

## Effects of Six Levels of Dietary 17 $\alpha$ -Methyltestosterone on Sex Reversal and Growth of *Oreochromis aureus* (Steindachner) and *O. niloticus* (Linnaeus)

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## 나일틸라피아와 청틸라피아의 성전환과 초기 성장에 미치는 17 $\alpha$ -Methyltestosterone 호르몬의 영향

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### ABSTRACT

The effects of six levels (0, 5, 10, 15, 20, and 25 ppm) of dietary 17 $\alpha$ -methyltestosterone (MT) on sex reversal and growth of *Oreochromis aureus* (Steindachner) and *O. niloticus* (L.) were evaluated. Fifteen ppm MT produced 100% male populations in both species. Both MT-treated and untreated *O. niloticus* grew significantly faster than their *O. aureus* counterparts ( $P < 0.05$ ).

### INTRODUCTION

The concentration of anabolic steroids used to sex reverse tilapia ranges from 30 to 60 ppm (Jalabert, *et al.* 1974; Guerrero 1975; Tayamen and Shelton 1978). Jo (1988) found that 10 ppm 17 $\alpha$ -methyltestosterone (MT) in the diet produced 99% male

*Oreochromis aureus* (Steindachner), which indicates that lower concentrations could be used.

*O. aureus* and *O. niloticus* (L.) are the two most widely cultured tilapia, but there have been few yield trials to compare growth of these species. Anderson and Smitherman (1978) found that normal male *O. niloticus* grew faster than normal male *O. aureus*, while sex-reversed *O. aureus* grew faster than sex-reversed *O. niloticus*.

The objectives of this study were to evaluate the sex reversing efficacy of 5 to 25 ppm MT on both *O. aureus* and *O. niloticus* and to compare growth of these species.

## MATERIALS AND METHODS

### 1. Fry Production

On June 12, 1987, six female and two male Auburn University strain *O. aureus* and six female and two male Auburn University-Egypt strain *O. niloticus* were each stocked into each of twenty-four 2 m<sup>3</sup> hapas that were suspended in 20 m<sup>2</sup> concrete tanks (3 hapas per tank) at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama. On June 23, swim-up fry of both species were collected.

### 2. Sex Reversal

On June 25, two hundred *O. aureus* or *O. niloticus* swim-up fry were each randomly stocked in each of twelve 2 m<sup>3</sup> hapas that were suspended in 20 m<sup>2</sup> outdoor concrete tanks (2 hapas per tank). Six treatments were randomly assigned to each of two replicate hapas within each species.

Fry were fed trout starter (50% protein) with channel catfish vitamin premix at 2% of total weight. Hormone-treated feeds were prepared as described by Shelton *et al.* (1978). Either 0 (control), 5, 10, 15, 20, or 25 ppm MT was added to the feed. The feed was then dried, and soybean oil was added at 5% of total weight to prevent leaching of MT in the water and to supply more energy. Control feed was treated in the same manner, except no MT was added. Feeds were stored in plastic bags and frozen. Fish were fed *ad libitum* three times daily.

After 30 days (July 24), fry were harvested and group weights from each hapa were obtained. Fish from the two replicate hapas for each treatment were then combined for the grow-out study.

### 3. Grow-Out

On July 27, fifty *O. aureus* and 50 *O. niloticus* fingerlings from each treatment were each randomly reallocated to each of two 20 m<sup>2</sup> concrete tanks. Fish were fed a commercial floating catfish starter feed (36% protein) *ad libitum* twice daily. On September 10, each fish was manually sexed and weighed to the nearest 0.1 g.

#### 4. Statistical Analysis

Growth rates were assessed by analysis of variance. Differences among group means were assessed by Duncan's new multiple range test and Student's t test (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

### 1. Sex Reversal

Sex ratios of *O. aureus* and *O. niloticus* are listed in Table 1. *O. aureus* has the WZ sex-determining system (Guerrero 1975), so males and females were assumed to be produced in equal numbers. Sex ratio in the control was not statistically different from a 1:1 sex ratio ( $\chi^2=1.30$ ;  $df=1$ ;  $P=0.25$ ). Sex ratio in the 5 ppm MT-treated group was 2.72 males: 1 female and was significantly different from a 1:1 ratio ( $\chi^2=19.88$ ;  $df=1$ ;  $P < 0.001$ ). In the 10 ppm treatment, 97% of the fish were males. These results are similar to those of a previous study where 10 ppm MT produced 97% males (Jo 1988). MT levels higher than 15 ppm produced 100% males.

Table 1. Percentages of male and female *Oreochromis aureus* and *O. niloticus* fed six different concentrations (ppm) of 17 $\alpha$ -methyltestosterone (MT) in the diet.

MT	<i>O. aureus</i>		<i>O. niloticus</i>	
	Females	Males	Females	Males
0	56	44	38	62
5	27	73	23	77
10	3	97	6	94
15	0	100	0	100
20	0	100	3	97
25	0	100	0	100

*O. niloticus* has the XY sex-determining system (Jalabert *et al.* 1974), so males and females were assumed to be produced in equal numbers. Sex ratio in the control was different than the expected 1:1 ratio ( $\chi^2=4.26$ ;  $df=1$ ;  $P < 0.025$ ). The greater than expected percentage of males was probably due to sampling error. The 5, 10, and 20 ppm treatments produced 77%, 94%, and 97% males, respectively. The 15 and 25 ppm, treatment produced 100% males.

These results suggest that 10 ppm MT is not sufficient to produce 100% males but that 15~25 ppm appears to be effective. These concentrations are lower than traditionally used to sex reverse tilapia.

The efficacy of the lower-than-usual dosages may have been due to the fact that the

tilapia were fed *ad libitum* three times daily. This feeding regime could have had two effects: One, by using this protocol the fish were less likely to eat natural food organisms, so virtually all food contained MT. Two, feeding *ad libitum* instead of feeding a fixed percentage body weight likely had the effect of increasing the amount of MT consumed, since the fish were able to eat as much as they wanted. For example, fish that eat 2 g of 10 ppm MT-treated feed consume as much hormone as fish that eat 1 g of 20 ppm MT-treated feed. Thus, fish eating 15~25 ppm MT-treated feed *ad libitum* could have consumed as much hormone as fish eating 60 ppm MT-treated feed when fed at a fixed percentage body weight. Research is needed to determine if sex reversal in tilapia is a function of total hormone consumption.

Mean weights at the end of the sex reversal period are listed in Table 2. Average mean weight of all MT-treated *O. aureus* groups combined was significantly heavier than that of untreated fish [ $P(t=2.45)=0.05$ ]. Average mean weight of all MT-treated *O. niloticus* groups combined was significantly heavier than that of untreated fish [ $P(t=3.15)=0.02$ ]. These results suggest that MT has an anabolic effect in both species during the sex reversal period. This is similar to that seen for *O. aureus* in a previous experiment (Jo 1988).

Table 2. Effects of six different concentrations (ppm) of 17 $\alpha$ -methyltestosterone (MT) in the diet on mean weight (g) of *Oreochromis aureus* and *O. niloticus* at the end of sex reversal period.

MT	<i>O. aureus</i>	<i>O. niloticus</i>
0	4.12	4.52
5	4.27	5.21
10	5.13	5.35
15	4.66	5.38
20	4.97	5.14
25	4.89	5.67

*O. niloticus* were significantly larger than *O. aureus* at the end of the sex reversal treatment [ $P(t=3.61)=0.01$ ]. Control *O. niloticus* were 9.7% heavier than control *O. aureus*, but the difference was not significant [ $P(t=1.20)=0.50$ ]. On the other hand, MT-treated (all treatments combined) *O. niloticus* were 11.8% heavier than MT-treated *O. aureus* and the difference was significant [ $P(t=3.40)=0.01$ ].

## 2. Grow-Out

Mean net weight gains of male, female, and male and female combined (overall) *O. aureus* and *O. niloticus* at the end of the grow-out period are listed in Table 3. Overall mean net weight gains of all MT-treated *O. aureus* except the 15 ppm treatment were significantly ( $P < 0.05$ ) greater than that of the control. The MT-treated groups gained

from 17.3% to 23.8% more than the control.

Overall growth differences between the control and the MT-treated groups of *O. aureus* were probably due to the comparatively poor growth of females in the control, because the males in the 5 ppm MT group were the only males that gained significantly more than those in the control. Control females gained significantly less weight than those in the 5 ppm MT group, and there were no females in any other group. Similar results were observed in a previous study (Jo *et al.* 1988).

Overall mean net weight gain of MT-treated *O. niloticus* was not significantly greater than that of the control. The likely reason that weight gain in the MT-treated *O. niloticus* was not greater than that of the control is that the sex ratio in the control group was significantly skewed toward males; there were too few control females to significantly depress overall net weight gain of the control group, as was the case with *O. aureus*. Observed mean net weight gain of the *O. niloticus* males in the 5 ppm treatment was greater than for all other groups, which is similar to that observed for *O. aureus*. However, mean net weight gains of treated and untreated *O. niloticus* males were not significantly different. *O. niloticus* females in the 5 ppm treatment had a greater observed mean net weight gain than control females but, unlike their *O. aureus* counterparts, the difference was not significant.

Table 3. Effects of six different concentration (ppm) of 17 $\alpha$ -methyltestosterone (MT) on mean net weight gains (g) of male, female, and male and female combined (overall) *Oreochromis aureus* and *O. niloticus* at the end of the grow-out period. Mean net weight gains in a column followed by the same letter are not statistically different ( $P < 0.05$ )

MT	<i>O. aureus</i>			<i>O. niloticus</i>		
	Overall	Male	Female	Overall	Male	Female
0	89.8b	99.5b	81.7b	114.9a	121.5a	104.5a
5	111.2a	116.4a	97.4a	123.6a	127.4a	110.7a
10	110.1a	110.9ab	—	118.6a	118.8a	—
15	105.3ab	105.3ab	—	122.1a	122.1a	—
20	113.5a	113.5b	—	120.3a	120.8a	—
25	108.6a	108.6ab	—	121.7a	121.7a	—

*O. niloticus* gained significantly more weight than *O. aureus* during the 45-day grow-out period [ $P(t=5.12)=0.001$ ]. Control *O. niloticus* gained 30% more than control *O. aureus* [ $P(t=5.38)=0.001$ ], and MT-treated *O. niloticus* (all treatments combined) gained 10.5% more than MT-treated *O. aureus* [ $P(t=3.91)=0.005$ ]. These results differ slightly from those of Anderson and Smitherman (1978). They found that untreated *O. niloticus* grew faster than untreated *O. aureus* but that sex-reversed *O. aureus* grew faster than sex-reversed *O. niloticus*. The difference may be due to the hormone used to sex reverse the tilapia. Anderson and Smitherman (1978) used 17 $\alpha$ -ethynyltestosterone. Future studies

are needed to determine species-specific residual effects of different sex-reversing hormones on growth in tilapia.

## 요 약

여섯 가지 농도(0, 5, 10, 15, 20, 30 ppm)의 17 $\alpha$ -methyltestosterone (MT)를 사료에 섞어서 청틸라피아 (*Oreochromis aureus*)와 나일틸라피아 (*O. niloticus*)에 30일간 먹여서 성전환율과 성장을 비교한 결과는 다음과 같다. 두 종류 모두 15 ppm의 MT는 100% 숫컷이 생산되었다.

MT를 먹인 것이나 먹이지 않은 나일틸라피아는 청틸라피아보다 빨리 성장하였다( $P < 0.05$ ).

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