bansaun Rungtawan, Nong Chok district, Bangkok within 2 hours. These larval fishes having an average weight of 0.0268g were randomized to rare in plastic containers (10 L) managed in a closed circulation system (CCS) by using 100 larval fishes per plastic container (Figure 1).

Experimental design for sex reversal

A completely randomized design with three replicates was used to evaluate the effect of *Moina* sp. immersion with 17alpha-methyl testosterone (17MT) on the sex reversal of Nile tilapia. This trial consisted of 4 treatments having (1) *Moina* sp. immersed in 17MT at concentrations of 50 mg/l: 50 (2) *Moina* sp. immersed in 17MT at concentrations of 100 mg/l: 100 (3) the positive group using 60 mg17MT/kg mixing commercial feed: PC and (4) the negative group without 17MT: NC.

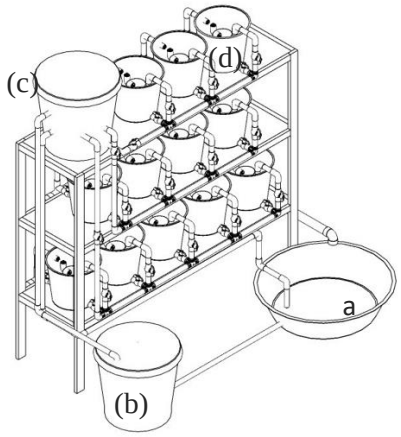


Figure 1. Schematic of closed circulation system; (a) water treatment, (b) water tank with water pump, (c) the water tank relies on the potential difference to distribute water to the nursery containers, and (d) plastic containers

Feed preparation

In 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 of absolute ethanol and this solution was kept at 4˚C (Barbosa *et al*., 2013).

Moina in this experiment was bought from Chatuchak Min Buri market, Bangkok. These were quickly disinfected with potassium permanganate ($KMnO_4$) and then washed twice with tap water. The totaled 60 g of *Moina* was immersed into 17MT concentrations at 50 and 100 mg/l, with a 300 ml solution volume for 60 minutes. Then immersed *Moina* was filtered, contained in the glass bottle, and kept away from light at 4˚C.

The positive control was prepared with 25% of 95% alcohol per kilogram feed at a concentration of 60 mg/kg. The solution was sprayed on powdered commercial feed (Prograde A 101 (Protein ≥40%, Fat≥5%, Fiber≥2%, Moisture≥12%)), and dry at room temperature. The negative group was only powdered commercial feed. These groups were kept in a dark container at 4˚C.

Fish rearing management

Tilapia Fries were fed with different diets (50, 100, PC, and NC groups) for 21 days by using Ad-lib four times per day. The CCS used an aquarium submerse pump (4000 L/h), which adjusted a speed of 30L/h to flow into each plastic container without aeration. The excreta and food leftovers at the bottom

of the plastic containers were siphoned off to remove every 3 days. Each experimental replicate was assessed daily for fish dried. At the end of the trial, the total weight of each replicate was recorded after 24 h starvation, and ten fishes of each replicate were randomly sampled to evaluate sex reversal.

Histological investigation

Tilapia samples were fixed in Davidson's fixative for 24 hr. and were decalcified in a decalcification solution containing $AlCl_3$-$6H_2O$ (12.67 g), HCl (8.5 ml), formic acid (5 ml), and distilled water (87 ml) for at least 4-8 hr. Then, samples were dissected in the gonadal tissue part (rear part of the body) and these sections were placed in a cassette within/ without sponges depending on sample sizes, which sample cassettes were placed into the tissue processor (Leica, TR 1020). The procedure involved immersing the samples in different percentages of graded alcohol (50, 70, 80, and 95%) and two containers each of absolute alcohol, xylene, and melted paraffin. This immersion program lasted for 1 hour in each container. Subsequently, the samples were embedded in paraffin blocks, trimmed, and sliced into 5-6 μm sections of transverse cross sections of the tilapia gonad using a semi-automatic microtome (Microm, Germany). To stain the sections, the samples were treated with hematoxylin and eosin (H&E) following the method described by Mumford (2004).

Statistical analysis

All parameters as average length and weight and survival rate and specific growth rate (SGR) were analyzed. The SGR at the end of the trial was calculated as described by Sveier *et al.* (2000) as follows:

$$\text{SGR (\%)} = \frac{\ln Final\ body\ weight - \ln Initial\ body\ weight}{Experiment\ peroid} \text{X100}$$

These parameters were analyzed to be significant at the level of significance $p<0.05$ by using One-way ANOVA with SYSTAT 13 version program.

Results

The results after 21 days at the end of the trial found a higher length, weight, and growth performance in the control group (PC and NC) than *Moina* feed group, which significant difference between the group that fed with *Moina* immersed with 17MT and all control groups (PC and NC). The length of 50 and 100 mg/l groups as 1.45±0.07 and 1.46±0.02 cm has no significant difference. The length of PC and NC groups as 2.67±0.08 and 2.86±0.17 cm has no significant difference (Figure 2). The weight of 50 and 100 mg/l as 0.08±0.10

and 0.07±0.01 g has no significant difference. The weight of the PC and NC has no significant difference of 0.40±0.07 and 0.51±0.08 g (Figure 3). The specific growth rate of 50 and 100 mg/l has no significant difference of 0.34±0.00 and 0.32±0.00%. The growth performance of PC and NC has a significant difference of 1.86±0.00 and 2.37±0.00% (Figure 4).

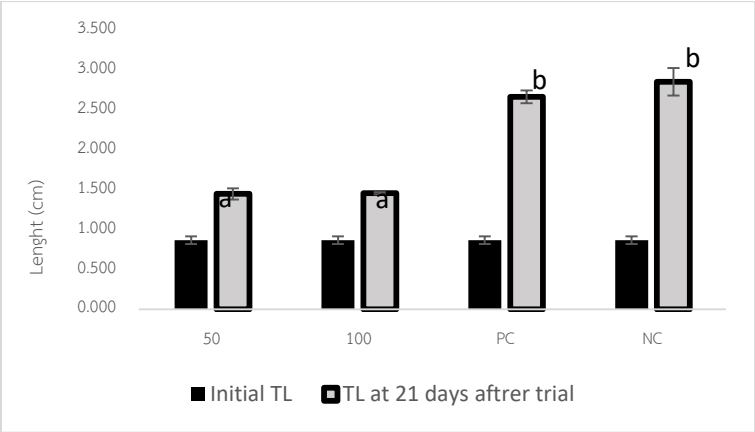


Figure 2. Average length at an initial length and the end of the trial (50= *Moina* sp. immersed in 17MT at concentrations of 50 mg/l; 100= *Moina* sp. immersed in 17MT at concentrations of 100 mg/l; PC= the positive group using 60 mg17MT/kg mixing commercial feed; and NC= the negative group without 17MT), Different letters are significantly different (P < 0.05)

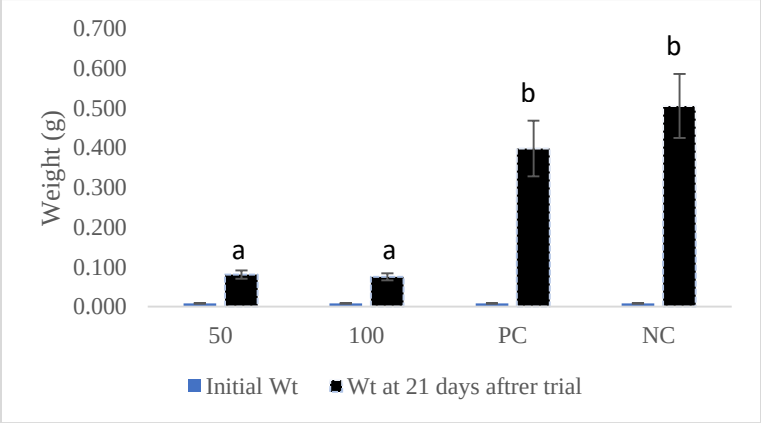


Figure 3. Average weight at an initial weight and the end of the trial(50= *Moina* sp. immersed in 17MT at concentrations of 50 mg/l; 100= *Moina* sp. immersed in 17MT at concentrations of 100 mg/l; PC= the positive group using 60 mg17MT/kg mixing commercial feed; and NC= the negative group without 17MT), Different letters are significantly different (P < 0.05)

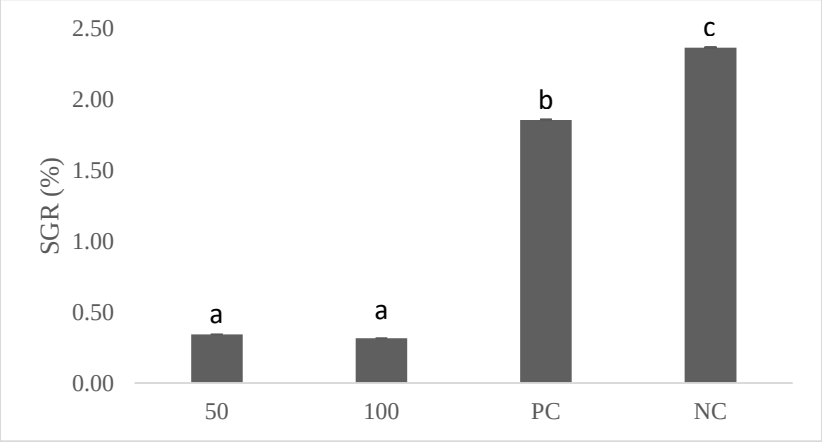


Figure 4. SGR (%) after the end of the trial (50= *Moina* sp. immersed in 17MT at concentrations of 50 mg/l; 100= *Moina* sp. immersed in 17MT at concentrations of 100 mg/l; PC= the positive group using 60 mg17MT/kg mixing commercial feed; and NC= the negative group without 17MT), Different letters are significantly different ($P < 0.05$)

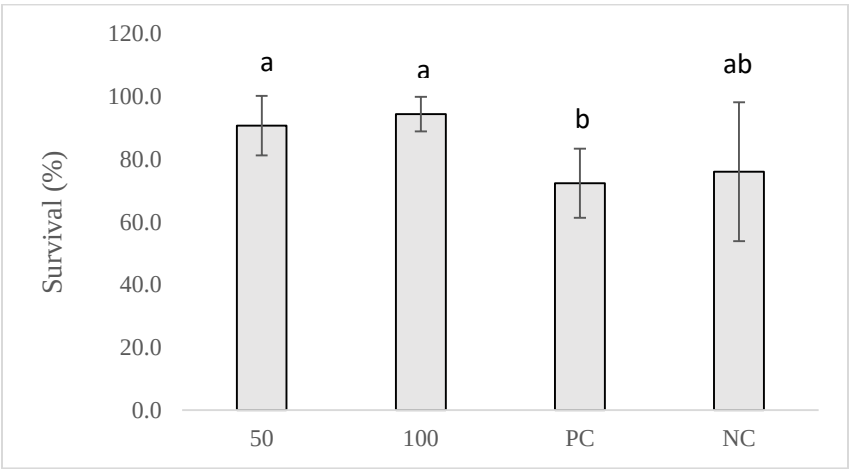


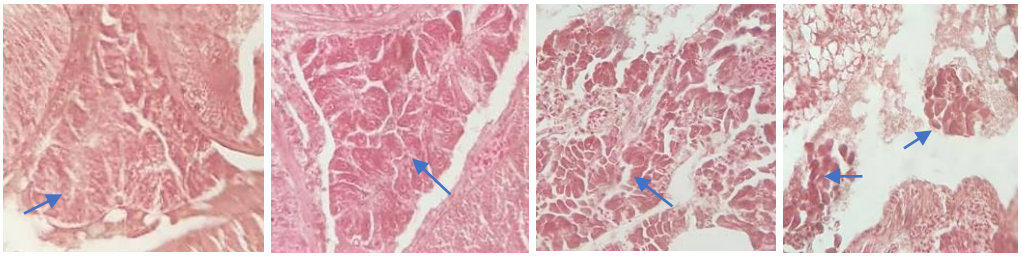
Figure 5. The survival rate after the end of the trial (50= *Moina* sp. immersed in 17MT at concentrations of 50 mg/l; 100= *Moina* sp. immersed in 17MT at concentrations of 100 mg/l; PC= the positive group using 60 mg17MT/kg mixing commercial feed; and NC= the negative group without 17MT), Different letters are significantly different ($P < 0.05$)

At the end of the trial, the survival percentage of tilapia after 21 days of 50, 100, PC, and NC groups were found 98±9.50, 98.3±5.50, 72.3±11.00, and 76±22.10, respectively. Two groups of immersed *Moina* with 17MT fed feed

displayed higher survival rates than PC and NC groups, which were not a significant difference in survival (Figure 5). The water quality in the system has shown a pH of 7.1-8.4, dissolved oxygen 5.5-6.5 mg/l and temperature were 27-28 ℃. Histological investigation to examine the sex reversal of all groups showed failure under the condition of this study.

Discussion

The present study was to study the possibility of sex reversal with immersed *Moina* with 17MT. The result of the study showed better growth performance in the positive control and negative control group than in feeding with immersed *Moina.* And immersed *Moina* with 17MT feeding has a higher survival rate than both the control groups. The lowest survival our studies have 72.3% in PC (the positive group using 60 mg17MT/kg mixing commercial feed). However, El-Greisy and El-Gamal (2012) reported that After 15 days post-hatching, the tilapia fry that received a diet containing 60 mg of 17MT per kilogram demonstrated the highest survival rate, reaching 93%. However, several studies have indicated that 17MT treatment does not have a positive effect on the survival of tilapia (Vera Cruz and Mair, 1994; Chakraborty *et al*., 2011). Thus, the cause of the low survival rate maybe not be the effect of 17MT. The growth performance SGR in the positive group using 60 mg 17MT/kg mixing commercial feed (PC) and the negative group without 17MT (NC) was 1.86 and 2.37. But Hassona *et al.* (2020) reported that the growth performance SGR in the positive group using 60 mg17MT/kg mixing commercial feed (PC) and the negative group without 17MT (NC) was 0.854 and 0.796. According to El-Greisy and El-Gamal (2012), the tilapia fry treated with a diet containing 60 mg of 17MT per kilogram exhibited the highest average weight (1.97 g) after 15 days of hatching, while the control group without 17MT had the lowest average weight (0.5 g). The weight of the positive control (PC) and negative control (NC) groups at 21 days old were measured at 0.34 g and 0.51 g, respectively. It is worth noting that some studies have reported that 17MT treatment leads to increased individual growth in tilapia (Little *et al*., 2003; El-Greisy and El-Gamal, 2012). Moreover, Islam *et al*. (2017) reported the growth performance value of feed conversion ratio, the specific growth rate, and the protein content of Tilapia fry, O. *niloticus* was better valued with *M. macrocopa* cultured. But our study was weak in the growth performance value in the *Moina* group. These may be affected by *Moina* being immersed in 17MT, which displayed deterioration to a solution without the shape of *Miona*.



(a) (b) (c) (d)

Figure 6. Histological investigation; *Moina* sp. immersed in 17MT at concentrations of 50 (a); *Moina* sp. immersed in 17MT at concentrations of 100 mg/l (b); the positive group using 60 mg17MT/kg mixing commercial feed and (c); and the negative group without 17MT (d); the arrow showed the hepatopancreas that misunderstanding to the gonad

In the present study, histological observations revealed that the initial indication of gonadal development was the formation of a genital ridge and the presence of primordial germ cells (PGCs), which served as evidence of the male proportion. However, our study could not determine the sex proportion accurately due to a misinterpretation. El-Greisy and El-Gamal (2012) reported that PGCs were located between the gut and kidney, a finding consistent with observations made by Nakamura (1984) in two salmonid species. Nonetheless, the administration of methyltestosterone had a suppressive effect on oogenesis. The extent of this inhibitory effect on oocyte development depended on the dosage of methyltestosterone. Despite initiating the administration of 17MT before ovarian differentiation, it was unable to completely prevent presumed genetically female individuals from producing at least a few young oocytes during the early stages of maturation. The ovary was mostly occupied by somatic elements (Wolf *et al*., 2004). Excessive hormone doses led to sterility or paradoxical feminization due to the aromatization of androgens into estrogens, while sub-optimal treatments resulted in intersex individuals (Popma and Green, 1990).

In conclusion, *Moina* immersion with 17MT can not be proven to induce male production. The group studies at the concentration of 50 and 100 mg/l have a high survival rate. Factors influenced the sex proportion in favor of males due to the administration of 17MT. The groups treated with 17MT exhibited a higher percentage of males. Further research on growth performance and position of sample to cross-section must be considered to use as a sex reversal feed and how to use live *Moina* after hormone immersion to rear tilapia larvae.

Acknowledgments

The author would like to thank KMITL Research and Innovation Services (KRIS), King Mongkut's Institute of Technology Ladkrabang (KMITL) for supporting this research and thank you to Bansuan Rungtawan for providing support to the research facility during the Covid situation. Special thanks to Assist. Dr. Praveena Taveekitjakan suggests histology studies.

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(Received: 27 August 2022, accepted: 10 July 2023)