EFFECTS OF LOW LEVELS OF THE STEROID 17 α METHYL TESTOSTERONE ON GROWTH, BODY COMPOSITION AND GONADAL DEVELOPMENT OF NILE TILAPIA (Oreochromis niloticus)

Henry K. M. Tolufase

Abstract

To study the effects of low levels of 17α- Methyl Testosterone on growth, body composition and sexual development of Oreochromis niloticus, fingerlings of the experimental fish were fed on 0.5, 2.0, 8.0 and 32.0 mg/kg feed of the steroid respectively for 56 days. Control diet without hormone was also fed. Results of the Percentage Weight Gain, Specific Growth Rate, Food Conversion Efficiency, Protein Efficiency Ratio, and Proximate Analysis of the carcass of the experimental fish before and after the experiment show improved growth and body composition in all hormone treated fish, with fish receiving 2.0 mg/kg feed of hormone producing the best growth response of 34.20% weight gain and body composition. Increased sexual development was observed in male fish for all hormone treated groups but there was no indication of weight difference between sexes in the study; therefore growth improvement could not be attributed to the androgenic effect of the hormone. These results show that low levels of the hormone 17 α - Methyl Testosterone have anabolic effects and its incorporation in the diet at 2.0mg/kg feed for a period of 8 weeks increased appetite, food conversion and body composition Oreochromis niloticus.

Over the years, dietary supplementation with hormone for accelerated growth in fish culture has become a common practice. This has triggered off a lot of research works on the use of hormone in fish nutrition (Donaldson, E.M., U.H.M Fagerlund, D. A. Higgs, and J.R. McBridge, 1979; Owusu-Frimpong and Nijjhar, B., 1981; Smith and Phelps, 1997; and Hossain M.A., 2005).

Hormones have variety of effects, some of which are potentially beneficial in culturing fishes. Of special interest are those hormones that may be added to fish diet without being destroyed in the digestive tract. Hormones used in fish culture could be categorized into three, based on their modes of action:

i. Those that influence growth and feed conversion.
ii. Those that influence sexual development.
iii. Those that affect Osmoregulation. (Halver, 1989)

In this aquacultural practice of dietary supplementation with hormones, androgens such as the male sex hormones 17 α – Methyl Testosterone, 17 α- Ethyl Testosterone or an estrogen such as the female sex hormone Ethynylestradiol are commonly added directly to fish diets to suppress sexual development and channel available energy towards growth. These cause sterility or sex reversal to a sex desired for culture. The use of sterile fish in aquaculture has the advantages of eliminating...
precociousness and the cessation of somatic growth that accompanies sexual maturity (Guerrero, 1975; Tayamen and Sheldon, 1978; Halver, 1989; Dan and Little, 2000; and Guerrero, R.D., 2008). Hormones which influence growth in fish include: Anabolic steroids, Thyroid hormones, Mammalian growth hormones, Pituitary extracts, Insulin and Testosterone. (Fagerlund, U.H.M., D.A. Higgs, J.R. McBridge, D.M. Ploinikoff and B.S Dosanjh, 1980; Refstie, 1982; and Rothbard, S., Z. Yaron and B MOAV, 1988). Of all these, 17 α – Methyl Testosterone (MT) has become prominently popular for its use as an androgen to produce single sex tilapia stocks to overcome the problems of excessive reproduction in most grow – out system (Clemens and Inslee, 1968; Nakamura, 1975; Abucay and Maier, 1997; and Contreras-Sanchez, 2001).

However, this usually involves the addition of high concentration of hormones to diets of fish for a relatively long period in order to achieve sex reversal to enhance growth. It has been reported that the highest sex reversal efficiency was obtained at 75 mg / kg feed of MT (Halver, 2009). This has increased the concern of many countries about hormone residues in products destined for the market place. Therefore in considering the commercial reality of hormones in aquaculture, the economic and health implications for the consumer should be considered (Rutten M.J.M., Komen H. and Bovenhuis H., 2005).

17 α - Methyl Testosterone is a white crystalline compound sold in powder or tablet forms and clinically used for hormone replacement therapy in man. It comes under the trade names such as Androids, Testred, Powertex, Pasoma strong etc. (Donald J., 2008)

While high dosages of exogenous male hormones, including MT, are known to cause side effects such as liver damage in human, lower levels have been reported to actually produce various health benefits including reduced risks from Cardio – Vascular diseases and Cancer (Valcour, 2001).

**Objective of Study**

The objective of the study is to determine the minimal effective dose of 17 α - Methyl Testosterone on Nile Tilapia (*Oreochromis niloticus*).

**Materials and Methods**

To achieve the above objective, the following experimental designs, materials and methods were formulated and carried out.

**The Synthetic Hormone Used**

The synthetic hormone used in this work was 17 α – Methyl Testosterone (MT). This was preferred being one of the most commonly used androgen for sex reversal in fish culture. It is relatively cheap and readily available in drug stores where they come under trade names such as powertex, pasoma strong etc. The hormone was administered orally by incorporation into the fish feed.

**Preparation, Treatment and Preservation of Feed**

Five experimental diets were formulated using cassava flour, corn flour, fish meal. Meat meal, Soya bean, groundnut oil, vitamin supplement and treated with hormones at 0.5, 2.0, 8.0, 32mg/kg feed respectively. The control diet was without any hormonal treatment. Hormonal treatment of the feed was by ethanol evaporation (Rothbard, S., Z. Yaron and B MOAV, 1988). The preferred concentrations of the hormone MT was dissolved in 95% ethanol and then mixed with the pellets at 1 litre ethanol per 2kg pellets. Control diet was similarly treated with ethanol, but without the hormone. The feeds were dried in the open air, packed in polyethylene bags, sealed, and marked according to treatment and stored in refrigerator until needed.
**Experimental System**
The tank and water quality
Ten plastic tanks with capacity of 24 litres were used to collect water from a well and arranged into five feeding treatments, labeled MT1, MT2, MT3, MT4 and CO, for 0.5, 2.0, 8.0, 32 mg / kg feed and control respectively. Each treatment was replicated.

**Collection, Acclimatization and Feeding of Experimental Fish**
About 150 fingerlings of *Oreochromis niloticus* were collected from Lewu farms, Kabba Kogi State, a distance of about 40km from the site of the study. Transportation was done in the morning to reduce thermal stress. The fish were sorted and preferred sizes of about 3-5cm were stocked at 12 fish per tank. They were acclimatized for 7 days and fed with the control diet 3 times daily. Uneaten food and faecal pellets were siphoned out of the water, and dead fish where promptly removed while water volume in each tank was maintained by adding fresh water daily.

At the end of the 7 days of acclimatization, the fish were weighed and starved for a day to empty their gastrointestinal tract before the commencement of feeding with experimental diet. A fish was also picked at random from each tank and prepared for a proximate analysis for moisture, ash, protein and lipid at the start of the experiment. Feeding commenced and fish were fed at 9:00 am, 1:00 pm and 6:00 pm, 7 days a week throughout the experimental period. Weighing was done weekly and all incidences of mortality were carefully recorded throughout the experiment. The experiment lasted 56 days (8 weeks)

**Weighing of Experimental Fish**
Weighing was done in water using a top loading scale. The weight of water and container was first taken without the fish, and then the fish were introduced and the new weight taken. The weight of the fish is the difference between the second and the first reading.

**Proximate Analysis of Experimental Fish and Feed**
Using the standard Association of Official Analytical Chemistry (AOAC) method (1980) the fish and formulated feed were analyzed to determine the moisture, ash, crude protein, crude Fat, crude Fibre and Carbohydrate contents.

**Determination of Indices of Growth and Feed Utilization**
Formulae of various growth and feed utilization parameters where used in the analysis of collected data (Jauncey, 1982).

**Percentage Weight Gain (%)**
\[
\text{% weight gain} = \frac{\text{weight gain (g)}}{\text{Initial weight (g)}} \times 100
\]

**Specific Growth Rate, S G R (%/ day):** This is the average percentage increase in body weight per day over any given time interval.
\[
\text{SGR} = \frac{\log W_2 - \log W_1}{T_2 - T_1} \times \frac{100}{1}
\]
Where \( W_1 \) = weight at time \( T_1 \) (initial weight)  
\( W_2 \) = weight at time \( T_2 \) (Final weight)  
\( T_2 - T_1 \) = Duration of growth trial

**Food Conversion Efficiency, FCE:** This is the new weight of fish produced per unit dry weight of food.
\[
\text{FCE} = \frac{\text{weight gain (g)}}{\text{Food consumed (g)}}
\]
**Henry K. M. Tolufase**

**Protein Efficiency Ratio, PER:** This is the weight of fish produced per unit weight of dietary protein.

PER = \( \frac{\text{Weight gain (g)}}{\text{Crude Protein fed (g)}} \)

**Statistical Analyses**

Analysis of variance (ANOVA) for completely randomized design was used at 5% level of significance to test if there was a significant difference between the range of treatment mean obtained for the growth and feed utilization parameters (% weight gain, SGR, FCE, PER)

**Histological Techniques**

Fish were randomly picked from the mixed population in each tank at the end of the feeding trial and dissected. The gonads were removed and weighed immediately with an analytical balance.

**TABLE 1:- Mean Weekly Weight Gain Values of Oreochromis niloticus Fed Low Levels of 17α – Methyl Testosterone (Grams)**

<table>
<thead>
<tr>
<th>DIET</th>
<th>INITIAL WEIGHT</th>
<th>WEEK 1</th>
<th>WEEK 2</th>
<th>WEEK 3</th>
<th>WEEK 4</th>
<th>WEEK 5</th>
<th>WEEK 6</th>
<th>WEEK 7</th>
<th>WEEK 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1</td>
<td>3.45</td>
<td>3.56</td>
<td>3.60</td>
<td>3.62</td>
<td>3.74</td>
<td>3.74</td>
<td>3.78</td>
<td>3.82</td>
<td>3.86</td>
</tr>
<tr>
<td>MT2</td>
<td>3.45</td>
<td>3.56</td>
<td>3.62</td>
<td>3.86</td>
<td>4.20</td>
<td>4.32</td>
<td>4.49</td>
<td>4.58</td>
<td>4.63</td>
</tr>
<tr>
<td>MT3</td>
<td>3.44</td>
<td>3.54</td>
<td>3.58</td>
<td>3.68</td>
<td>3.72</td>
<td>3.81</td>
<td>3.95</td>
<td>4.07</td>
<td>4.16</td>
</tr>
<tr>
<td>MT4</td>
<td>3.45</td>
<td>3.50</td>
<td>3.58</td>
<td>3.65</td>
<td>3.68</td>
<td>3.70</td>
<td>3.84</td>
<td>3.87</td>
<td>4.05</td>
</tr>
<tr>
<td>CO</td>
<td>3.44</td>
<td>3.48</td>
<td>3.50</td>
<td>3.55</td>
<td>3.61</td>
<td>3.66</td>
<td>3.68</td>
<td>3.70</td>
<td>3.74</td>
</tr>
</tbody>
</table>
**Effects of Low Levels of the Steroid 17 α Methyl Testosterone on Growth, Body Composition and Gonadal Development of Nile Tilapia (Oreochromis niloticus)**

**Fig 1: Effect of 17 α – Methyl Testosterone on growth of Oreochromis niloticus**

![Graph showing the effect of different doses of 17 α Methyl Testosterone on the growth of Nile Tilapia over 8 weeks.](image)

**TABLE 2:** Mean Proximate Composition of Experimental Fish Before and After Treatment (% Weight)

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>INITIAL</th>
<th>MT1</th>
<th>MT2</th>
<th>MT3</th>
<th>MT4</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.60</td>
<td>5.90</td>
<td>5.82</td>
<td>5.74</td>
<td>5.76</td>
<td>5.82</td>
</tr>
<tr>
<td>Ash</td>
<td>30.00</td>
<td>26.50</td>
<td>30.50</td>
<td>24.50</td>
<td>21.50</td>
<td>26.00</td>
</tr>
<tr>
<td>Protein</td>
<td>48.00</td>
<td>61.50</td>
<td>81.00</td>
<td>75.00</td>
<td>78.50</td>
<td>56.50</td>
</tr>
<tr>
<td>Lipids</td>
<td>6.10</td>
<td>8.80</td>
<td>10.50</td>
<td>12.00</td>
<td>16.00</td>
<td>8.10</td>
</tr>
</tbody>
</table>

**TABLE 3:** Mean Wet Weights of the Gonads of Fish Receiving Various Doses of 17 α – Methyl Testosterone

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>GONADS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MALE (TESTIS)</td>
</tr>
<tr>
<td>MT1</td>
<td>0.032</td>
</tr>
<tr>
<td>MT2</td>
<td>0.035</td>
</tr>
<tr>
<td>MT3</td>
<td>0.039</td>
</tr>
<tr>
<td>MT4</td>
<td>0.043</td>
</tr>
<tr>
<td>CO</td>
<td>0.032</td>
</tr>
</tbody>
</table>
TABLE 4: Table of Means for Growth and Food Utilization of Experimental Diets

<table>
<thead>
<tr>
<th>Exp. Diets</th>
<th>Initial Weight (G)</th>
<th>Final Weight (G)</th>
<th>Weight Gain (G)</th>
<th>Percentage Weight Gain</th>
<th>SGR (% Per Day)</th>
<th>FCE</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1</td>
<td>3.45</td>
<td>3.86</td>
<td>0.41</td>
<td>11.88</td>
<td>0.13</td>
<td>0.015</td>
<td>0.050</td>
</tr>
<tr>
<td>MT2</td>
<td>3.45</td>
<td>4.63</td>
<td>1.18</td>
<td>34.20</td>
<td>0.35</td>
<td>0.045</td>
<td>0.146</td>
</tr>
<tr>
<td>MT3</td>
<td>3.44</td>
<td>4.16</td>
<td>0.72</td>
<td>20.93</td>
<td>0.23</td>
<td>0.027</td>
<td>0.089</td>
</tr>
<tr>
<td>MT4</td>
<td>3.45</td>
<td>4.05</td>
<td>0.60</td>
<td>17.39</td>
<td>0.19</td>
<td>0.023</td>
<td>0.074</td>
</tr>
<tr>
<td>CO</td>
<td>3.44</td>
<td>3.74</td>
<td>0.30</td>
<td>8.72</td>
<td>0.10</td>
<td>0.012</td>
<td>0.037</td>
</tr>
</tbody>
</table>

- SGR (Specific Growth Rate), FCE (Food Conversion Efficiency), PER (Protein Efficiency Ratio).

a) Percentage Weight Gain (F = 5.19, P < .05)
b) Specific Growth Rate (SGR) (F = 5.19, P < .05)
c) Food Conversion Efficiency (FCE) (F = 5.19, P < .05)
d) Protein Efficiency Ratio (PER) (F = 5.19, P < .05)

Result

The weights of fish throughout the experiment are presented in Table 1 and plotted in Fig. 1. The results of the proximate analysis of the fish carcass before and after feeding are presented in Table 2. The weights of the male and female gonads are presented in Table 3. The calculated values for the Percentage Weight Gain, Specific Growth Rate (SGR), Food Conversion Efficiency (FCE), Protein Efficiency Ratio (PER), are shown in Table 4.

Discussion of the Result

Growth: Growth rates obtained with MT incorporated at 0.5, 2.0, 8.0 and 32.0 mg/kg feed (MT1, MT2, MT3 and MT4) were significantly higher (P < .05) than with the control. Food conversion efficiency and protein efficiency ratio of the hormone treated fish also showed higher values than the control. More food was consumed by hormone treated fish and more protein was also converted to body tissue, resulting in the highest percentage weight gain of 34.2 observed in the group receiving diet MT2, containing 2.0mg/kg feed of MT.

Body Composition: Results of the proximate composition of the experimental fish show that there was no significant difference in the moisture content. The protein and lipids produced highly significant differences. The ash decreased with the increase in the dose of MT, while protein and lipid contents increased. There is a significantly higher increase in the protein and lipid content of all treated groups compared to the control. This is an indication of nutrient retention in fish flesh, and suggests that dietary incorporation of MT in feed influence metabolic activities in Oreochromis niloticus.

Sexual Development: Result obtained from the wet weight of the gonads show significant differences in weight among the male testes obtained from different treatment, while the female ovum showed no significant differences in weight among treatment. The steady increase in weight of male testes with increase in doses of MT could be attributed to androgenic effect of the hormone on the male Oreochromis niloticus.

However, the fish with the largest testes did not show the best growth performance. This indicates that the growth produced at a low dose of MT (2 mg / kg feed) was more as a result of the anabolic effect of the steroid than the androgenic effect.
Conclusion

Results from the various growth parameters used (Percentage Weight Gain, Specific Growth Rate, Food Conversion Efficiency, and Protein Efficiency Ratio) as shown in table 4, and the proximate analysis of the tissue composition of the experimental fish, show that dietary inclusion of a low level of 17 α – Methyl Testosterone at 2mg/kg feed increased appetite, food utilization, and metabolism, thereby improving growth and body composition of Oreochromis niloticus.

It can therefore be concluded that, in addition to its effects on sexual development in the male Oreochromis niloticus, the hormone MT also has anabolic effects which led to a weight gain of 34.2% and an increase in body composition when fed to the fish at a low level of 2.0 mg/kg of feed. The use of 17 α – Methyl Testosterone at this safe dosage should therefore be encouraged as it produces a relatively high growth increase in the experimental fish without the usual sex reversal which involves the use of higher and unsafe doses of MT.

Recommendations

Results obtained in this research shows that improved growth and body composition was obtained by dietary supplementation with 17 α- Methyl Testosterone at a low dosage of 2 mg/kg feed. This concentration is considered safe for the market without the fear of hormone residue in harvested fish. It is therefore recommended for use in fish culture.

Also, the improved growth recorded at this concentration of MT was without sex reversal which usually accompanies androgenic growth produced with high doses of the steroid. Therefore it is recommended for growth improvement in aquacultural situations where mixed sexes are required for culture.

Increase in growth was recorded within a short period of administering the low dose of MT. It is therefore recommended for use by fingerling culturists. The low concentration of 2mg/kg feed of MT improved appetite and food conversion. So it is recommended as feed supplement to improve appetite in Oreochromis niloticus.

17α- Methyl Testosterone is cheap and readily available in the market. It is economical since only a little quantity produced the remarkable growth improvement recorded. Its use in fish culture should therefore be encouraged and more studies carried out to improve its applicability.

References


