

## Effects of Dietary 17 $\alpha$ -Methyltestosterone on Body Composition and Gonosomatic Indices in Blue Tilapia, *Oreochromis aureus* (Steindachner)

Jae-Yoon Jo, R. Oneal Smitherman\* and Douglas Tave\*

National Fisheries University of Pusan, Department of Aquaculture, Pusan 608-737, Korea

\*Department of Fisheries and Allied Aquacultures, Alabama Agricultural

Experiment Station, Auburn University, Alabama 36849 USA

## 청틸라피아의 체조성과 생식소 중량 지수에 미치는 17 $\alpha$ -Methyltestosterone 호르몬의 영향

조재윤 · 알 오닐 스미들먼\* · 더글러스 테이브\*

부산수산대학교 양식학과

\*어른대학교 수산양식학과

### ABSTRACT

Blue tilapia, *Oreochromis aureus* swim-up fry were fed, 0, 1, 10, or 60 ppm 17 $\alpha$ -methyltestosterone (MT) for 30 days (sex reversal period). Fish that had been fed 0 ppm MT during the sex reversal period were subsequently fed 0 ppm MT (0-0), 10 ppm MT (0-10) or 60 ppm MT (0-60) for the next 57 days (post sex reversal period); fish that had been fed 60 ppm MT during the sex reversal period were subsequently fed 0 ppm MT (60-0), 10 ppm MT (60-10), or 60 ppm MT (60-60) during the post sex reversal period. One group was fed 1 ppm MT (1-1), while another group was fed 10 ppm MT (10-10) during the entire 87-day experiment. There was a significant inverse relationship between concentration of MT and percentage body protein and percentage body fat at the end of the sex reversal period, while there was a significant positive relationship between concentration of MT and percentage body moisture. MT treatment during the post sex reversal period had greater effects on body composition of males at the end of the post sex reversal period than did MT treatment during the sex reversal period. There was a significant inverse relationship between MT concentrations, during both the sex reversal and post sex reversal periods, and gonosomatic indices (GSI) in males. In females, there was a significant inverse relationship between MT concentrations during the post sex reversal period and GSI.

## INTRODUCTION

The androgen, 17 $\alpha$ -methyltestosterone (MT), is used to sex-reverse tilapias for the production of all-male populations (Hunter and Donaldson 1983). Consumption of MT during the sex reversal period has recently been found to enhance growth rate of blue tilapia, *Oreochromis aureus* (Jo et al. 1988), and Nile tilapia, *O. niloticus* (Muhaya 1985).

Several studies have shown that body composition can be affected by MT. Percentage body protein increased in MT-treated common carp, *Cyprinus carpio* (Matty and Lone 1979), European eel, *Anguilla anguilla* (Degani 1985), and channel catfish, *Ictalurus punctatus* (Gannam 1988). On the other hand, protein levels of pink salmon, *Oncorhynchus gorbucha*, steelhead trout, *Salmo gairdneri* (Fagerlund and McBride 1975), and coho salmon, *O. kisutch* (Fagerlund et al. 1980) were not affected by MT.

Percentage body fat can also be affected by MT treatments. Fat content of common carp (Matty and Lone 1979), steelhead trout, pink salmon (Fagerlund and McBride 1977), and coho salmon (Fagerlund and McBride 1975) were increased by MT treatments. Conversely, fat content of European eel (Degani 1985, 1986) and channel catfish (Gannam 1988) decreased as a result of MT treatments.

Percentage body protein and fat levels in *Oreochromis mossambicus* were not affected by MT (Howerton et al. 1988). Body composition of *O. aureus* under the influence of MT has not been evaluated.

The objectives of this study were to evaluate the effects of different concentrations of MT in the diet and of different treatment durations on body composition and on gonosomatic indices of blue tilapia *O. aureus*.

## MATERIALS AND METHODS

### Fry Production

On June 29, 1986, three female and two male Auburn University strain blue tilapia, *Oreochromis aureus* were stocked into each of twelve 2 m<sup>3</sup> hapas (mesh size : 1.5 $\times$ 2 mm) suspended in 3.05 m diameter circular plastic pools (one hapa per pool) at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama. On July 6, eggs and/or fry were collected and placed in McDonald hatching jars for incubation. Sixteen sib-lots, totalling approximately 20,000 fry of the same developmental stage, were produced.

### Sex Reversal Period

On July 12, one hundred and fifty swim-up fry were randomly allocated to each of

twenty-four 2 m<sup>3</sup> hapas suspended in eight 20 m<sup>2</sup> concrete tanks (3 hapas per tank). Fry were fed finely ground trout chow with 0 (control), 1, 10, or 60 ppm MT. Groups fed 1 ppm and 10 ppm MT-treated feed had three replications, while groups fed 0 ppm and 60 ppm MT-treated feed had 9 replications. Treatments were randomly assigned to the hapas. After 30 days (August 11), fry were harvested and group weights were taken in each hapa. The fish from the replicate hapas for each treatment were then pooled and held in hapas until stocking for the grow-out study.

### Post Sex Reversal Period

On August 14, fry from each treatment were randomly reallocated to 2 m<sup>3</sup> hapas that were suspended in a 0.1 ha earthen pond. The group that had been fed 0 ppm MT-treated feed during the sex reversal period was divided into three sub-groups ; for the next 57 days, the 3 sub-groups were fed either 0, 10, or 60 ppm MT-treated feed. These groups were designated 0–0, 0–10, and 0–60, respectively. The group that had been fed 60 ppm MT-treated feed during the sex reversal period was also divided into three sub-groups ; for the next 57 days, the three sub-groups were fed either 0, 10, or 60 ppm MT-treated feed. These groups were designated 60–0, 60–10, and 60–60, respectively. The groups that had been fed 1 ppm or 10 ppm MT-treated feed during the post sex reversal period received 1 or 10 ppm MT-treated feed respectively. They were designated 1–1 and 10–10, respectively. Each treatment had 6 replications, and 15 fish were randomly assigned to each replicate hapa. On October 10, each fish was manually sexed and weighed to the nearest 0.1 g.

### Experimental Feed

During the sex reversal period, fry were fed a commercial trout chow (40% protein). Trout chow was ground and screened through a 1 mm sieve, and vitamin premix for domestic animals was added at 2% of total feed. Fish were fed *ad libitum* three times daily.

During the post sex reversal period, fish were fed a commercial floating catfish fingerling feed (36% protein). Fish were fed *ad libitum* twice daily.

Hormone-treated feed was prepared as described by Shelton et al. (1978). After appropriate amounts of MT were added to the feed, it was dried, and soybean oil was added at 5% of total weight to prevent MT from leaching and to supply more energy. Control feed was treated in the same manner except no MT was added. Feed was stored in plastic bags and kept in a freezer.

### Proximate Analysis

At the end of the sex reversal period, 50 fish per treatment were randomly sampled

for proximate analysis. At the end of the post sex reversal period, 18 fish per treatment were sampled for proximate analysis. Percentage body protein, percentage body fat, and percentage body moisture of whole fish were ascertained. Percentage nitrogen was determined using the standard Kjeldahl method (AOAC 1950) ; percentage protein was then determined by multiplying percent nitrogen by 6.25. Percentage fat was determined by the Gerver method (AOAC 1984). Percentage moisture was determined as described by AOAC (1975).

### Gonosomatic Index

When fish were harvested at the end of the post sex reversal period, gonads were collected from 48 randomly chosen fish per treatment and were stored in 15% formalin. Gonads were weighed to the nearest milligram. Gonosomatic index (GSI) was calculated by using the following formula : (gonad weight/body weight)  $\times$  100.

### Statistical Analysis

Regression analysis (Steel and Torrie 1980) was used to determine the relationships among MT concentration and percentage body protein, percentage body fat, percentage body moisture, and GSI.

Table 1. Effects of various concentrations (ppm) of 17 $\alpha$ -methyltestosterone (MT) in the diet on mean percentage body protein, mean percentage body fat, and mean percentage body moisture of blue tilapia, *Oreochromis aureus* at the end of the sex reversal period

MT	Protein	Fat	Moisture
0	14.6	5.7	75.1
1	14.5	5.1	75.7
10	14.6	4.6	76.2
60	13.9	3.4	78.1

## RESULTS AND DISCUSSION

Mean percentage body protein, percentage body fat, and percentage body moisture at the end of the sex reversal period are listed in Table 1. Protein and fat contents of the 60 ppm MT-treated group were the lowest, while its moisture content was the highest. Mean protein and fat contents decreased significantly as MT levels increased, while average moisture content increased significantly (Fig. 1).

Proximate analyses at the end of the post sex reversal period are listed in Table 2, and the results were similar to those obtained after the sex reversal period. There

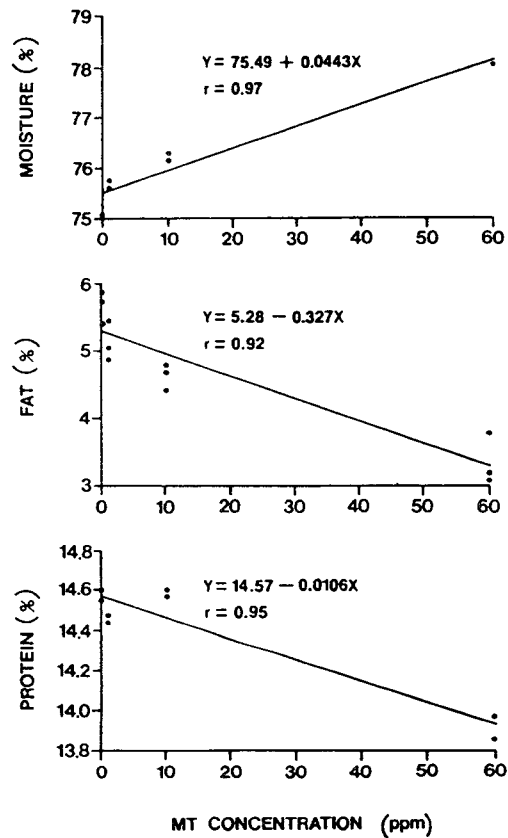


Fig. 1. Relationships between 17 $\alpha$ -methyltestosterone (MT) concentrations in the diet and mean percentage body moisture, mean percentage body fat, and mean percentage body protein in blue tilapia, *Oreochromis aureus* at the end of the sex reversal period.

was a significant inverse relationship between concentration of MT in the post sex reversal period and percentage body protein and body fat at the end of the post sex reversal period, while there was a significant positive relationship between concentration of MT in the post sex reversal period and percentage body moisture at the end of the post sex reversal period (Fig. 2).

Percentage body protein ranged from 16.4% for groups fed 0 ppm MT during the post sex reversal period to 15.6% for groups fed 60 ppm MT. Percentage body fat ranged from 11.4% for groups fed 0 ppm MT during the post sex reversal period to 9.4% for groups fed 60 ppm. Percentage moisture ranged from 68.4% for groups fed 0 ppm MT during the post sex reversal period to 70.7% for groups fed 60 ppm.

MT concentration during the sex reversal period had little effect on percentage body

fat and percentage body moisture at the end of the post sex reversal period (Fig. 3). On the other hand, percentage body protein decreased significantly. These results indicate that MT treatment during the post sex reversal period had a greater effect on body composition at the end of the post sex reversal period than did MT concentration during the sex reversal period.

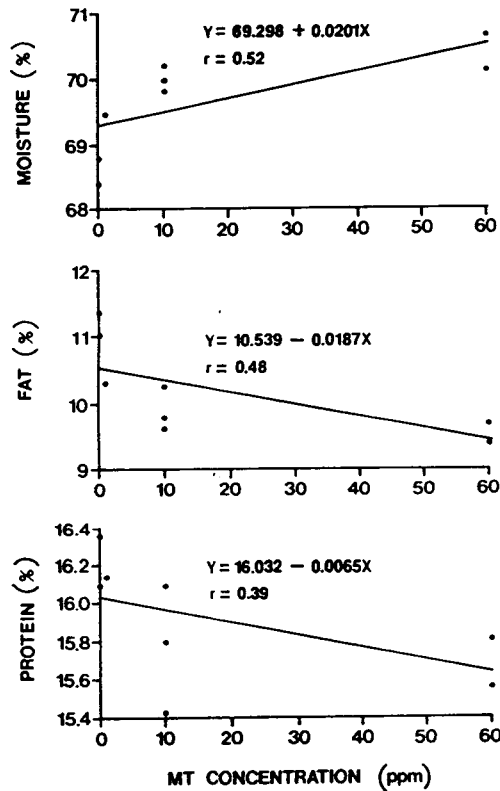


Fig. 2. Relationships between 17 $\alpha$ -methyltestosterone (MT) concentrations in the diet during the post sex reversal period and mean percentage body moisture, mean percentage body fat, and mean percentage body protein in blue tilapia, *Oreochromis aureus* at the end of the post sex reversal period.

Jo et al. (1988) showed that MT had significant positive anabolic effects on weight gain of *Oreochromis aureus*. These results suggest that the significant increase in body weight observed by Jo et al. (1988) were due to an increase in moisture content, not protein or fat.

In contrast, Fagerlund and McBride (1975, 1977) found that percentage fat increased in MT-treated steelhead trout, pink salmon, and coho salmon, but percentage protein

was unaffected. Matty and Lone (1979) found that fat and protein content of MT-treated common carp increased. Degani (1985) reported that MT-treated European eel had less fat, but an increased protein level. Gannam (1988) found that MT-treated channel catfish gained less fat but more protein than untreated controls. The inconsistency of these results may be due to species-specific differences or differences in experimental protocols.

Table 2. Effects of various concentrations and durations of 17 $\alpha$ -methyltestosterone (MT) on mean percentage body protein, mean percentage body fat, and mean percentage body moisture of blue tilapia, *Oreochromis aureus* at the end of the grow-out period

Treatment*	Protein	Fat	Moisture
0-0	16.4	11.4	68.4
0-10	16.1	9.8	70.0
0-60	15.8	9.7	70.1
1-1	16.1	10.3	69.5
10-10	15.4	9.6	70.2
60-0	16.1	11.1	68.8
60-10	15.8	10.3	69.8
60-60	15.6	9.4	70.7

\* The first number refers to ppm MT during the sex reversal period ; the second number refers to ppm MT during the post sex reversal period.

Table 3. Effects of various concentrations and durations of 17 $\alpha$ -methyltestosterone (MT) on mean gonosomatic indices (GSI) of male and female blue tilapia, *Oreochromis aureus*

Treatments*	Male GSI	Female GSI
0-0	0.097	0.160
0-10	0.051	0.075
0-60	0.051	0.076
1-1	0.091	0.114
10-10	0.048	—
60-0	0.083	—
60-10	0.035	—
60-60	0.023	—

\* The first number refers to ppm MT during the sex reversal period ; the second number refers to ppm MT during the post sex reversal period.

Effects of MT on GSI's of males and females are listed in Table 3. Consumption of MT adversely affected gonadal development of both sexes. These results are similar to those observed in brown trout (Ashby 1957), coho salmon (McBride and Fagerlund 1973 ; Fagerlund and McBride 1975 ; Billard et al. 1982), and *Oreochromis aureus* (Eckstein and Spira 1965).

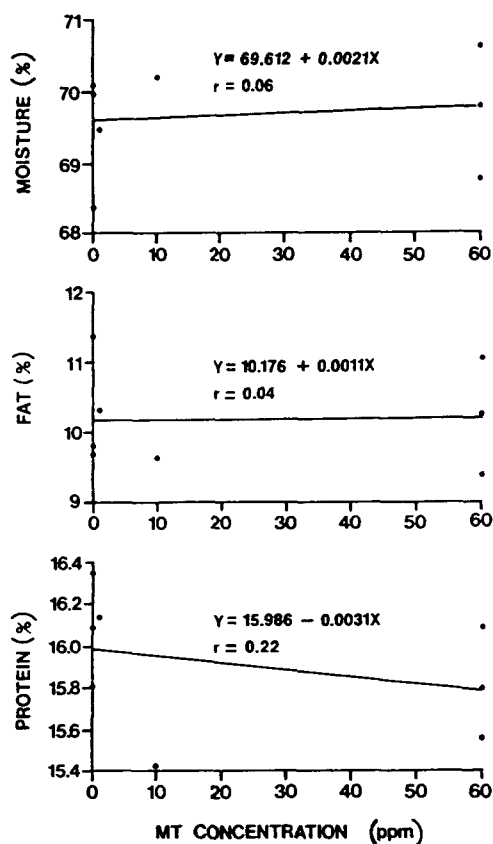


Fig. 3. Relationships between 17 $\alpha$ -methyltestosterone (MT) concentrations in the diet during the sex reversal period and mean percentage body moisture, mean percentage body fat, and mean percentage body protein in blue tilapia, *Oreochromis aureus* at the end of the post sex reversal period.

There was a significant inverse relationship between MT concentrations, during both the sex reversal and post sex reversal periods, and GSI's of males (Fig. 4). Mean GSI for the 0-0 group was 1.9 times greater than those of the 0-10 and 0-60 groups. These groups were treated identically during the sex reversal period, but not during the post sex reversal period. Mean GSI's of the 60-10 and 60-60 groups were 58% and 72% less than that of the 60-0 group, respectively. These groups were treated identically during the sex reversal period, but were treated differently during the post sex reversal period. These results indicate that increasing concentrations of MT during the post sex reversal period adversely affected gonadal development.

Consumption of MT during the sex reversal period also adversely affected gonadal development. Mean GSI of the 0-10 group was 5% and 44% greater than those of



10–10 and 60–10 groups, respectively. Mean GSI of the 10–10 group was 37% greater than that of the 60–10 group. Mean GSI of the 0–0 group was 18% greater than that of the 60–0 group.

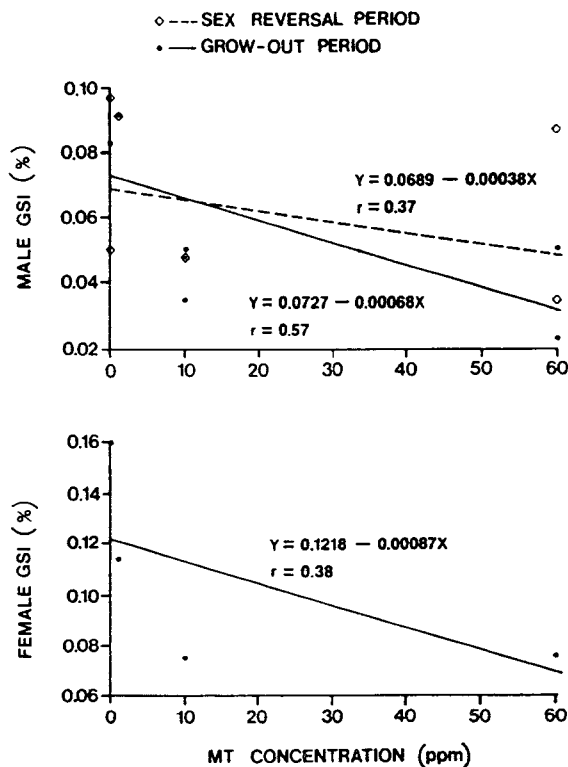


Fig. 4. Relationships between 17 $\alpha$ -methyltestosterone (MT) concentrations, during both the sex reversal and post sex reversal periods, and gonosomatic indices (GSI) of male blue tilapia, *Oreochromis aureus* and the relationship between MT concentrations during the post sex reversal period and GSI of female blue tilapia.

There was an inverse relationship between MT concentrations during the post sex reversal period and GSI's of females (Fig. 4). GSI of the 0–0 group was 1.4, 2.1, and 2.1 times greater than those of the 1–1, 0–10, and 0–60 groups, respectively. Because only two MT treatments during the sex reversal period produced females, the regression between MT concentration during the sex reversal period and female GSI was not determined.

#### ACKNOWLEDGMENTS

This research was done while the senior author was on a Fulbright Scholarship. Additional

support for the research was provided by New Jersey Marine Sciences Consortium (USAID) Project No. AID/NE-CA-1706. We thank J. C. Williams for assistance with data analysis and Katherine B. Tave for critical review of the manuscript. This paper is AAES Journal No. 8-881930P.

## 요 약

청틸라피아 (*Oreochromis aureus*)의 자어에 0, 1, 10, 60 ppm의 17 $\alpha$ -methyltestosterone (MT)을 30 일간 사료에 섞어 먹인 후 다음 57 일간을 0 ppm을 먹인 그룹은 다시 세그룹으로 나누어 0 ppm MT (0-0), 10 ppm MT (0-10), 및 60 ppm MT (0-60)을 먹였고, 60 ppm 을 먹인 그룹은 다시 3 그룹으로 나누어 0 ppm MT (60-0), 10 ppm MT (60-10), 및 60 ppm MT (60-60)을 먹였으며, 초기 30 일간 1 ppm과 10 ppm MT를 먹인 것은 계속해서 1과 10 ppm을 먹여서 체조성과 생식소 중량지수에 미치는 영향을 조사하였다.

초기 30 일간 어체의 조단백과 조지방은 MT 농도와 역상관 관계를 보였고, 수분 함량은 순상관 관계를 보였다. 후기 57 일간에 먹인 MT는 초기 30 일간 먹인 MT 보다도 숫컷의 체조성에 더 큰 영향을 미쳤다. 사료 중의 MT는 초기 30 일간이나 후기 57 일간 모두 숫컷의 생식소 중량지수에 나쁜 영향을 보인 반면에, 암컷의 경우에는 후기 57 일간에 사료 중의 MT 양이 생식소 중량지수에 나쁜 영향을 나타내었다.

## REFERENCES

- Ashby, K. R., 1957. The effect of steroid hormones on the brown trout (*Salmo trutta* L.) during the period of gonadal differentiation. J. Embryol. Exp. Morph. 5 : 225~249.
- AOAC (Association of Agricultural Chemists), 1950. Official methods of analysis, seventh edition, Washington, D.C., USA.
- AOAC (Association of Official Analytical Chemists), 1975. Official methods of analysis, twelfth edition, Washington, D.C., USA.
- AOAC (Association of Official Analytical Chemists), 1984. Official methods of analysis, twelfth edition, Washington, D.C., USA.
- Billard, R., M. Richard and R. Rombauts, 1982. Inhibition of spermatogenesis and vitellogenesis in rainbow trout by hormonal additives in the diet. Prog. Fish-Cult. 44 : 15~18.
- Degani, G., 1985. The influence of 17 $\alpha$ -methyltestosterone on body composition of eels (*Anguilla anguilla* (L.)). Aquaculture 50 : 23~30.
- Degani, G., 1986. Effect of dietary 17 $\beta$ -estradiol and 17 $\alpha$ -methyltestosterone on growth and body composition of slow-growing elvers (*Anguilla anguilla* L.). Comp. Biochem. Physiol. 85A : 243~247.
- Eckstein, B. and M. Spira, 1965. Effect of sex hormones on gonadal differentiation in a cichlid, *Tilapia aurea*. Biol. Bull. 129 : 482~489.
- Fagerlund, U. H. M. and J. R. McBride, 1975. Growth increments and some flesh and gonad characteristics of juvenile coho salmon receiving diets supplemented with 17 $\alpha$ -methyltestosterone. J. Biol. 7 : 305~314.

- Fagerlund, U. H. M. and J. R. McBride. 1977. Effects of 17 $\alpha$ -methyltestosterone on growth, gonad development, external features, and proximate composition of muscle of steelhead trout, coho, and pink salmon. Fish. Mari. Serv. Tech. Rep. 716 : 37.
- Fagerlund, U. H. M., D. A. Higgs, J. R. McBride, M. D. Plotnikoff and B. S. Dosanjh, 1980. The potential for using the anabolic hormones 17 $\alpha$ -methyltestosterone and (or) 3, 5, 3', -triiodo-L-thyronine in the fresh water rearing of coho salmon (*Oncorhynchus kisutch*) and the effects on subsequent seawater performance. Can. J. Zool. 58 : 1424~1432.
- Gannam, A. L., 1988. Hormones as feed supplements for growth enhancement in channel catfish. Doctoral dissertation, Auburn University, Alabama, USA.
- Howerton, R. D., D. K. Okimoto and E. G. Grau, 1988. Changes in the growth rate of the tilapia, *Oreochromis mossambicus*, following treatment with the hormones, triiodothyronine (T<sub>3</sub>) and testosterone. In : Pullin, R. S. V., T. Bhukasawan, K. Tonguthai, and J. L. Maclean, Editors. The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines. p. 598.
- Hunter, G. A. and E. M. Donaldson, 1983. Hormonal sex control and its application to fish culture. Pages 223~303. In : Hoar, W. S., D. J. Randall, and E. M. Donaldson, editors. Fish Physiology, Vol. 9, Reproduction, Part B, Behavior and Fertility Control, Academic Press, New York, New York, USA.
- Jo, J.-Y., R. O. Smitherman and L. L. Behrends, 1988. Effects of dietary 17 $\alpha$ -methyltestosterone on sex reversal and growth of *Oreochromis aureus*. In : Pullin, R. S. V., T. Bhukasawan, K. Tonguthai, and J. L. Maclean, Editors. The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines. pp. 203~207.
- Matty, A. J. and K. P. Lone, 1979. The effect of androgenic steroids as dietary additives on the growth of carp (*Cyprinus carpio*). Proc. World Maricult. Soc. 10 : 735~745.
- McBride, J. R. and U. H. M. Fagerlund, 1973. The use of 17 $\alpha$ -methyltestosterone for promoting weight increases in juvenile pacific salmon. J. Fish. Res. Board. Can. 30 : 1099~1104.
- Muhaya, B. B. M, 1985. Growth comparisons of *Tilapia nilotica* males produced through oral administration of methyltestosterone at varying levels and durations. Master's thesis, Auburn University, Alabama, USA.
- Shelton, W. L., K. D. Hopkins and G. L. Jensen, 1978. Use of hormones to produce monosex tilapia for aquaculture. Pages 10~33. In : Smitherman, R. O., W. L. Shelton, and J. H. Grover, editors. Symposium on Culture of Exotic Fishes. Fish Culture Section, American Fisheries Society, Auburn, Alabama, USA.
- Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics : A Biometrical Approach. McGraw-Hill Book Co., New York, USA.