Original Article

LIMNOLOGY FRESHWATER BIOLOGY

Influence of 17α-Methyl Testosterone Infused Diets on Growth and Tissue Integrity in *Oreochromis niloticus* (Linnaeus, 1758): A Laboratory Study

Rahman H.B., Khan M.A., Sultana S.*

Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

ABSTRACT. In this study, the effects of varying concentrations of 17α-methyl testosterone (17α-MT) in the diet on the growth performance, survival rate, and histopathology of Nile tilapia - *Oreochromis niloticus* (Linnaeus, 1758) were investigated. Four different hormone doses, 0 mg/g (control), 0.5 mg/g, 0.7 mg/g, and 0.9 mg/g were administered with the diet of *O. niloticus* for 120 days. Growth performance, as measured by the increase in total length (12.98 ± 1.26 cm) and total weight (18.99 ± 2.36 g), was highest in *O. niloticus* receiving 0.9 mg/g 17α-MT diet. The total weight (TW) increase was significantly more pronounced than that observed in total length (TL). In the average growth of length (31.13%) and weight (43.16%), the 0.7 mg/g 17α-MT diet had the highest percentage. However, the hormone treatments had no serious effect on the survival rates of the fish in the study. The histopathological changes in the liver showed sinusoid dilation, vacuolization of hepatocytes, enlargement of central veins, and hemorrhages with increased hormone doses. In the kidney, treatment of 0.7 mg/g and 0.9 mg/g of the 17α-MT diet resulted in changes in the renal tubules, necrosis, degenerated kidney tubules, and dilation of the glomerulus. The present findings show that 17α-MT treatments notably enhance the overall length, weight, and general growth performance of *O. niloticus*. Therefore, carefully structured experiments employing suitable hormone doses in fish feed may prove advantageous for the commercial cultivation and conservation of this popular fish species in the country.

Keywords: 17α-methyl testosterone, growth performances, survival rate, histopathology, *Oreochromis niloticus*

For citation: Rahman H.B., Khan M.A., Sultana S. Influence of 17α-Methyl Testosterone Infused Diets on Growth and Tissue Integrity in *Oreochromis niloticus* (Linnaeus, 1758): A Laboratory Study // Limnology and Freshwater Biology. 2025. - № 1. - P. 107-112. DOI: 10.31951/2658-3518-2025-A-1-107

1. Introduction

Fishery resources are one of the most important sources of animal protein. Compared to other animal protein sources, fish is considered a highly nutritious food source that provides high-quality animal protein at a comparatively low cost and is often more readily available and inexpensive (Khan et al., 2024). A typical person in Bangladesh consumes about 63 grams of fish per day as part of their diet due to the country's high population density (DoF, 2020). Therefore, much attention has been paid to the growth and production of freshwater fish in aquaculture (Juin et al., 2017). Hence, various growth promoters are used in fish farms. The use of hormones in aquaculture is effective for growth performance, reproduction, and monosex male production. The administration of androgenic hormones during sex reversal has an anabolic effect that increases growth and protein synthesis, leading to an increase in muscle mass (Robles Basto et al., 2011).

According to multiple studies, different supplements can be added to fish feed to improve growth metrics (Ajiboye, 2015; Kumar et al., 2016). Numerous anabolic steroids, which include both androgenic and estrogenic types, have demonstrated efficacy in improving growth and feed conversion when included in the diet (Jensi et al., 2016). Testosterone is a well-studied natural androgen that promotes fish development. 17α-methyl testosterone (17α-MT) is a synthetic compound that acts as both an anabolic and androgenic steroid hormone that promotes muscle growth and manifests male sexual characteristics (Nagaraju and Devi, 2019). 17α-MT is used in fish fingerlings to induce sex reversal and/or produce mono-sex populations of Nile tilapia (*Oreochromis* spp.) and climbing perch (*Anabas* spp.) in commercial fish farms, both domestically (Rima et al., 2017; Hossain et al., 2018; Hasan et al.,

*Corresponding author.
E-mail address: sabinasultana019@gmail.com (S. Sultana)

Received: December 05, 2024; ***Accepted:*** February 14, 2025; ***Available online:*** February 26, 2025

2022) and internationally (Jensi et al., 2016; Singh et al., 2018; Rivero-Wendt et al., 2020). Fish farmers are interested in it as it has become increasingly popular in recent years due to its ability to accelerate growth parameters and reduce feed costs in aquaculture.

Commercial production of Nile tilapia (*O. niloticus*) (Linnaeus, 1758) is expected to increase because of its widespread cultivation. Bangladesh ranks third in Asia and fourth in the world in Nile tilapia production (FAO, 2022). According to Rahman et al. (2012), Nile tilapia is considered the most significant aquaculture species of the twenty-first century. It is a popular fish farmed in Bangladesh to meet the growing demand for protein. This species tolerates low-protein food, shows physiological and immunological tolerance to a wide range of climatic conditions, and has a high degree of resistance to stress and infection (El-Sayed, 2006; Ng and Hanim, 2007). These characteristics make Nile tilapia a perfect species for laboratory research, especially for studies on growth and reproductive biology (Stickney, 2000; Lacerda et al., 2018). These studies can provide valuable information that can be used to improve the growth rates of other aquacultured fish species.

Although the use of 17α-MT for growth enhancement and sex reversal is well-documented worldwide, region-specific research on the effects of 17α-MT, particularly through infused diets, is limited in Bangladesh. Existing studies focus primarily on growth performance or sex reversal and neglect critical aspects such as tissue health and food safety. Additionally, with increasing concerns about sustainable aquaculture practices, understanding the physiological effects of 17α-MT is crucial to ensure consumer safety and minimize environmental risks. Therefore, the current study aims to evaluate the effects of 17α-MT on growth performance, survival rates, and histopathological changes in the liver and kidney of *O. niloticus* in a controlled laboratory environment. The present study has the potential to help in the development of *O. niloticus* monoculture technology that could pave the way for the widespread cultivation, conservation, and economic benefits of this fish species in the country.

2. Materials and Methods

2.1. Collection of the Experimental Fish

A total of 28 *O. niloticus* fish (7 fish for each treatment) were collected from local fishermen for the experiment. The average length of the fingerlings was 10.82 ± 0.22 cm, and the weight was 12.77 ± 0.28 g, respectively. The investigation included four treatment groups: C (control), T1 (Treatment 1), T2 (Treatment 2), and T3 (Treatment 3). The juveniles were reared on hormone-enriched fish feed for 120 days.

2.2. Experimental Design

For the experiment, four semi-circulatory aquariums (45 cm × 30 cm × 30 cm) were used in the laboratory for 120 days (March to June) with four treatments and three replicates. The air pump of the aquarium allowed a daily water exchange (25%) for aeration, while a water heater kept the water temperature stable (between 25–30°C). Four treatment groups were formed to evaluate growth performance and conduct histopathological examinations: 0.5 mg/g (T1), 0.7 mg/g (T2), 0.9 mg/g (T3), and 0.00 mg/g (C) of the hormone per gram of fish diet. Due to the limited size of the aquarium and the relatively small number of fish, we used this specific concentration of testosterone. The hormone was administered orally by mixing it with the diet. To mix 17-αMT with fish food, the required doses were first dissolved in 5 ml of pure alcohol, as it is insoluble in water. All treatments were allowed to air dry for 12-16 hours before being added to the aquariums. The experimental fish were fed twice a day, with the treated food (~2g pellets) given once daily.

2.3. Growth Studies and Survival Rate

The study involved assessing the growth rate and mortality of *O. niloticus* when 17α-MT hormones were administered or not. As described by Roy et al. (2019) and Nushy et al. (2020), the evaluation of fish growth performance, measured in length (cm) and weight (g) every month, along with their survival rate, was conducted as follows:

$$\text{Growth percentage} = \frac{\text{Final data - Initial data}}{\text{Initial data}} \times 100$$

$$\text{Survival rate} = \frac{\text{Number of alive fish}}{\text{Number of fish utilized}} \times 100$$

2.4. Histopathological Studies

For histopathological examination, the transverse sections of the kidney and liver tissues were obtained from both untreated and 17α-MT-treated fish. Two individuals were taken from each treatment. According to the methods described by Islam et al. (2008), Nasiruddin et al. (2012) and Uddin et al. (2019), these tissues were processed using standard microtome techniques. The selected organ tissues were meticulously cut into tiny fragments and subsequently preserved in alcoholic Bouin's solution. They were then dehydrated through a series of alcohol concentrations ranging from 70% to 100%, with the alcohol removed by xylene. The tissues were then immersed in liquefied paraffin wax. A rotary microtome was used to cut the wood blocks at a thickness of 6 μm after they were attached to the microtome chuck, allowing for the paraffin block to be sliced and expose the embedded tissues within. The sections of tissues were subsequently stained with hematoxylin and eosin. A compound microscope was used to closely examine the sections.

2.5. Statistical Analysis

The data were analyzed using ANOVA followed by Tukey's HDS post hoc analysis for multiple comparisons. The data were expressed as mean ± SD and analyzed using IBM SPSS Statistics version 20.0 statistical software, with a significance threshold of 5% ($p < 0.05$).

3. Results

3.1. Growth performance

The growth performance of *O. niloticus* was evaluated by applying varying concentrations of 17 α-methyl testosterone. The effects of 17-αMT on the total length (TL) and total weight (TW) of *O. niloticus* in the control (C), T1, T2, and T3 groups in a controlled laboratory environment are shown in Table 1. Despite a significant difference ($p < 0.05$) in TL between the control and T1 groups as well as between the T2 and T3 groups, the overall effect of the hormone on TL was not statistically significant ($p = 0.520$) based on the results. The same pattern was found for TW; however, the overall effect of 17-αMT on TW was highly significant ($p < 0.015$), indicating that hormone therapy of the experimental fish resulted in a much greater increase in TW than TL. The fish fed with a 0.9 mg/g 17-αMT dosage showed the best results in terms of total length (12.98 ± 1.26) cm and total weight gain (18.99 ± 2.36) g. As the dosage of 17-αMT was increased, the length and weight of the experimental fish also progressively increased.

The monthly gain in average length and weight was found to increase gradually from the start of the study period to the end. During the study, Nile tilapia were found to gain the highest average percentage of 31.13% in length and 43.16% in weight when treated with 0.7 mg/g of 17-αMT (Table 1). As predicted, all three treatment groups showed an increase in both morphometric parameters in *O. niloticus* compared to the control group (Fig. 1). Compared to the increase in total length (TL), the increase in total weight (TW) was more noticeable.

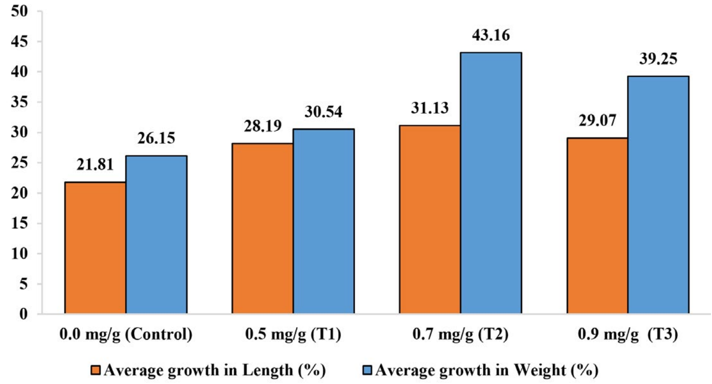

Fig.1. Changes in average growth performance (%) of *O. niloticus* induced 17α-MT under laboratory conditions.

3.2. Survival rate

Table 2 shows the survival rate of the experimental fish after 17α-MT therapy. In the control group, 100% of the fish were alive. Two of the fish from the T3 group and one from the T1 and T2 groups had died during the entire observation period. As a result, the hormone treatments had no discernible effect on the survival of the fish in this investigation.

3.3. Histopathology

3.3.1. Potential Effects of 17α-MT on the Liver

The transverse sections of the liver of the untreated control fish (C) showed a normal, regular structure (Fig. 2a). Similarly, the T1 group of treated fish showed no conspicuous changes. The hepatocytes and other liver cells were systematically arranged (Fig. 2b). In contrast, the histopathological alterations in the T2 group were characterized by the presence of more epithelial cells scattered among the hepatocytes, sinusoid dilation, vacuolization of hepatocytes, enlargement of central veins (Fig. 2c). The T3 group showed more severe alterations, including hemorrhage, vacuolization of hepatocytes, and severe necrosis (Fig. 2d) in *O. niloticus* examined.

3.3.2. Potential Effects of 17α-MT on the Kidney

The current investigation revealed that the control group of fish had an abundance of renal corpuscles and a network of renal tubules (Fig. 3a). Mild necrosis was observed in the T1 group (Fig. 3b). Visible changes occurred in the T2 treatment that included changes in the epithelial cells of the renal tubule, necrosis and degenerated kidney tubules (Fig. 3c). More conspicuous changes were found in the T3-treated kidney, which included necrosis and hemorrhage with degeneration of the kidney tubules on microscopic study (Fig. 3d).

4. Discussion

The current investigation was conducted to determine the performance of 17α-methyl testosterone hormone concentrations in fish feed required to promote better growth. The findings of the present experiment indicate that dietary supplementation with 17α-MT at a concentration of 0.9 mg/g in the artificial diet was a more effective anabolic steroid for the growth of *O. niloticus* than the doses of 0.0, 0.5, and 0.7 mg/g. Sultana and Farha (2022) reported that 0.7 mg/g 17α-

Table 1. Changes in total length and total weight of *O. niloticus* induced by 17-αMT under laboratory conditions.

Doses of 17- αMT	Total length (TL) cm (mean ± SD)	Total weight (TW) g (mean ± SD)	Average growth in length (%)	Average growth in weight (%)
0.0 mg/g (Control)	11.82 ± 0.94	14.18 ± 1.36	21.81	26.15
0.5 mg/g (T1)	11.91 ± 1.13	14.29 ± 1.48	28.19	30.54
0.7 mg/g (T2)	12.05 ± 1.40	15.67 ± 2.35	31.13	43.16
0.9 mg/g (T3)	12.98 ± 1.26	18.99 ± 2.36	29.07	39.25

Note: N = 7 for each treatment, *data are presented as mean ± standard deviation (SD) at significant level $p < 0.05$.

Table 2. The survival rate of *O. niloticus* after 17-αMT therapy in laboratory settings during the study period.

Doses of 17α-MT	March		April		May		June	
	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)
0.0 mg/g (Control)	7	100	7	100	7	100	7	100
0.5 mg/g (T1)	7	100	7	100	6	85.71	6	85.71
0.7 mg/g (T2)	7	100	7	100	6	85.71	6	85.71
0.9 mg/g (T3)	7	100	6	85.71	5	71.43	5	71.43

MT was more effective for the growth of *H. fossilis*. Jensi et al. (2016) provided evidence of the most significant increases in weight and length in the same species at a dose of 60 mg 17α-MT. The maximum weight (14.62 ± 0.59 g) and length (92.18 ± 3.01 mm) were observed at a dosage of 60 mg/kg in *O. niloticus* by Sarker et al. (2022). Singh et al. (2018) provided five variety dosages of the hormone to *O. niloticus* for 30 days in Rajasthan, India, and found that the highest rate of sex alteration rate occurred at 50 mg/kg of feed, and the fish in the treated groups grew faster than the control group. Rima et al. (2017) indicated that a dosage of 50 mg/kg of feed was best for both growth and male enhancement in Nile tilapia. Muniasamy et al. (2018) found in India that dosages of 60 mg/kg achieved optimal results in *C. mrigala* and 100 mg/kg in *C. punctatus.*

The experimental *O. niloticus* showed a markedly superior growth performance in the treated groups compared to the control group. At a dosage of 0.7 mg/g, the percentage increases in length and weight were 31.13% and 43.16%, respectively, whereas the control group had only 21.81% and 26.15% increases, which is almost consistent with the result of Sultana and Farha (2022). Nushy et al. (2020) conducted a comparative analysis of the effects of prepared feed versus commercial feed on the growth performance of this fish over 5 months in a farmer's pond located in Gazipur.

The survival rate of the control group was superior to that of the treatment group. The survival rate in the control group was unequivocally 100%, 85.71% in the 0.5 mg/g and 0.7 mg/g treatment group, and 71.43% in the 0.9 mg/g 17α-MT treatment group, respectively. Celik et al. (2011) reported a survival rate of 80% for *O. niloticus,* whereas Jensi et al. (2016) reported 81.6% and 82%, respectively. Sultana and Farha (2022) reported that the survival rate of *H. fossilis* treated with 0.5 mg/g and 0.9 mg/g 17α-MT was 85.71%, whereas, at 0.7 mg/g, 100% survived.

While hormone therapy promotes growth metrics in experimental fish, it also influences the internal organs by causing alterations and deterioration in the liver and kidney function and structure. With the increase of 17α-MT doses, several changes, including sinusoid dilation, hemorrhage, vacuolization of hepatocytes, and severe necrosis, were observed in the liver. These changes are similar to those induced by heavy metals and pesticides, as described by Mohamed (2009) and Mekkawy et al. (2013). Gayão et al. (2013) discovered higher protein levels in fish treated with steroid hormones, followed by considerable hepatic changes. Sayed et al. (2018) found hydropic degeneration in liver cells and blood congestion in the central veins of tilapia treated with 17α-MT. Khater (1998) documented that the hepatic parenchyma showed diffuse vacuolar degeneration accompanied by congestion in the central veins and hepatic sinusoids of *Tilapia nilotica*.

As an osmoregulatory organ, the kidney plays an important role in excretion in all vertebrates, including fish. Among the changes in the kidney tubules, necrosis, degenerated kidney tubules, and hemorrhages were found with increasing doses of 17α-MT, which is consistent with the findings in *H. fossilis* (Sultana and Farha, 2022). Nurmalita et al. (2020) documented the presence of hemorrhage, lymphocyte, and neutrophil

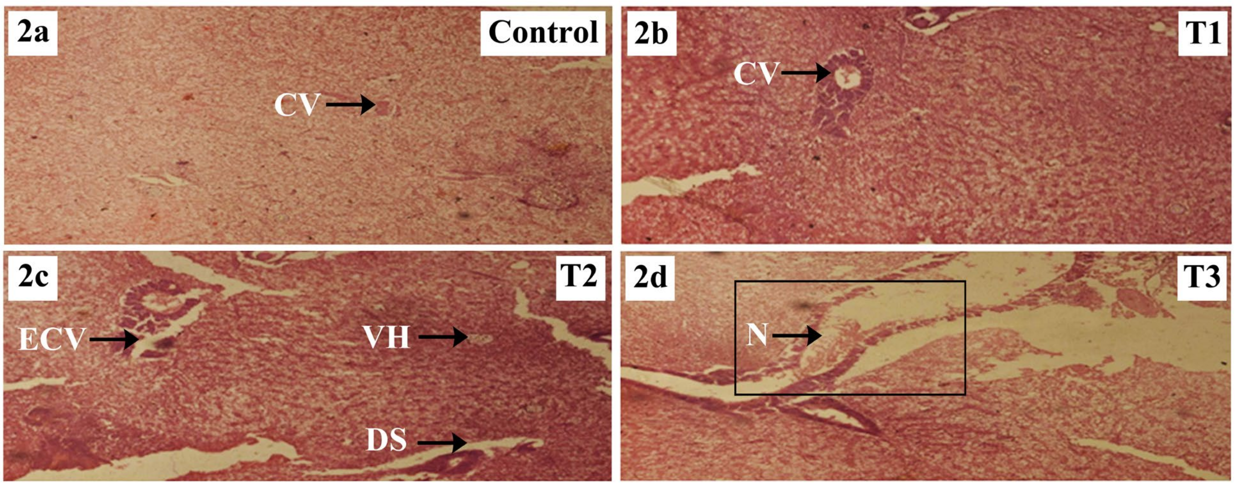

Fig.2. Transverse sections of the liver at 10× magnifications (a-d) taken from the control (C, 0.0 mg/g), T1 (0.5 mg/g), T2 (0.7 mg/g), and T3 (0.9 mg/g) groups of 17 α-MT-treated fish. (CV = central vein, ECV = Enlarged Central Vein, DS = Dilation of sinusoid, VH = Vacuolization of Hepatocyte, N - Necrosis and hemorrhage area).

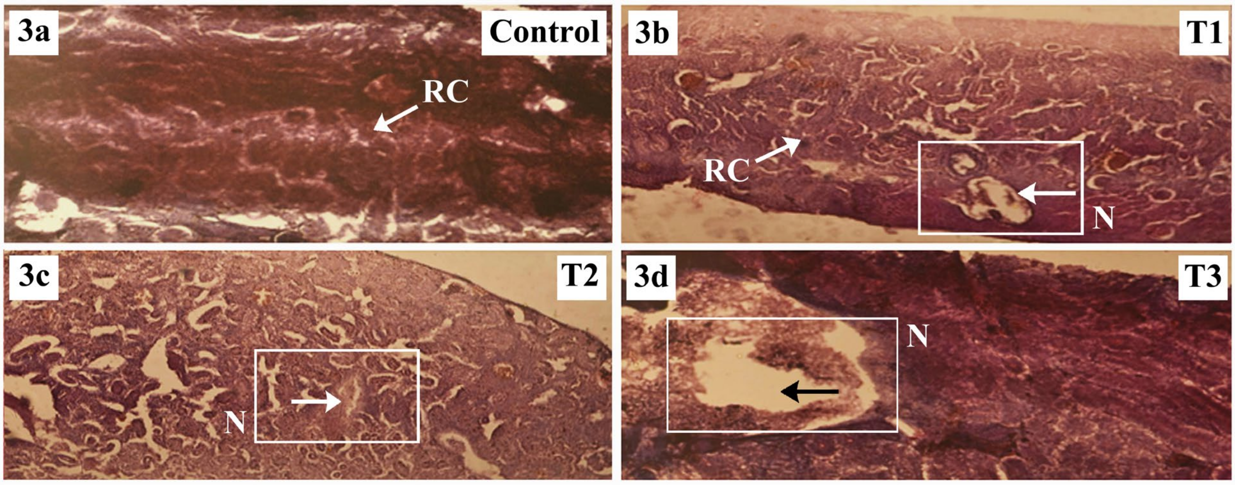

Fig.3. Transverse sections of the kidney at 10× magnifications (a-d) taken from the control (C, 0.0 mg/g), T1 (0.5 mg/g), T2 (0.7 mg/g), and T3 (0.9 mg/g) groups of 17 α-MT-treated fish. (RC= Renal corpuscle, N - Necrosis and hemorrhage area).

infiltration, inflammation, and necrosis in the renal tissue of *O. niloticus*. Sayed et al. (2018) detected glomerular enlargement, renal corpuscle rupture with disruption of the glomerular tuft, unclear lumen, isolated epithelial cells of renal tubule degradation, and tubular necrosis in *O. niloticus.* Katsiadaki et al. (2006) reported kidney hypertrophy in the three-spined stickleback (*Gasterosteus aculeatus*) due to the administration of 17α-MT.

5. Conclusion

The study demonstrated that the inclusion of 17α-MT in the diet of *O. niloticus* significantly improved growth performance under laboratory conditions. The treated fish showed a significant increase in both total length and total weight compared to the control group, with the optimal dose being 0.9 mg/g feed. Although the hormone treatment effectively promoted growth, it also had negative effects on the internal organs, particularly the kidneys and the liver, indicating potential health risks associated with prolonged use. These findings suggest that while 17α-MT can be an effective tool for enhancing growth in aquaculture, it should be used with caution to avoid unfavorable health effects. Uncontrolled or excessive use of 17α-MT could have unintended consequences, not only affecting the physiology and health of the target species but also potentially introducing residual hormones into the aquatic environment. This could disrupt local ecosystems, harm non-target organisms, and pose risks to human health through the food chain. Although this study provides important insights, further research is essential to explore the long-term effects of 17α-MT on the environment and to establish safe, sustainable practices for its use in aquaculture.

Acknowledgement

The authors sincerely thank the pertinent authority at the Fisheries Research Laboratory, Department of Zoology, University of Rajshahi, for permitting access to their laboratory facilities.

Conflict of interest

The authors declare no conflict of interest.

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