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Effect of 17α-Methyltestosterone Hormone on Certain Biochemical Parameters of Fish, *Tilapia Mossambica*

M. Nagaraju[1] and G. Sunitha Devi[2]
Department of Zoology, University College of Science, Osmania University,
Hyderabad-500 007, Telangana State, India.

ABSTRACT

Aquaculture is one of the main food production sectors to deal with the high demand for food due to the human population explosion. The demand for fish has increased in recent years due to population growth and the constant search for a healthy diet. The present study was conducted to assess an anabolic impact of an androgenic hormone, 17α-Methyltestosterone (MT) on RNA and DNA levels in certain tissues *i.e.* skeletal muscle, liver and gonad of fish, *Tilapia mossambica*. The three different dosages of 17α-MT hormone such as 2 µg, 5 µg, and 10 µg/10 g body weight (b.w) of fish, were dissolved in 0.1 ml alcohol and administrated via, injection mode for 24 hours, 48 hours, 7 and 14 days. The highest increment in RNA and DNA levels was observed under 10 µg/10 g body weight (b.w) followed by 5 µg/10 g body weight (b.w) and 2 µg/10 g body weight (b.w) in skeletal muscle, liver and gonad of fish. The more increase in RNA levels was observed after 14 days of the 17α-MT injection route of administration. Hence, it is clear that the anabolic hormones such as 17α-MT hormone play an important role in enhancing the RNA and their by tissue protein content for nutritional purposes. There was not much change in DNA levels.

Keywords: 17α-Methyltestosterone; RNA; DNA and *Tilapia mossambica*.

INTRODUCTION

Aquaculture, probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish[5]. One of the major sources of animal protein for human consumption is fisheries source. Therefore, considerable attention has been given to the production and growth of freshwater fish in aquaculture[9]. Aquaculture plays a leading role in the fight against food insecurity, malnutrition, and poverty globally [15,18]. Primary fish culture on a large scale for a commercial purpose is to obtain faster tissue growth on a low budget. The fact that anabolic promoting agents had been using it in husbandry to enhance growth rate at least cost production promoted the investigations into the use of sex hormones and their synthetic analogues in fish[12].

17α-Methyltestosterone is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and therefore the development of male sexual characters. The growth enhancement was studied in *Tilapiamossambica* due to anabolic effects among 17α-Methyltestosterone treated and control fish[14]. Growth is largely protein accretion and RNA levels may be used as a measure of growth rate[6]. However, the ratio of RNA to DNA is generally considered a more accurate index of growth (protein synthesis) than RNA alone since this ratio is not affected by the size of the tissue sampled for analysis[7]. Based on many reports, different supplementations can be added to feed in order to stimulate the growth parameters of fishes[1,10]. A number of anabolic steroids both androgenic and estrogenic increase growth and food conversion efficiency when administered via food[8,13].

The present study was undertaken in order to know about the growth-promoting efficiency of the steroid hormone, 17α-MT on *Tilapia mossambica*, and its impact on some biochemical Parameters as well.

MATERIAL AND METHODS

The fish, *Tilapia mossambica*used in the present study were procured from State fisheries culture tanks acclimatized to laboratory conditions for 10 days. Fish of uniform size of about 22±1 g were selected for each set of experiments. Fish were divided into four groups and kept in circular plastic troughs having a capacity of 20 liters. The fish were fed with commercial feed once a day at a rate of 2% of body weight both before and during the experimental period. The temperature was maintained at 27 ± 1°C and water in the containers renewed every 24 h.

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Preparation of 17α-MT Injection Dosages

In Present Investigation, hormone powder (Sigma Chemical) was used to make essential injection dosages of 17α-MT. The fish were maintained separately in circular plastic troughs, the group I (control) 10 fishes received only 0.1 ml ethyl alcohol/10 g. b.w of fish,whereas the other three groups of fish received 0.1 ml of ethyl alcohol containing different dosages of 17α-MT injected intraperitoneal to the fish. After injecting the hormone regularly for a period of 24 hours, 48 hours, 7 days and 14 days. Then, the fish were sacrificed by decapitation and the tissues muscle, liver, and gonad dissected out and used for bio-chemical analysis (RNAand DNA).

Injection mode: The experiments were conducted in triplicate with one control and three treatments.
Group - I Control - 0 $\mu g$of MT/ 10 g body weight (b.w) of fish.
Group - II T1 - 2 $\mu g$of MT/ 10 g b.w of fish.
Group - III T2 - 5 $\mu g$of MT/ 10 g b.w. of fish.
Group - IV T3 - 10 μg *of* MT/ 10 g b.w. of fish.
Injection experiments were conducted for a short-term period (24 hours, 48 hours) and a long-term period (7 days, 14 days).

Tissue Biochemical Analysis

The biochemical parameters such as RNA and DNA were estimated in Muscle, Liver, and Gonads of the fish, *Tilapia mossambica*. These biochemical parameters were estimated by using the following methods.

- ✓ **Nucleic acids** (RNA & DNA) were extracted by the method[16].
- ✓ **RNA** (Ribose Nucleic acid) was estimated by - Orcinol method.
- ✓ **DNA** (Deoxyribose Nucleic acid) was estimated by - Diphenylamine method.

Statistical Analysis

All the data were presented as Mean±SE. For each experimental Treatment, three replicates of 10 individuals were taken. For eachreplicate, under each Treatment Mean was calculated. The three Mean values thus obtained were used to calculate experimental treatment Mean and Standard Errors of Mean. The student's 't'-test was applied between control values and each of the experimental treatment values. p values were determined using the t-statistics and degrees of freedom (df) and denoted as NS- Not Significant; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$. The experimental data were analysed statistically by adopting valid statistical methods[23](Pillai and Sinha, 1968), the standard deviation and probability test i.e. 't' test were calculated. The Student's t-test was carried out to know the levels of significance.

RESULTS AND DISCUSSION

In the present study, the impact of Anabolic androgenic steroid, 17α-Methyltestosterone (Injection mode) on tissue RNA and DNA of fish, *Tilapia mossambica,* was studied. The different dosages of 17α-Methyltestosterone (2 $\mu g$of MT/10 g, 5 $\mu g$of MT/10 g, and 10 $\mu g$of

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MT/10 g b.w) were given to fish and studied the bio-chemical analysis after 24 hours, 48 hours, 7 days, and 14 days of exposure.

RNA levels in different tissues

The Nucleic Acids play a decisive role in regulating cellular activities so that they serve as important biomarkers of the metabolic potential of cells in the organism[3]. Ribonucleic acid or RNA is a nucleic acid polymer consisting of nucleotide monomers that play several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products; RNA acts as a messenger between DNA and therefore the protein synthesis complexes referred to as ribosomes form vital portions of ribosomes, and acts as an important carrier molecule for amino acids to be utilized in protein synthesis. RNA is a polymer with a ribose and phosphate backbone and four different bases: adenine, guanine, cytosine, and uracil[20].

In the present investigation, the Nucleic acid levels (RNA) in different tissues like Muscle, Liver, and Gonad were observed under different dosages of 17α-MT. There was a significant increase in RNA levels in all tissues at all durations and in all different concentrations were observed. The order of increase in different tissues when exposed to different concentrations was observed as Muscle (58.70%, $p < 0.001$) > Gonad (56.89%, $p < 0.001$) > Liver (38.93%, $p < 0.001$) of fish compared to control. An increase of RNA amount was more at a higher concentration of 17α-MT (10µg MT/10 g b.w) and a higher duration (14 days). Similar results were observed that the RNA arising in larger quantity is instrumental in turning out a greater quantity of protein[17].

A raise in RNA content in the tissues of *Tilapia mossambica* would reflect the induced synthesis of nucleic acids. This clearly evidences that the protein synthesis machinery of the fishes has been adversely affected. The same result showed that increased growth is represented by an increase in protein and with constant DNA and increased RNA[4].

Table 1.RNAlevels in fish Muscle after injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	2.770	2.850	2.990	3.100
	SE	± 0.144	± 0.169	± 0.146	± 0.171
2 µg / 10g.b.w	Mean	2.980 NS	3.120 NS	3.420 NS	3.780 *
	SE	± 0.164	± 0.153	± 0.142	± 0.160
	%V	7.81	9.48	14.54	21.66
5 µg / 10g.b.w	Mean	3.210 *	3.460 *	3.830 *	4.240 **
	SE	± 0.178	± 0.174	± 0.121	± 0.183
	%V	16.03	21.42	28.10	36.69
10 µg / 10g.b.w	Mean	3.350 *	3.610 *	4.110 **	4.930 ***
	SE	± 0.149	± 0.122	± 0.175	± 0.224

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	%V	20.85	26.68	37.58	58.70

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of RNA/gram wet weight of tissue.
SE - Standard Error, %V- Percent variation, NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Figure 1. RNA levels in fishMuscle after injection of different dosages of 17α-Methyltestosterone(Mean ± SE).

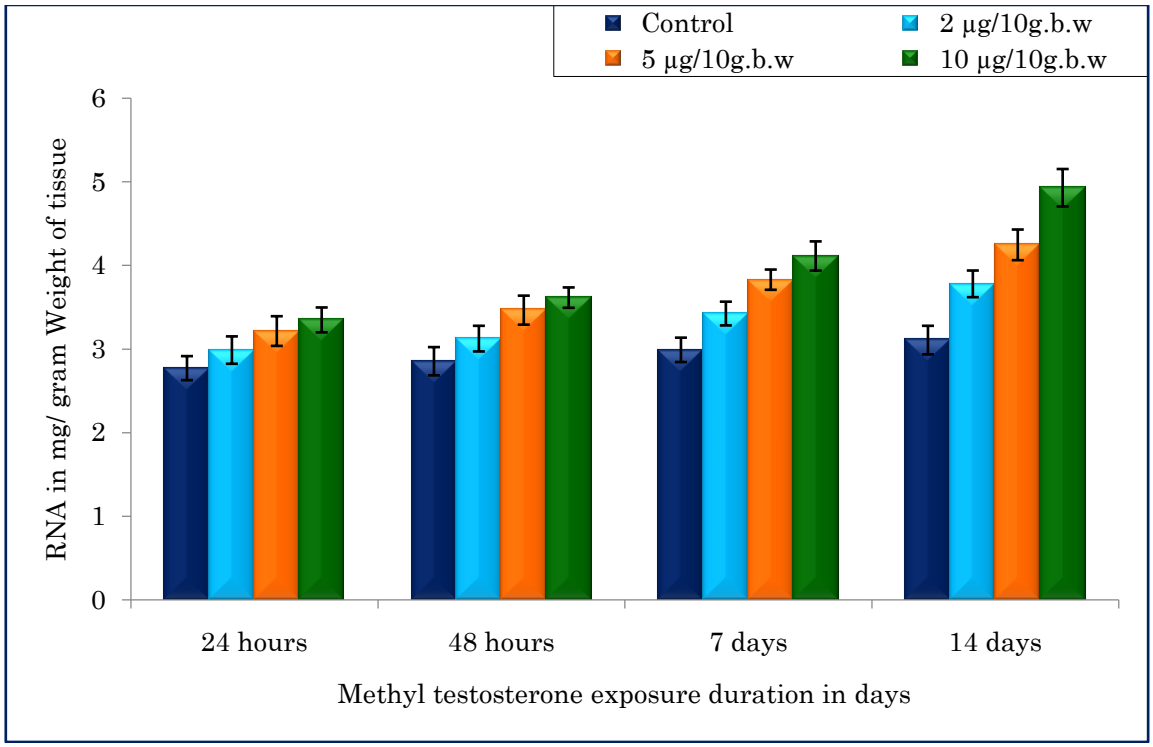

Table 2.RNAlevels in fish Liver after injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	3.990	4.000	4.110	4.280
	SE	± 0.170	± 0.120	± 0.135	± 0.151
2 µg / 10g.b.w	Mean	4.110 NS	4.190 NS	4.420 NS	4.960 *
	SE	± 0.132	± 0.142	± 0.171	± 0.165
	%V	2.84	4.78	7.78	15.76
5 µg / 10g.b.w	Mean	4.220 NS	4.330 NS	4.710 *	5.430 **
	SE	± 0.162	± 0.155	± 0.158	± 0.184

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	%V	5.73	8.32	14.89	26.69
10 µg / 10g.b.w	Mean	4.410 NS	4.560 *	4.980 **	5.910 ***
	SE	± 0.187	± 0.170	± 0.178	± 0.166
	%V	10.46	14.03	21.47	38.93

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of RNA/gram wet weight of tissue.
SE - Standard Error, %V- Percent variation, NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Figure 2. RNA levels in fish Liver after injection of different dosages of 17α-Methyltestosterone(Mean ± SE).

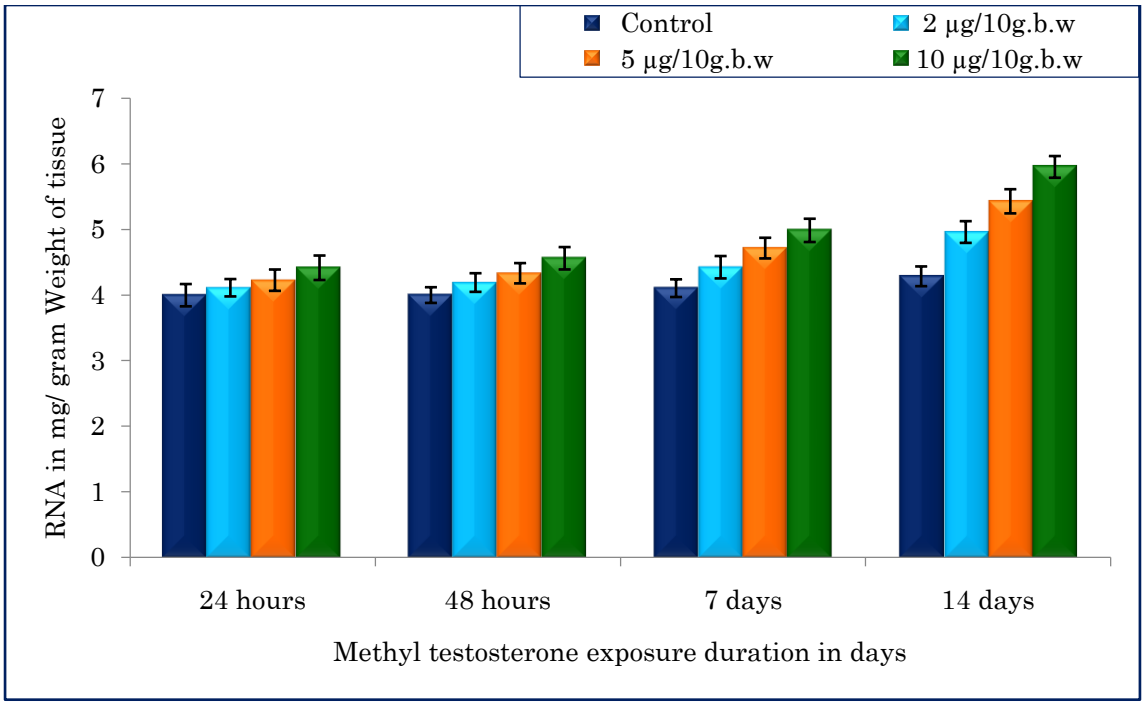

Table 3.RNAlevels in fish Gonadafter injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	1.800	1.820	1.950	2.020
	SE	± 0.088	± 0.098	± 0.067	± 0.091
2 µg / 10g.b.w	Mean	1.930 NS	1.990 NS	2.220 *	2.470 **
	SE	± 0.099	± 0.077	± 0.100	± 0.082

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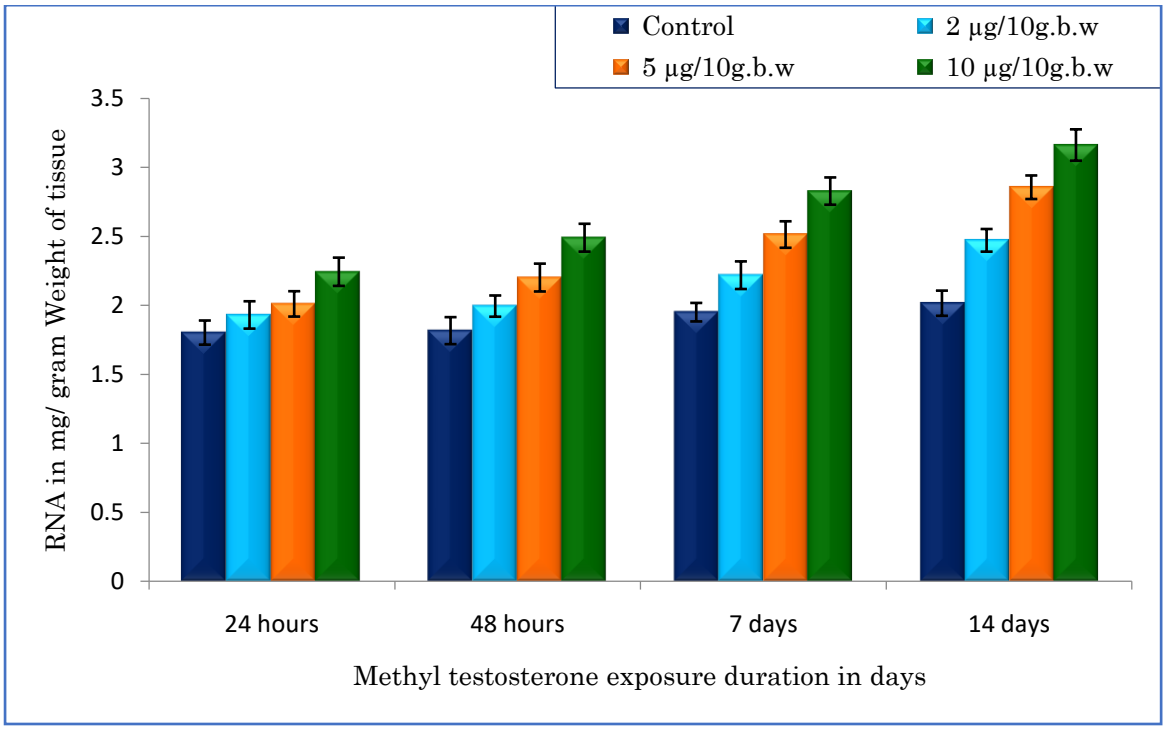
	%V	7.10	9.80	13.74	22.62
5 µg / 10g.b.w	Mean	2.010 NS	2.200 *	2.510 **	2.860 ***
	SE	± 0.092	± 0.101	± 0.096	± 0.085
	%V	11.54	21.19	28.86	41.72
10 µg / 10g.b.w	Mean	2.240 *	2.490 **	2.830 ***	3.160 ***
	SE	± 0.102	± 0.101	± 0.099	± 0.114
	%V	24.46	37.09	45.00	56.89

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of RNA/gram wet weight of tissue.
SE - Standard Error, %V- Percent variation, NS: Not Significant, * P < 0.05, ** P < 0.01, *** P < 0.001

Figure 3. RNA levels in fish Gonad after injection of different dosages of 17α-Methyltestosterone(Mean ± SE).



DNA levels in different tissues

Deoxyribonucleic acid (DNA) may be a molecule that carries the genetic information utilized in the expansion, development, functioning, and reproduction of all living organisms. The two DNA strands are called polynucleotides since they are composed of simpler monomer units called nucleotides[2,11].

The present study revealed that DNA levels in different tissues like Muscle, Liver, and Gonads were not changed much under differentdosages of 17α-MT but at higher

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concentrations (10µg MT/10 g b.w) and higher duration (14 days), there was a less increase in DNA content. The quantitative changes in DNA and RNA levels in *Cyprinuscarpio* would be due to increased activity of DNA and the inhibition of RNA polymerase function[3].

Table 4.DNAlevels in fish Muscle after injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	0.750	0.778	0.767	0.775
	SE	± 0.018	± 0.013	± 0.019	± 0.015
2 µg/10 g.b.w	Mean	0.769 NS	0.763 NS	0.735 NS	0.724 NS
	SE	± 0.012	± 0.019	± 0.017	± 0.016
5 µg/10 g.b.w	Mean	0.771 NS	0.786 NS	0.745 NS	0.751 NS
	SE	± 0.016	± 0.023	± 0.01	± 0.036
10 µg/10 g.b.w	Mean	0.782 NS	0.798 NS	0.778 NS	0.801 *
	SE	± 0.013	± 0.023	± 0.015	± 0.026

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of DNA/gram wet weight of tissue.
SE - Standard Error, NS: Not Significant, * $P < 0.05$

Table 5.DNAlevels in fish Liver after injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	1.149	1.117	1.153	1.125
	SE	± 0.024	± 0.027	± 0.039	± 0.042
2 µg/10 g.b.w	Mean	1.152 NS	1.135 NS	1.115 NS	1.159 NS
	SE	± 0.037	± 0.029	± 0.041	± 0.038
5 µg/10 g.b.w	Mean	1.163 NS	1.170 NS	1.147 NS	1.185 NS
	SE	± 0.032	± 0.022	± 0.022	± 0.035
10 µg/10	Mean	1.165 NS	1.147 NS	1.121 NS	1.174 NS

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g.b.w	SE	± 0.019	± 0.016	± 0.024	± 0.020

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of DNA/ gram wet weight of tissue.
SE - Standard Error, NS: Not Significant.

Table6.DNAlevels in fish Gonadafter injection of different dosages of 17α-Methyltestosteronecompared to control.

17α-MT		Days of exposure			
		24 hours	48 hours	7 days	14 days
Control (0 µg / 10g.b.w)	Mean	1.211	1.212	1.228	1.231
	SE	± 0.015	± 0.025	± 0.028	± 0.020
2 µg/10 g.b.w	Mean	1.223 NS	1.235 NS	1.245 NS	1.249 NS
	SE	± 0.022	± 0.034	± 0.026	± 0.032
5 µg/10 g.b.w	Mean	1.236 NS	1.227 NS	1.254 NS	1.255 NS
	SE	± 0.021	± 0.018	± 0.017	± 0.026
10 µg/10 g.b.w	Mean	1.244 NS	1.241 NS	1.250 NS	1.262 NS
	SE	± 0.014	± 0.023	± 0.031	± 0.019

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of DNA/ gram wet weight of tissue.
SE - Standard Error, NS: Not Significant.

CONCLUSION

Based on the results obtained from the present investigation, it was concluded that 17α-MT elevated RNA levels in different tissues of *Tilapia mossambica*. Hence, it is clear that the anabolic hormones such as 17α-MT hormone play an important role in enhancing the tissue protein content in fish for the nutritional purpose of Man.

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