



Effect of 17 α -Methyl-Testosterone Hormone on Growth Performance, Feed Utilization, and Gonads Histology of Different Ages of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Three different weights of the Nile tilapia (*Oreochromis niloticus*) were used in this study, namely 7.7g (T1), 18g (T2), and 26g (T3) to investigate the effects of the 17 α -methyl-testosterone hormone (17a-MT) on the growth performance, feed utilization, and gonads histology. Varying concentrations of hormones were added based on the weight of the fish. Specifically, 6, 12, and 18 ml of 60mg of 17a-MT/ kg feed were administered to the respective weight groups. Control treatments (C1, C2, and C3) were also included for each weight category. The fish were provided with a commercially manufactured pellet feed containing 30% protein, 3% fat, and 4% fiber. This food was administered to the fish twice daily, with a quantity equivalent to 4% of their body weight. The fish density was subjected to a stocking strategy, where the sex ratio was maintained at a ratio of 1 male to 2 females for 12 weeks. The T3 group achieved a statistically higher significant survival rate and final body weight ($P < 0.05$). The T2 and T3 groups exhibited the lowest recorded values of PPV, respectively. The ovaries of the fish in the control groups had a substantial quantity of mature vitellogenic oocytes (MVO). The testes of fish exhibited the presence of normal spermatogenic cells (SC) and demonstrated a typical process of spermatogenesis. The ovaries of fish that were administered 17 α -methyl-testosterone hormones exhibited significant ovarian atrophy, characterized by the presence of severe atresia in several oocytes. While the testes of fish exhibited a moderate level of testicular degeneration characterized by degenerative alterations in the spermatogenic cells. The findings indicate that the hormone exhibits efficacy across various age and weight categories of fish. Therefore, hormonal treatment at these ages can be a means of controlling the ovulation process in tilapia fish.

INTRODUCTION

Aquaculture has emerged as a promising solution to tackle the problem of food shortages, particularly in the developing nations such as Egypt, where it has gained a significant attention and backing from experts. Aquaculture involves the practice of cultivating aquatic organisms, including fish, crustaceans, and mollusks in controlled

environments, such as ponds, tanks, and raceways (**Jennings *et al.*, 2016**). This method presents several advantages, viz. sustainable production, reduced environmental impact, and decreased dependence on wild populations due to reduced fishing activities.

Aquaculture has shown a great potential in addressing food security concerns in the underdeveloped countries, going beyond its current prevalence in wealthier nations. By providing affordable, nutritious, and high-quality food, aquaculture can significantly improve the socio-economic conditions of the populations in these regions, provided that there is an adequate infrastructure and support. **Assefa and Abunna (2018)** suggest that with the right assistance, aquaculture could help alleviate food consumption deficits in these areas, contributing to the United Nations' Sustainable Development Goals.

Egypt's population is projected to reach 110 million by 2023, as stated by **Abdalla *et al.* (2022)**. With this demographic growth, there is a corresponding need for an increase in food production. However, this poses a challenge due to the limited quality and quantity of land and water resources. To address this issue, the aquaculture industry in Egypt has expanded significantly. According to **El -Gayar (2003)** and **Badrey *et al.* (2019)**, the industry has the potential to fulfill the increasing need for protein. In fact, **Kaleem and Sabi (2021)** reported that Egypt has the largest aquaculture industry in Africa, producing over 1.8 million tons of fish. As a result, it is considered the primary source of fish in the region. The use of advanced technology, such as extruded feed, water circulation systems, and enhanced farm management practices, has led to a significant increase in the fish output. According to **GAFRD (2020)**, fish production increased from 0.54 million tons in 2005 to 1.59 million tons in 2020.

Egypt has been quite successful at the tilapia farming thanks to the implementation of strategies that are focused on producing monosex male tilapia through sex reversal techniques utilizing 17-methyl testosterone. These techniques have proven to be quite effective and have contributed significantly to the success of the tilapia farming in the country (**El-Sayed, 2006**).

The tilapia is an ideal fish species for aquaculture, especially in countries that are economically struggling due to its several beneficial characteristics. These characteristics include its ability to grow and develop rapidly, adapt to different environmental conditions, withstand stress and diseases, reproduce in captivity, have a short reproductive cycle, feed on a lower trophic level, and accept artificial feeds. These features make the tilapia fish an attractive option for fish farmers looking to maximize their production (**Pullin & Lowe-McConnell, 1982; Pullin & Capili, 1988**).

However, it is important to note that despite the numerous advantages of tilapia fish, they exhibit early sexual maturity and can reproduce without restrictions. This reproductive behaviour often leads to overcrowding of immature fish in the production system, which can negatively affect fish growth and overall production (**Geletu & Zhao, 2023**).

The inclusion of monosex production exclusively including males is of utmost importance in the context of culture since it serves to enhance the productivity per unit time of *O. niloticus* (Mair & Little, 1991). Male monosex cultures are often favored over female monosex cultures due to the differential growth that occurs in favor of males, as a result of the allocation of metabolic energy toward growth (Tran-Duy *et al.*, 2008; Angienda *et al.*, 2010). One additional concern pertaining to females is the allocation of a greater proportion of their metabolic energy toward reproductive processes. This phenomenon leads to the overcrowding of tilapia ponds and subsequently hampers their growth (Fashina-Bombata & Megbowon, 2012). To address this issue, the monosex culture technique can be employed to manage the undesired reproduction of tilapia. This technique involves the cultivation of exclusively male tilapia in the pond.

The current study aimed to determine the effect of treating different weights of the Nile tilapia with 17 α -methyltestosterone hormone on growth performance, food utilization, and histopathological changes in the gonads.

MATERIALS AND METHODS

In this study, three different weights of the Nile tilapia (*Oreochromis niloticus*) were utilized, namely 7.7, 18, and 26g. Additionally, varying concentrations of the 17 α -methyl-testosterone hormone were introduced, specifically 6, 12, and 18ml of a solution containing 60mg of 17 α -MT per kilogram of feed. These concentrations were administered based on the weight of the fish. Control treatments were also included for each weight category.

The fish were provided with a commercially available pellet diet containing 30% protein, 3% fat, and 4% fiber. Feeding was offered twice daily, with the amount of food given being 4% of the body weight of fish. During a period of 12 weeks, the fish samples were introduced into a population with a sex ratio of 1 male to 2 females. Prior to conducting the trials, the fish had a thorough examination to assess their overall health condition and detect the presence of any parasites.

Dechlorinated tap water was provided to each tank, and a constant supply of aeration was maintained through the use of an air compressor. Throughout the study period, we regularly monitored water quality parameters, such as temperature (27.5°C), pH (8.7), and dissolved oxygen concentration (5mg/ L) in all tanks using a mercury-in-glass thermometer, a pH meter (Hanna model H198106), and a dissolved oxygen meter (JPP-607 model), respectively.

Experimental design

The experimental design employed in this study was carefully planned and executed. The fish that had undergone acclimatization were divided into six groups, with each group consisting of 15 fish placed in 300L of water.

C1 group (7.7 grams) control group without any hormone

T1 group (7.7 grams), which consisted of 6 millilitres derived from a 60-gram sample of the 17 α -methyl-testosterone hormone.

C2 group (18 grams), control group without any hormone

T2 group (18 grams), which consisted of 12 milliliters derived from a 60-gram sample of the 17 α -methyl-testosterone hormone.

C3 group (26 grams) control group without any hormone.

T3 group (26 grams), which consisted of 18 milliliters derived from a 60-gram sample of the 17 α -methyl-testosterone hormone.

Preparation, feeding treated feeds by hormone

10g of the hormone was dissolved in one liter of ethyl alcohol 95%, and this standard solution is deemed to be usable for three months if stored in the refrigerator. 6 , 12 & 18ml of the previously prepared standard solution were mixed with half a liter of pure alcohol. Afterward, the mixture was added to 1kg of soft feed to increase the rate of spread of the hormone, as well as to ensure its distribution over the entire feed. Subsequently, the feed was spread on a clean plastic sheet in a well-ventilated area that was not exposed to direct sunlight to ensure that the thickness of the feed layer was not more than 2cm until the alcohol was volatilized.

All growth parameters were measured every two weeks. The weight was measured and data were recorded. Growth performance was calculated using the following equations:

$$\text{Weight gain} = W_t - W_o$$

$$\text{Specific growth rate (SGR) \% / day} = (\ln W_t - \ln W_o) 100 / n$$

Since [n: number of days; W_O: Initial weight at the beginning; W_t: Final weight at the end of period].

At the end of the experiments, the survival percentage was calculated based on the following equation:

$$\text{Survival (\%)} = 100 * (\text{final fish number} / \text{initial fish number}).$$

Feed utilization

Feed conversion ratio (FCR), protein efficiency ratio (PER), and protein productive value (PPV) were estimated according to the following equations:

$$\text{Feed conversion ratio (FCR)} = \text{feed intake} / \text{weight gain}.$$

$$\text{Protein efficient ratio (PER)} = \text{weight gain} / \text{protein intake}.$$

$$\text{Protein productive value (PPV)} = \text{protein gain} / \text{protein intake}.$$

Histopathological studies

Specimens from the gonads of the fish (two fish from each replicate) after days 28 and at the end of the experiment were collected and rapidly fixed in 10% neutral buffered formalin. The fixed specimens were dehydrated using ascending concentrations of ethanol, cleared in xylene, and embedded in paraffin wax at 60°C. Five-micron thick paraffin sections were prepared. These sections were stained using hematoxylin and eosin, following the method outlined by **Bancroft and Gamble (2013)**.

Analytical statistics

The analysis of variance (ANOVA) was employed to examine the presence of statistically significant variations ($P < 0.05$) among the means of the different treatments in relation to growth, feed utilization, and histopathological studies. The statistical analyses were performed using (IBM-SPSS v. 28) software.

RESULTS

1. Growth performance and feed utilization

Table (1) displays the values for survival percentage (SUR%), final body weight (FBW), and specific growth rate (SGR). The T1 group exhibited the lowest rate of survival, regardless of whether the hormonal treatment was administered or not. Conversely, the findings suggest that the administration of hormonal treatment in isolation yielded the best rate of survival ($P < 0.05$). The experimental group denoted as T3 had the most substantial increase in ultimate body weight when subjected to hormone intervention. The group with the lowest weight, and the group treated with the hormone had a considerably higher specific growth rate compared to the other groups ($P < 0.05$).

Table (2) shows the values for feed conversion ratio (FCR), protein productive value (PPV%), and protein efficiency ratio (PER). The data indicate that group C2 had the greatest levels of FCR, whereas group T2 demonstrated the lowest values. The impact of varying hormone concentrations and weights on protein retention/ feed (PPV) and protein gain/ feed (PER) was shown to be statistically significant ($P < 0.05$). The data revealed that group C2 had the highest positive predictive value (PPV), while groups T2 and T3 demonstrated the lowest PPV values, respectively. The study revealed that group T2 exhibited the highest values of PER, whereas group C2 displayed the lowest values.

Table 1. Survival % (SUR%), final body weight (FBW), and specific growth rate (SGR) for different weights of the Nile tilapia (*Oreochromis niloticus*) treated with 17 α -Methyltestosterone hormone for 90 days

HER	Weight	Sur %	FBW	SGR
Interactions				
NH	C1	80 \pm 0.0 ^b	32.40 \pm 0.40 ^d	1.59 \pm 0.01 ^b
	C2	90 \pm 3.3 ^{ab}	43.0 \pm 1.0 ^{cd}	0.96 \pm 0.02 ^d
	C3	93.3 \pm 0.0 ^a	71.75 \pm 2.75 ^b	1.12 \pm 0.04 ^d
H	T1	83.3 \pm 3.3 ^b	46.50 \pm 3.50 ^c	1.99 \pm 0.08 ^a
	T2	90 \pm 3.3 ^{ab}	58.05 \pm 5.45 ^c	1.29 \pm 0.10 ^{cd}
	T3	96.6 \pm 3.3 ^a	89.0 \pm 6.0 ^a	1.36 \pm 0.07 ^{bc}

Hormone level			
NH	80.0±2.21 ^a	49.05±2.19 ^b	1.23±.038 ^b
H	82.21±2.21 ^a	64.51±2.19 ^a	1.55±.038 ^a
Weight			
T1	71.67±2.71 ^c	39.45±2.68 ^c	1.79±.047 ^a
T2	81.65±2.71 ^b	50.52±2.68 ^b	1.13±.047 ^b
T3	90.0±2.71 ^a	80.37±2.68 ^a	1.24±.047 ^b

Significant differences ($P < 0.05$) exist between means that are in the same column but do not share the same superscript.

Table 2. Feed conversion ratio (FCR), protein productive value (PPV%), and protein efficiency ratio (PER) for different weights of the Nile tilapia (*Oreochromis niloticus*) treated with 17 α -Methyltestosterone hormone for 90 days

HER	Weight	FCR	PPV	PER
Interaction				
NH	C1	2.21±0.09 ^{ab}	95.20±4.27 ^{ab}	1.50± 0.06 ^{abc}
	C2	2.40±0.03 ^a	111.07±1.96 ^a	1.38±0.02 ^c
	C3	2.27±0.19 ^{ab}	94.05±7.42 ^{ab}	1.47±0.12 ^{bc}
H	T1	1.88±0.09 ^{bc}	93.92±4.70 ^{ab}	1.77±0.09 ^{ab}
	T2	1.80±0.15 ^c	81.95±6.69 ^b	1.85±0.16 ^a
	T3	1.91±0.11 ^{bc}	90.51±5.33 ^b	1.74±0.10 ^{abc}
Hormone level				
NH		2.29±.07 ^a	100.11±3.09 ^a	1.45±.06 ^b
H		1.87±.07 ^b	88.79±3.09 ^b	1.79±.06 ^a
Weight				
	T1	2.05±.09 ^a	94.56±3.79 ^a	1.64±.07 ^a
	T2	2.10±.09 ^a	96.51±3.79 ^a	1.62±.07 ^a
	T3	2.09±.09 ^a	92.28±3.79 ^a	1.61±.07 ^a

Means in the same column having the same letter are not significantly different ($P < 0.05$).

2. Ovarian and testicular histology

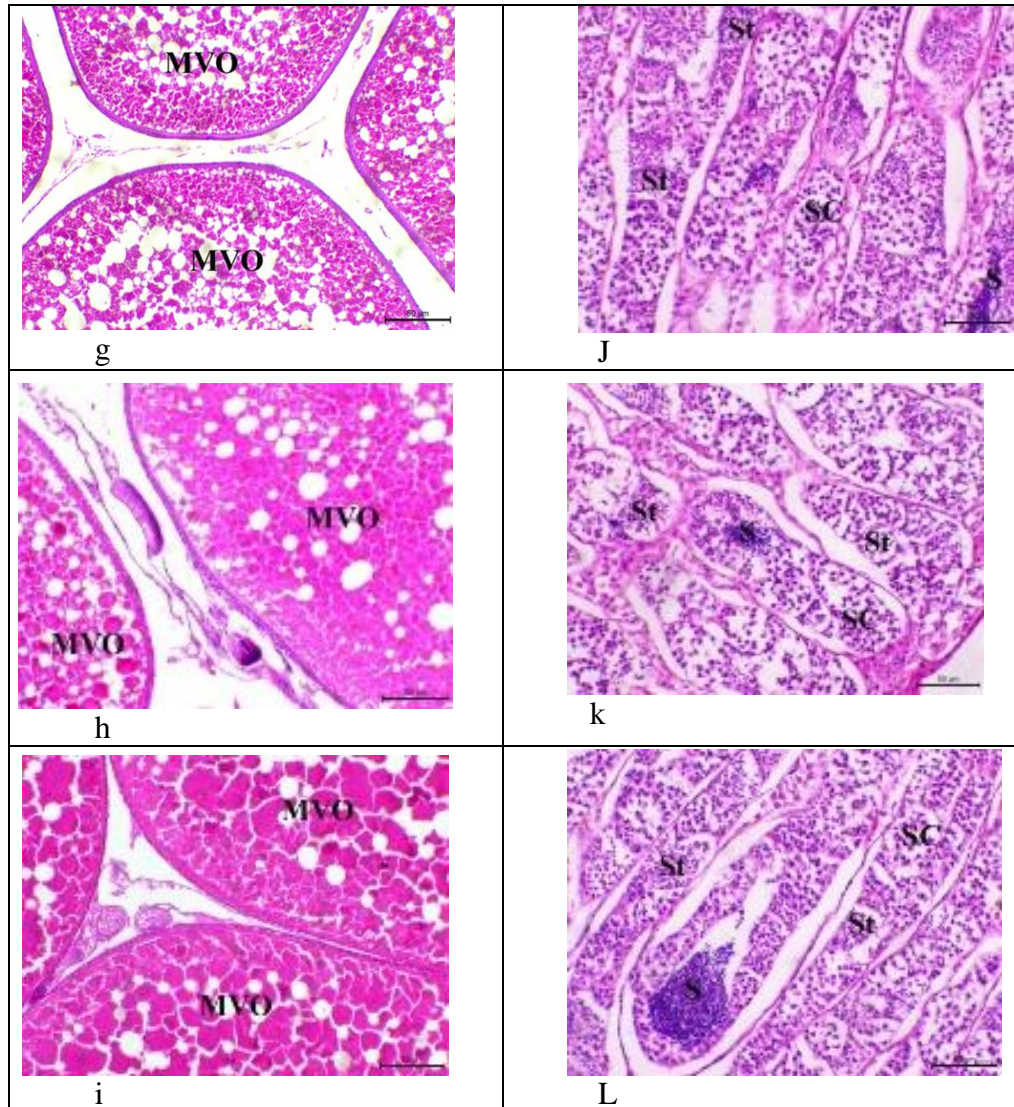


Fig. 1. Sections of the ovary and testis in *Oreochromis niloticus* control groups showing: g- The ovary of *Oreochromis niloticus* fed C1 showing stage IV of the vitellogenesis process associated with the presence of a large number of mature vitellogenic oocytes (MVO), j- The testis of fish fed C1 showing a normal spermatogenesis process (S indicates spermatozoa and St indicates spermatids), h- The ovary of fish fed on C2 showing the presence of a large number of mature vitellogenic oocytes (MVO), i- The ovary of an *Oreochromis niloticus* fed on C3 showing stage IV vitellogenesis, with a lot of mature vitellogenic oocytes (MVO) filled with a lot of yolk globules, k and l- The testis of fish fed on C2 and C3 showing normal spermatogenic cells (SC) in the testis with a normal spermatogenesis process (S indicates spermatozoa and St indicates spermatids)

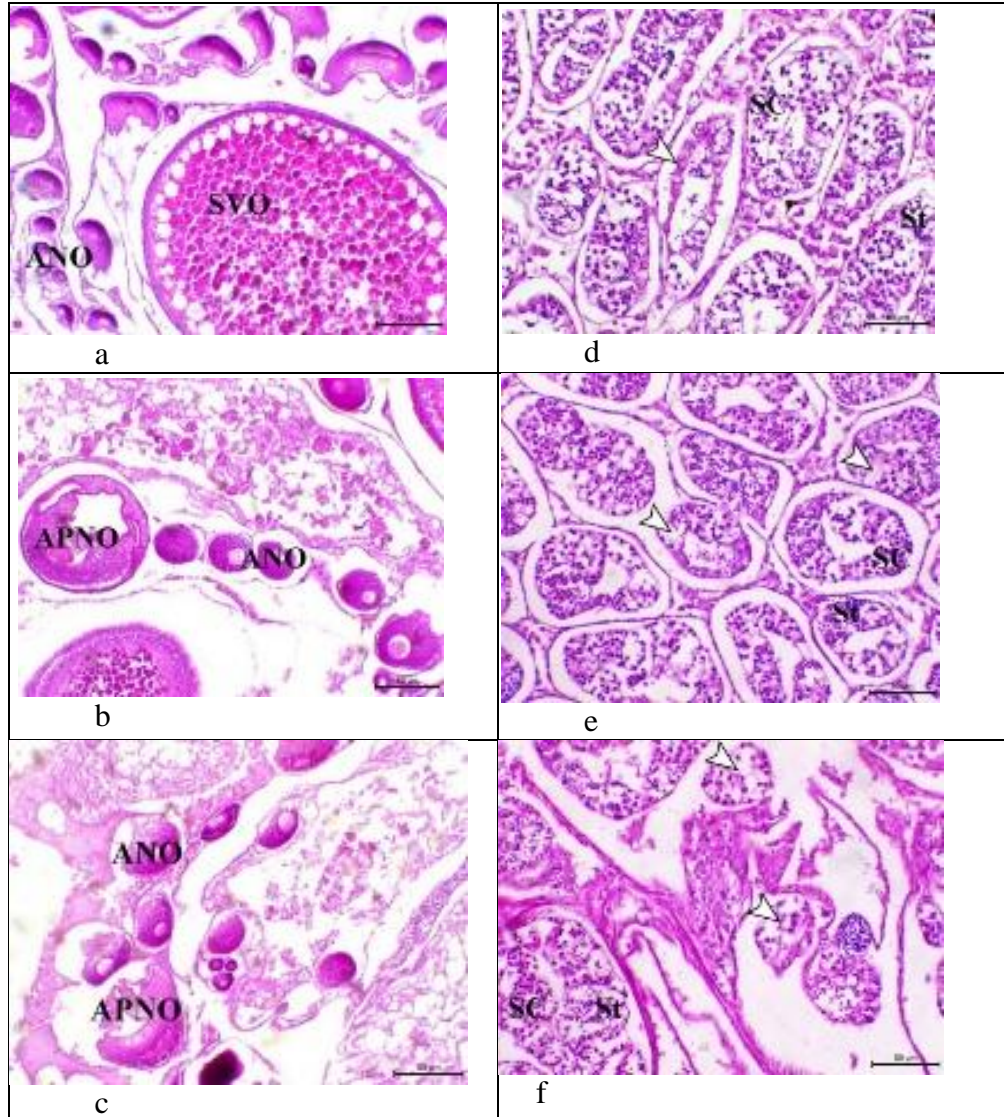


Fig. 2. Sections of the ovary and testis in *Oreochromis niloticus* fed on 17 α -methyl-testosterone hormones a, b and c- The ovary of the fish fed with (T1, T2, and T3) showing a marked degree of an ovarian atrophy associated with a severe atresia of different oocytes (CN indicates chromatin nuclear oocytes) and (SVO indicates secondary growing vitellogenic oocytes), d, e and f- The testis of fish fed on T1, T2, and T3 showing a mild degree of testicular degeneration associated with degenerative changes within the spermatogenic cells (arrowhead) (St indicates spermatids).

The testicles and ovaries of *Oreochromis niloticus* were examined from the gonadal tissue through a histological investigation conducted twice, once after 28 days and then at the end of the experiment. After 28 days, significant morphological occurrences are depicted in Figs. (1, 2). *Oreochromis niloticus* fed on C1 and C3 exhibited stage IV of the vitellogenesis process associated with the presence of a large number of mature vitellogenic oocytes (MVO) in the ovary and testis, showing a normal spermatogenesis process.

The ovary of the fish fed on C2 exhibited a presence of a large number of mature vitellogenic oocytes (MVO). The testis of fish fed C2 and C3 demonstrated normal spermatogenic cells (SC) in the testis with a normal spermatogenesis process.

The ovary of the fish fed on 17 α -methyl-testosterone hormones showed a marked degree of ovarian atrophy associated with severe atresia of different oocytes. Additionally, the testis of the fish expressed a mild degree of a testicular degeneration associated with degenerative changes within the spermatogenic cells.

DISCUSSION

The quantity of hormone that is actually taken by each person is an important component in determining whether or not sex reversal therapy will be successful. In the present study, varying weights of the Nile tilapia were offered commercial diets combined with different volumes of a 17 alpha-methyl testosterone solution for 28 days. Subsequently, the fish were fed solely on commercial diets for two more months in order to track the effects of the hormone.

In this study, the potential for different volumes of 17 α -MT (6– 12– 18ml of 60mg of 17 α -MT/ kg feed) and different weights (7.7– 18– 26g) was examined and compared to the control group. Survival rate, final body weight, and specific growth rate of tilapia treated with hormone were significantly greater than those of the control group ($P < 0.05$). The anabolic action of 17 α -MT is responsible for the greater weight values of the fish treated with 6– 18ml of 60mg of 17 α -MT per kg of feed for the 28 days of the treatment compared to the control group (Jo *et al.*, 1995). In this regard, studies by Mair *et al.* (1995), Dan and Little (2000) and Little *et al.* (2003) have indicated that the 17 α -MT treatment enhances the individual growth of tilapia. In another research by Chakraborty and Banerjee (2010), it was reported that, the higher mean weights of sex-reversed *Oreochromis niloticus* fry were attributed to their improved food conversion efficiency. Furthermore, studies by Chakraborty *et al.* (2011), Githukia *et al.* (2015) and Gómez-Márquez *et al.* (2015) have demonstrated the greater development of male monosex Nile tilapia. Monosex male tilapia grow more quickly when they don't have to expend as much energy on courtship, nest preparation, and territorial defense during reproductive activities (Tran-Duy *et al.*, 2008; Mukti *et al.*, 2020). The average survival rate rises with the age of the larvae. Some investigations; however, found that 17 α -methyltestosterone therapy had no effect on tilapia survival (Vera Cruz & Mair, 1994; Chakraborty *et al.*, 2011).

A higher FCR in the male monosex group suggests that they were more effective at using the given feed by nutrient-extracting from the meal and turning it into meat. However, the FCR values of both groups were within the 1.4 to 2.5 range that was recommended for tilapia cage aquaculture systems in Africa (Alhassan *et al.*, 2018).

However, Komen *et al.* (1989) reported that eating common carp fry with 17 α -MT in diets at a rate of 50 and 100mg/ L caused severe deformities in the fish, as well as high

rates of sterile fish. **Amer *et al.* (2021)** in their study treated the red tilapia fry with the hormone 17 α -MT, which led to the occurrence of degenerations in eggs. Additionally, relapses in the ovary and testicles were noted by **Hirose and Hibiya (1968a, b)** when the hormone was added to the goldfish and rainbow trout diet at a dose of 2.5mg/ kg.

The ovary of fish fed on control diets showed the presence of a large number of mature vitellogenic oocytes (MVO). The testis of the fish fed C2 and C3 showed normal spermatogenic cells (SC) in the testis with a normal spermatogenesis process.

After 28 days, the fish treated with the 17 α -methyl-testosterone hormone didn't spawn, despite being treated with the hormone after the stage of sexual differentiation. The ovary of fish fed on 17 α -methyl-testosterone hormones showed a marked degree of ovarian atrophy associated with severe atresia of different oocytes. Furthermore, the testis of fish showed a mild degree of testicular degeneration associated with degenerative changes within the spermatogenic cells.

Treatment with 17 α -MT should commence on the second or third day following the discharge of fry from their mother's care. Other studies, however, recommend beginning the treatment on the seventh day after hatching and continuing through the 30th day (**Nakamura & Iwahashi, 1982**). On the other hand, other studies recommend that the production of monosex tilapia with 17-methyltestosterone (MT) is well-established and that various concentrations of MT hormone could be incorporated into a starter feed (**Poppa & Green, 1990**).

CONCLUSION

At the end of the study, the results showed that treating tilapia fish with 17 α -methyl-testosterone hormone after the end of the sex determination stage and at different ages leads to a noticeable degree of ovarian atrophy associated with severe atresia of the various eggs. Furthermore, the fish testes showed a mild degree of testicular degeneration associated with degenerative changes within the spermatogenic cells. However, it turns out that this is a non-permanent change, hence a hormonal treatment at these ages could be a means of controlling the ovulation process in tilapia fish.

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