## ANABOLIC EFFECT OF 17α- METHYLTESTOSTERONE HORMONE ON CERTAIN BIOCHEMICAL PARAMETERS OF FISH, *TILAPIA MOSSAMBICA*

M. Nagaraju and G. Sunitha Devi

Department of Zoology, University College of Science, Osmania University, Hyderabad-500 007, Telangana State, India.

**ABSTRACT:** One of the major sources of animal protein for human consumption is a fisheries resource. The demand for fishhas 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,  $17\alpha$ -Methyltestosterone (MT) on Protein contentand RNA levels in certain tissues *i.e.* skeletal muscle, liver and gonad of fish, *Tilapia mossambica*. The hormone was incorporated in the feed and fed to the fish up to four weeks, in the form of three pelleted diets containing 4, 8 and 16 mg MT/ kg diet along with a fourth control group without hormone. The highest increment in tissue Protein content, RNA and DNA levels was observed under 16 mg MT/ kg diet followed by 8 mg MT/ kg diet and 4 mg MT/ kg diet in skeletal muscle, liver and gonad of fish. The more increase in Protein content, RNA and DNA levels was observed after 28 days of the  $17\alpha$ -MT oral route of administration. Hence, it is clear that the anabolic hormones such as  $17\alpha$ -MT hormone play an important role in enhancing the tissue protein content for nutritional purposes.There was not much change in DNA levels.

KEYWORDS: 17α-Methyltestosterone; Total proteins; RNA; DNA and Tilapia mossambica.

#### **INTRODUCTION**

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<sup>15</sup>. Aquaculture is one of the main food production sectors to deal with the high demand for food due to the human population explosion. Aquaculture, probably the fastest growing food-producing sector, now accounts for nearly 50 percent of the world's food fish<sup>10</sup>. Aquaculture plays a leading role in the fight against food insecurity, malnutrition, and poverty globally<sup>24,27</sup>. 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<sup>21</sup>. The literature on the hormonal enhancement of growth in fish has been reviewed by many authors<sup>9,30</sup>. Steroid hormones used as growth promoters in fish where they enhanced the weight gain of fish and enhanced the rate of muscle protein accretion<sup>20,29</sup>.

 $17\alpha$ -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 17a-Methyltestosterone treated and control fish<sup>23</sup>. Growth is largely protein accretion and RNA levels may be used as a measure of growth rate<sup>11</sup>. 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<sup>13</sup>. Based on many reports, different supplementations can be added to feed in order to stimulate the growth parameters of fishes<sup>2,17</sup>. A number of anabolic steroids both androgenic and estrogenic increase growth and food conversion efficiency when administered via food<sup>14,22</sup>.

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

### MATERIALS 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  $12\pm1$  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 litters. 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  $27 \pm 1^{\circ}$ C and water in the containers renewed every 24 h.

# Preparation of control and steroid containing diets

A control diet was prepared by mixing 30% of fish meal, 30% of soya bean meal, 18% of wheat bran, 13% of yellow corn, 6% of corn oil, 2% of vitamins and minerals premix, and 1% of carboxymethyl cellulose. In addition to the control diet, the other three experimental diets were prepared with the addition of 4, 8, and 16 mg of  $17\alpha$ -MT/kg of diet. The diets were prepared by spraying the hormone dissolved in 50 ml of 95% ethyl alcohol and mixed well. Glycerine was added at 0.5%/kg by volume to render the harmful effect of the alcohol. The mixture of diet has been completely dried at room temperature and then sealed in an airtight black container and stored in the refrigerator until use to avoid bacterial or fungal contamination. The diets containing  $17\alpha$  -MT were characterized as follows:

**Oral mode:** The experiments were conducted in triplicate with one control and three treatments.

Diet (1): Control diet (without  $17\alpha$  - MT) Diet (2): 4 mg of  $17\alpha$  - MT/kg of control diet.

Diet (3): 8 mg of 17 $\alpha$  - MT/kg of control diet.

Diet (4): 16 mg of  $17\alpha$  - MT/kg of control diet.

### **Tissue Biochemical Analysis**

The biochemical parameters such as Protein, 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.

- ✓ Estimation of **Total Protein** levels by the method<sup>6</sup>.
- ✓ Nucleic acids (RNA & DNA) were extracted by the method<sup>25</sup>.
- **RNA**(Ribose Nucleic acid) was estimated by - Orcinol method.
- ✓ DNA(Deoxyribose Nucleic acid) was estimated by -Diphenylamine method.

**Statistical Data:**Analysed by using Student's t-test.

### **RESULTS AND DISCUSSION**

The biochemical contents such as total proteins, RNA, and DNA levels were estimated in response to the different dosages of  $17\alpha$ -Methyltestosterone for 7 days, 14 days, 21 days, and 28 days.

**Proteins** play an important role in the metabolism and regulation of water balance<sup>12</sup>. It is the basic building nutrient of any growing animal and also used as an indicator of their state of health<sup>18</sup>. Protein

plays a central role in cell function and cell structure and these are high molecular weight polypeptides. They are not onlyresponsible for comprising the structure of cells but areinvolved in all the aspects of cell physiology such ascatalysis, tissue repair, building, defense reactions, etc., through hormones, they regulate the metabolic aspects of the animal<sup>31</sup>. In the present study, the effect of Anabolicandrogenic steroid Methyl Testosterone (MT) to on Total Proteins, RNA (Ribose Nucleic acid), DNA (Deoxyribose Nucleic acid) in Skeletal Muscle, Liver and gonad of fish, Tilapia mossambica were studied up to four weeks (1,2,3 & 4 weeks). The variations of Total Proteins, RNA, and DNA in different tissues were given in Tables (1 to 9) and Figures (1 to 6) in terms of SEM (Mean± SE) along with control. The results were statistically analyzed using Student's t-test, all data were presented as Mean ± SE. P values were determined using the t statistics and denoted as NS- Not Significant, \* p <0.05; \*\* p <0.01; \*\*\* p < 0.001.

**Protein levels in different tissues:** At the end of experimental durations, Protein levels in different tissues were significantly increased. The increase of protein levels was more at a higher dosage of  $17\alpha$ -Methyltestosterone ( $17\alpha$ -MT) hormone (16mg/kg) and a higher duration (28 days). The order of increase was observed at higher dosage (16mg/kg) and longer duration (28 days) as Muscle (80.38%) (P < 0.001) > Liver (60.18%) (P < 0.001) Gonad (55.01%) (P < 0.001) of fish.

- Protein activity in fish was significantly increased with high Methyltestosterone doses of 20 and 40 mg/kg<sup>1</sup>.
- \* Several studies are in agreement that

testosterone produces muscle increasing muscle protein synthesis<sup>5</sup>. The change in protein levels may be due to the fact that androgens regulate protein synthesis by binding to cytosolic or nuclear receptors for steroids that than modulates transcription<sup>28</sup>.

- Androgens promote male sex characteristics development as well as anabolic activity that is expressed as an increase in body weight due to increased protein biosynthesis<sup>8,16</sup>.
- The present study revealed that improvement in the quality of flesh in terms of higher protein after the fed of 17α-Methyltestosterone up to 28 days.

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	91.260	91.500	91.990	92.430
Control	SE	± 2.252	$\pm 2.003$	$\pm 1.678$	± 1.957
	Mean	94.540 <sup>NS</sup>	98140 *	104.610 **	112.580 ***
4 mg/kg	SE	$\pm 1.887$	± 2.041	$\pm 2.188$	$\pm 1.821$
	%V	3.60	7.25	13.72	21.79
	Mean	100.300 *	109.440 **	118.600 ***	139.780 ***
8 mg/kg	SE	$\pm 1.784$	± 1.940	± 1.752	± 1.973
	%V	9.92	19.60	28.93	51.22
16 mg/kg	Mean	107.950 **	116.430 ***	138.130 ***	166.730 ***
	SE	$\pm 1.985$	± 2.026	± 1.893	± 2.120
	%V	18.29	27.24	50.17	80.38

**Table-1.** Protein content in fish Muscle after fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control

Each value is the Mean  $\pm$  SE of six individual observations.

Values are expressed as mg of protein / 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. Protein content in fish Muscle after fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control (Mean  $\pm$  SE)

**Table-2.** Protein content in fish Liver after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	60.260	60.410	61.090	61.430
control	SE	± 1.742	± 1.635	± 1.467	± 1.592
	Mean	64720 <sup>NS</sup>	67.450 *	73.720 **	77.220 ***
4 mg/kg	SE	$\pm 1.800$	$\pm 1.816$	$\pm 2.090$	± 1.966
	%V	7.40	11.66	20.67	25.71
	Mean	70.110 *	77.610 **	83.780 ***	92.300 ***
8 mg/kg	SE	$\pm 1.807$	± 1.945	$\pm 2.028$	$\pm 1.788$
	%V	16.34	28.47	37.13	50.26
16 mg/kg	Mean	76.610 **	83.220 ***	89.110 ***	98.390 ***
	SE	± 1.747	$\pm 1.687$	± 1.806	± 1.935
	%V	27.13	37.76	45.85	60.18

Values are expressed as mg of protein / 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. Protein content in fish Liver after fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control (Mean  $\pm$  SE)

**Table-3.** Protein content in fish Gonad after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	99.020	99.240	99.740	100.060
Control	SE	$\pm 1.858$	± 1.706	± 1.617	± 1.751
	Mean	103.300 <sup>NS</sup>	106.800 *	109.630 **	117.330 ***
4 mg/kg	SE	± 1.791	± 1.675	± 1.860	± 1.829
	%V	4.32	7.61	9.92	17.26
	Mean	108.800 *	117.480 **	122.980 ***	131.510 ***
8 mg/kg	SE	$\pm 1.945$	$\pm 2.071$	± 1.743	± 1.936
	%V	9.87	18.37	23.30	31.43
16 mg/kg	Mean	118.160 **	129.070 ***	142.310 ***	155.100 ***
	SE	± 1.896	± 1.981	$\pm 2.073$	$\pm 1.784$
	%V	19.33	30.06	42.68	55.01

Values are expressed as mg of protein / 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. Protein content in fish Gonad after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control (Mean $\pm$ SE)

### **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<sup>4</sup>. 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<sup>32</sup>.

In the present investigation, the Nucleic acid levels (RNA) in different tissues like Muscle, Liver, and Gonad were observed under different dosages of  $17\alpha$ -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 Gonad (73.23%, p < 0.001) > Liver (61.63%, p < 0.001) > Muscle (58.52%, p < 0.001) of fish compared to control. An increase of RNA amount was more at a higher concentration of  $17\alpha$ -MT (16mg) and a higher duration (28 days). Similar results were observed that the RNA arising in larger quantity is instrumental in turning out a greater quantity of protein<sup>26</sup>.

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<sup>7</sup>.

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	3.040	3.190	3.260	3.330
Control	SE	$\pm 0.090$	$\pm 0.081$	$\pm 0.097$	$\pm 0.087$
	Mean	3.320 <sup>NS</sup>	3.580 *	3.740 **	4.0540***
4 mg/kg	SE	$\pm 0.098$	$\pm 0.096$	$\pm 0.102$	± 0.130
	%V	9.23	12.19	14.70	21.51
	Mean	3.560 *	3880 **	4.260 ***	4.690 ***
8 mg/kg	SE	$\pm 0.106$	± 0.125	± 0.145	± 0.125
	%V	17.19	21.66	30.53	41.04
16 mg/kg Mean	3.840 **	4.310 ***	4.710 ***	5.280 ***	
	SE	$\pm 0.104$	$\pm 0.110$	$\pm 0.108$	$\pm 0.100$
	%V	26.57	35.25	44.27	58.52

Table-4. RNA content in fish Muscle after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control

Values are expressed as mg of protein / gram wet weight of tissue.

SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001



Figure-4. RNA content in fish Muscle after fed of different dosages of  $17\alpha$ -Methyltestosterone ompared to control (Mean  $\pm$  SE)

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	4.200	4.220	4.230	4.250
Control	SE	± 0.166	± 0.162	$\pm 0.184$	± 0.159
	Mean	4.440 <sup>NS</sup>	4.600 *	4.950 *	5.260 **
4 mg/kg	SE	$\pm 0.202$	$\pm 0146$	$\pm 0.201$	$\pm 0.228$
	%V	5.51	9.06	17.35	24.61
	Mean	4.710 <sup>NS</sup>	5.210 **	5.730 ***	6.330 ***
8 mg/kg	SE	± 0.199	$\pm 0.186$	± 0.193	$\pm 0.217$
	%V	11.77	23.51	35.74	49.95
16 mg/kg	Mean	4.980 *	5.690 ***	6.070 ***	6.820 ***
	SE	$\pm 0.185$	± 0.164	± 0.219	$\pm 0.195$
	%V	18.30	34.91	43.90	61.63

**Table-5.** RNA content in fish Liver after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control

Values are expressed as mg of protein / gram wet weight of tissue. SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001



Figure-5. RNA content in fish Liver after fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control(Mean  $\pm$  SE)

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	1.960	1.980	2.040	2.080
Control	SE	$\pm 0.076$	$\pm 0.089$	$\pm 0.085$	$\pm 0.078$
4 mg/kg	Mean	2.170 *	2.260 *	2.430 *	2.630 **
	SE	$\pm 0.045$	$\pm 0.075$	$\pm 0.098$	$\pm 0.095$
	%V	10.80	14.29	19.20	26.43
8 mg/kg	Mean	2.380 **	2.590 **	2.880 ***	3.160 ***
	SE	$\pm 0.087$	$\pm 0.102$	$\pm 0.095$	$\pm 0.096$
	%V	20.99	30.54	41.45	52.29
16 mg/kg Mean		2.680 ***	2.890 ***	3.170 ***	3.600 ***
	SE	$\pm 0.089$	$\pm 0.118$	$\pm 0.106$	$\pm 0.085$
	%V	36.37	40.59	55.65	73.23

**Table-6.** RNA content in fish Gonadafter fed of different dosages of $17\alpha$ -Methyltestosterone compared to control

Values are expressed as mg of protein / gram wet weight of tissue.

SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001



Figure-6. RNA content in fish Gonadafter fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control(Mean  $\pm$  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<sup>3,19</sup>. levels in different tissues like Muscle, Liver, and Gonads were not changed much under differentdosages of  $17\alpha$ -MT but at higher concentrations (16 mg/kg) and higher duration (28 days), there was a less increase in DNA content. The quantitative changes in DNA and RNA levels in *Cyprinus carpio* would be due to increased activity of DNA and the inhibition of RNA polymerase function<sup>4</sup>.

The present study revealed that DNA

$17\alpha$ -MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	0.851	0.855	0.867	0.872
Control	SE	$\pm 0.010$	$\pm 0.014$	$\pm 0.026$	$\pm 0.022$
4 mg/kg	Mean	0.808 <sup>NS</sup>	0.839 <sup>NS</sup>	0.854 <sup>NS</sup>	0.848 <sup>NS</sup>
	SE	± 0.015	$\pm 0.023$	± 0.032	± 0.046
8 mg/kg	Mean	0.843 <sup>NS</sup>	0.844 <sup>NS</sup>	0.865 <sup>NS</sup>	0.894 *
	SE	± 0.029	$\pm 0.018$	$\pm 0.042$	± 0.034
16 mg/kg	Mean	0.837 <sup>NS</sup>	0.859 <sup>NS</sup>	0.895 <sup>NS</sup>	0.907 *
	SE	$\pm 0.013$	$\pm 0.025$	$\pm 0.027$	$\pm 0.029$

**Table-7.** DNA content in fish Muscle after fed of different dosages of 17α-Methyltestosterone compared to control

Each value is the Mean  $\pm$  SE of six individual observations.

Values are expressed as mg of protein / gram wet weight of tissue.

SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05,

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	0.418	0.403	0.415	0.422
Control	SE	$\pm 0.011$	$\pm 0.022$	$\pm 0.027$	$\pm 0.013$
4 mg/kg	Mean	0.421 <sup>NS</sup>	0.462 <sup>NS</sup>	0.424 <sup>NS</sup>	0.441 <sup>NS</sup>
	SE	± 0.015	$\pm 0.017$	$\pm 0.012$	± 0.019
8 mg/kg	Mean	0.433 <sup>NS</sup>	0.444 <sup>NS</sup>	0.472 <sup>NS</sup>	0.464 *
	SE	± 0.012	$\pm 0.014$	$\pm 0.018$	± 0.022
16 mg/kg	Mean	0.404 <sup>NS</sup>	0.459 <sup>NS</sup>	0.433 *	0.489 *
	SE	± 0.019	± 0.024	$\pm 0.020$	$\pm 0.027$

**Table-8.** DNA content in fish Liver after fed of different dosages of $17\alpha$ -Methyltestosterone compared to control.

Values are expressed as mg of protein / gram wet weight of tissue.

SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05,

17α-MT/Kg diet		Days of exposure			
		7 days	14 days	21 days	28 days
Control	Mean	1.011	1.014	1.012	1.010
Control	SE	$\pm 0.029$	$\pm 0.043$	$\pm 0.018$	$\pm 0.027$
4 mg/kg	Mean	1.012 <sup>NS</sup>	1.011 <sup>NS</sup>	1.009 <sup>NS</sup>	1.012 <sup>NS</sup>
	SE	± 0.038	$\pm 0.027$	± 0.039	± 0.046
8 mg/kg	Mean	1.024 <sup>NS</sup>	1.028 <sup>NS</sup>	1.015 <sup>NS</sup>	1.037 <sup>NS</sup>
	SE	± 0.039	± 0.041	± 0.053	± 0.034
16 mg/kg	Mean	1.017 <sup>NS</sup>	1.045 <sup>NS</sup>	1.032 *	1.049 *
	SE	± 0.024	± 0.029	± 0.045	$\pm 0.052$

**Table-9.** DNA content in fish Gonadafter fed of different dosages of  $17\alpha$ -Methyltestosterone compared to control

Each value is the Mean  $\pm$  SE of six individual observations.

Values are expressed as mg of protein / gram wet weight of tissue.

SE - Standard Error, %V- Percent variation, NS: Not Significant, \* P < 0.05,

### CONCLUSION

Based on the results obtained from the present study, it was concluded that  $17\alpha$ -MT induced Protein synthesis in different tissues of *Tilapia mossambica*. Hence, it is clear that the anabolic hormones such as 17á-MT hormone play an important role in enhancing the tissue protein content in fish for the nutritional purpose of Man.

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