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Effects of 17 Alpha-Methyltestosterone on Growth, Survival and Sex Reversal of Thai Walking Catfish, *Clarias macrocephalus*

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ABSTRACT

Fry of *Clarias macrocephalus* were fed at first feeding with water fleas (*Moina* sp.) immersed for 20 minutes in 200 ppm or 400 ppm MT for 15 days. The groups fed water flea (200 ppm MT) were fed a diet containing 30 mg MT and the groups fed water flea (400 ppm MT) were fed 60 mg MT, for the additional 15 or 45 days. Results indicated that administration of MT did not affect growth ($P>0.05$) at 33 days but retarded growth at 63 days of all treated groups. Growth of treated groups was increasingly suppressed with increased dose and duration of treatment. Administration of MT lowered survival rate at 33 days ($P<0.05$). Survival from day 33-63 was unaffected by the hormone application. Ovotestes were found in the groups treated with 30 and 60 mg MT for 60 days. In general, sex reversal to maleness was not accomplished, and with increasing dose and duration there appeared to be paradoxical feminization. Alternatively, MT may have prevented gonads of genetic males from differentiation or caused the formation of ovotestes in genetic females.

Keywords : sex-reversal, *Clarias macrocephalus*, 17 α -methyltestosterone

INTRODUCTION

Thai walking catfish, *Clarias macrocephalus* is the most acceptable among 3 species of *Clarias* being cultured in Thailand due to its superior meat quality. Slow growth rates and disease susceptibility of Thai walking catfish make or hinder culture of this species difficult. Selection for fast growth (Jarimopas *et al.*, 1988) and increased disease resistance (Na-Nakorn, unpublished) have not improved these traits in Thai walking catfish.

First generation gynogenetic stocks of Thai walking catfish have been produced at Fish Genetics Laboratory, Department of Aquaculture. Crossbreeding of highly inbred gynogenetic common carp, *Cyprinus carpio*, lines can result in high yielding crossbreeds (Nagy *et al.*, 1984). The gynogenetic lines of Thai walking catfish might also be crossed to produce fast growing crossbreed.

However, gynogenetic individuals of this species are all females (Na-Nakorn, unpublished). Sex reversal of a portion of these females (presumed genotype of XX) to males will be necessary to allow crossing of the gynogenetic lines.

A synthetic steroid, 17 alpha-methyltestosterone (MT) is an androgen which has been frequently and successfully used for sex reversal of fish to all-male populations. Different results were reported depending on dosage of MT, route of administration, initiation time, treatment duration (for review see Hunter and Donaldson 1983; Chan and Yeung, 1983) as well as experimental environment (Nagy *et al.*, 1981).

The objectives of this study were to evaluate the effects of dosage and duration of treatment of 17 alpha-methyltestosterone on growth, survival rate and sex reversal of Thai walking catfish.

MATERIALS AND METHODS

Fish Fry

Thai walking catfish fry were obtained from artificial fertilization of eggs and sperm collected from hormone injected brooders (3000 IU HCG/kg in females; and 300 IU HCG/kg in males). A female was stripped 16 hours later while a male was dissected and testis was collected.

Culture Conditions

Thai walking catfish fry from one family were

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stocked in three replicates of 250 l fiber glass tanks per treatment at the rate of 250 fry/tank. At day 34 stocking density was adjusted to 40 fish/tank due to high mortality of MT treated groups.

Fish were reared in stagnant water (28-30°C) and 30 % of the water was changed daily. Water level was 30 cm in the first month. It was increased monthly to 60 and 90 cm.

Hormone Treatment

Treatments included fry fed diets supplemented with either 30 or 60 mg MT/kg for either 30 or 60 days. Controls received the same diets without supplement of MT. Beginning at 3 days post-hatch fish in each tank were fed *ad libitum*, twice daily, with live cladocerans (*Moina* sp.) immersed in either 200 or 400 ppm MT for 20 minutes since Thai walking catfish fry do not accept artificial feed until 2 weeks post-hatch. This procedure was successfully used to sex reverse Siamese fighting fish using dihydrotestosterone (Tangtrongpairoj *et al.*, 1987). The fry were fed the cladocerans for 15 days. Then the fry fed the cladocerans treated with 200 ppm MT were fed a locally available 40 % protein powdered feed for either 15 or 45 additional days which 30 mg MT/kg of feed had been added by dissolving the MT in ethanol, mixing the feed followed by air drying. Fry feed cladocerans treated with 400 ppm MT were then fed the same feed treated with 60 mg MT/kg feed for either 15 or 45 additional days. At the end of the treatment period, fry were fed the same feed without MT for 30 days. Gonads of the fish were histologically examined at this time.

Histological studies were made at 1 month after termination of hormone administration to investigate permanent effects of hormone. Fifteen fish were randomly sampled from each treatment.

Samples were obtained at day 63 in controls and the groups treated for 30 days and at day 93 in the groups treated for 60 days and control. The fish were fixed in Bouin's solution and stored in 50 % isopropyl alcohol for histological examination. The tissues were embedded in paraplast paraffin and section (5 μm) were stained with hematoxylin and eosin for examination.

Data Analysis

Body weight of 25 % of the experimental fish in each replicate tank was measured at day 30 and 60 and survival rate was determined accordingly. Mean body weight and survival rates were analyzed using a one-way analysis of variance. Comparison was made using Duncan's new multiple range test (Steel and Torrie, 1980). Sex ratios were analyzed using chi-square.

RESULTS

Growth

At day 33, mean body weight of MT treated fish did not differ ($P>0.05$) from that of the controls (Table 1). Growth retardation ($P<0.05$) of the MT treated groups was observed at day 63 compared to the controls. As the level of MT in diets increased mean body weight decreased ($P<0.05$). The observed means for the longer durations of hormone administration were smaller than those for the shorter duration (Table 1). The growth reduction ranged from 32 % for the 30 mg MT treatment for 30 days to 69 % for the 60 mg MT treated for 60 days compared to the controls. Survival rate of MT treated groups at day 33 was lower ($P<0.05$) than that of the control (Table 1). The reduction in survival ranged from 64-80 % in the MT treated groups compared to the controls. No difference in survival rate was observed in the treated and untreated groups from day 33 to day 63.

Ratio of sex phenotypes

Number of sex phenotypes observed in each treatment are shown in Table 2. Sex ratio of the control and treated groups at 63 and 93 days did not deviate from the expected 1:1 ratio. Deviation from the expected 1:1 ratio was observed only in the 60 mg-60 D group where 9 individuals with female gonads were identified and no male individuals were found. Undifferentiated gonads were observed in all groups sacrified at day 63 (the 30 mg treatments) including the control. The largest number of undifferentiated individuals was found in the 60 mg MT treatment. At day 93 every fish in the control group could be identified sexually while undifferentiated gonads were still found in the MT treated groups. Two intersex gonads were observed in the 30 mg and 60 mg-groups treated for 60 days.

Histology of gonads at day 63

Testes of the untreated group were not visible macroscopically and characterized by the long club shape appearance with a stalk attached to peritoneum. Cell differentiation was not observed. Ovaries of control group were much larger than testes. Most of germ cells were previtellogenic oocytes, a few oogonia were observed.

The fish treated with 30 mg MT/kg diet for 30

Table 1 Effects of 17 alpha methyltestosterone on mean body weight and survival rate of Thai walking catfish, *Clarias macrocephalus*, grown in 250 liter tanks.

Dosage and duration	mean body weight (g)* (days post-hatch)		survival rate (%) (days post-hatch)	
	day 33	day 63	day 3-33	day 33-63
Control	0.51 ± 0.30 a	6.97 ± 2.93 a	81.80 ± 0.85 a	85.00 ± 0.00 a
30 mg/kg -30D	0.47 ± 0.15 a	4.74 ± 1.91 b	29.73 ± 11.71 b	80.00 ± 17.50 a
30 mg/kg -60D	0.56 ± 0.17 a	2.53 ± 1.04 c	17.40 ± 5.94 b	83.75 ± 19.44 a
60 mg/kg -30D	0.42 ± 0.11 a	3.25 ± 1.28 bc	16.00 ± 6.61 b	74.17 ± 29.19 a
60 mg/kg -60D	0.41 ± 0.15 a	2.16 ± 1.17 c	17.86 ± 8.77 b	62.50 ± 31.80 a

* Means in the same column followed with different letters were different ($P<0.05$)

days had normal testes (Figure 1) although relatively small compared to controls. Ovaries of this group were smaller than those of control individuals. Normal anatomy of the ovary was characterized by pear shape appearance with 2 stalks attached to peritoneum (Figure 2). Ovaries contained few previtellogenic oocytes. A large amount of early stage-germ cells was observed relatively to the control indicating retardation of development in the treated fish.

Testes of the 60 mg-30 D groups were normal and did not differ from the control testes. Ovaries of this groups were much reduced in size. Histology of ovary did not differ from the 30 mg-30 D group.

Histology at day 93

At day 93, both testes and ovaries of the control groups were visible macroscopically. Testes were well developed. Every stage of germ cells were found including a few spermatozoa. Ovaries contained mostly previtellogenic oocytes and a few vitellogenic oocytes surrounded by well developed follicular layer.

In the 30 mg-60 D group, few ovaries were well developed. They contained every stage of germ cell development and included the advanced stage of vitellogenic oocytes characterized by a small nucleus in the middle, yolk granule occupying most of the area of the oocytes and the peripheral yolk vesicle. Follicular layers were well developed.

Most of ovaries in this group were extremely small and contained a few previtellogenic oocytes and no oocytes beyond this stage. Two regressed small in size and without any germ cells inside ovaries were identified.

Two types of testes in the 30 mg-60 D group were found. One was visible macroscopically. Histological studies showed vascularized area with a few developing spermatogenic cells and a few undeveloped oocytes were unexpectedly found (Figure 3). This was identified as an ovotestes and the individuals termed as intersex. Another type of testes was very tiny and visible only with the aid of an microscope without germ cell differentiation.

Ovaries of the 60 mg-60 D group were small, and retarded development of oocytes was marked. Vitellogenesis was not found. One ovary was found having an oocyte with nuclei undergoing irregular division as indicated by a cluster of 4 small nuclei with distinctly pink nucleoli (Figure 4).

An ovotestes was found containing male and female germinal cells which were intermingled. Every stage of germ cells undergone spermatogenesis was found including spermatozoa. The oocytes were in the previtellogenic stage (Figure 5). No testes were found.

DISCUSSION

Methyltestosterone which has been reported to enhance growth of various fish species such as Pacific salmon, *Oncorhynchus tschawytscha* (McBride and Fagurlund, 1973), common carp (Lone and Matty, 1980) and Nile tilapia, *Oreochromis niloticus* (Tayamen and Shelton, 1978) was shown to retard growth of the Thai walking catfish. Growth reduction has also been reported for channel catfish, *Ictalurus punctatus*, fed 5 and 10 mg MT/kg diet for 12 weeks. This is because the hormone inhibited protein synthesis and/or increased protein catabolism in the body (Simone, 1990). In our study the effects of this metabolic change were evident after the termination of hormone administration since the decreased growth of the groups treated for 30 days were observed at day 63 but not at day 33.

Degeneration of the renal tubules and many of

Table 2 Effects of 17 alpha-methyltestosterone on sex ratio of Thai walking catfish, *Clarias macrocephalus*.

Dosage and duration	total	male	female	intersex	undifferentiated
Control (for 30D-group)	15	5	7	-	3
30 mg-30D	11	4	5	-	2
60 mg-30D	13	1	6	-	6
Control (for 60D-group)	15	7	8	-	-
30 mg-60D	13	4	6 (2)**	1	2
60 mg-60D	13	-	9*(2)**	1	3

Note : * Significantly different from the phenotype ratio of the control ($P<0.05$)
** Number in parentheses indicates regressed (small without identifiable germ cells) ovaries

the renal corpuscles of MT treated channel catfish were also reported (Simone, 1990). Similar changes might have occurred in Thai walking catfish and thus caused the high mortality of the treated fish during the first 30 days period.

Presence of ovotestes generally indicates partial sex-reversal provides that a particular species follows a differentiated gonochorism type of reproduction (Chan and Yeung, 1983). Therefore, this revealed that oral administration of either dosage of 30 or 60 mg MT/kg diet for 60 days caused partial sex reversal of Thai walking catfish, however, only one ovotestes of 13 gonads inspected was found in each group. Direction of sex reversal can not be absolutely determined since original sexual phenotype could not be identified. The two possibilities are either a transition from ovary to testes or vice versa. The most likely explanation is that the testes were being feminized, paradoxical feminization (Goudie *et al.*, 1983). When compared sex-ratio of the 60 mg-30 D treatment with that of the control, less male but more undifferentiated gonads were found in the first group while numbers of females in each group were comparable. MT may have been broken down into an estrogenic compound preventing differentiation of the genetic males which should have been observed at this time. By day 63 all of the controls had differentiated, 47 % females and 53 % males. However, the 60 mg-60 D treatment yielded no males, nine females, one intersex and three undifferentiated. There was a significant ($P<0.05$) shift from the 1:1 sex ratio to femaleness, intersex individuals and undifferentiated individuals. A definite trend away from maleness existed indicating possible feminization.

Paradoxical feminization was also observed in African catfish, *Clarias gariepinus*, when 17 alpha - methyltestosterone was administered as a bath, 1-100 µg/l, for 28 days at the early development, 14 days post-hatch (Van Den Hurk *et al.*, 1989). The effect of alpha-methyltestosterone was altered in *C. gariepinus* by timing of administration. When treatment of 3-300 µg/l of 17 alpha-methyltestosterone were initiated 28 days post-hatch the sex ratio shifted slightly towards maleness (Van Den Hurk *et al.*, 1989).

Again, similar to our results, dosage and duration of treatment were important on paradoxical feminization of rainbow trout, as this phenomenon occurred only (Solar *et al.*, 1984) when MT was applied at high dosage for prolonged periods. Paradoxical feminization has also been observed in channel catfish (Goudie *et al.*, 1983), and in various cichlids (Reinboth, 1969; Hackmann and Reinboth, 1974; Nakamura, 1975) when androgens were applied.

An alternative explanation for the observed sex ratios and phenotypes in the current study is that MT has partially sex - reversed genetic females to males resulting in individuals with ovotestes. Ovotestes of 60 mg - 60 D group was active, mature germ cells were present and spermatogenesis observed. Previtellogenic oocytes found may be the remnant of the female germinal cells as has been shown in the maturing - male phase of protogynous serranids, *Epinephelus akaara* (Chan and Yeung, 1983). This explanation is partially confirmed by observation of the ovotestes in the 30 mg - 60 D group which had regressed oocytes that were almost extinct.

However, there was a possibility that the ovotestis found in the 60 mg-60 D treatment may not be an indication of sex reversal since an ovotestis histologically resembled the one found in this treatment was recently observed among 50 fish in the untreated population of Thai walking catfish at Department of Aquaculture (Na-Nakorn, unpublished). More incidences are needed to confirm this explanation.

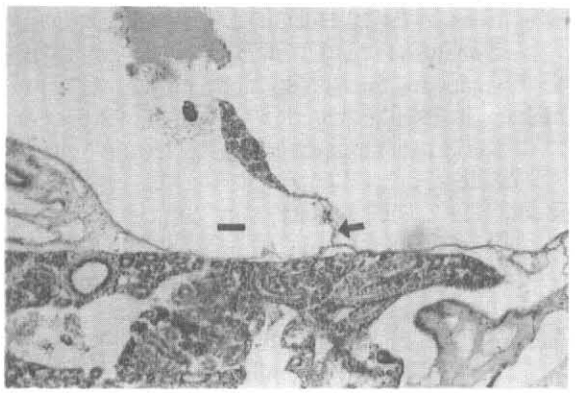

Figure 1 Testes at day 63, of *Clarias macrocephalus* treated with 30 mg of 17 alpha-methyltestosterone for 30 days showing typical anatomy of testes. Notice a stalk (arrow) attached to peritoneum. Scale = 10 microns

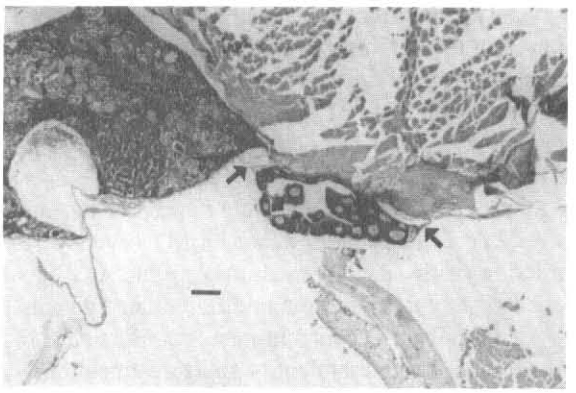

Figure 2 Ovary at day 63 of *Clarias macrocephalus* treated with 30 mg of 17 alpha-methyltestosterone for 30 days showing typical anatomy of the ovary with two stalks (arrows) attached to peritoneum. Scale = 10 microns

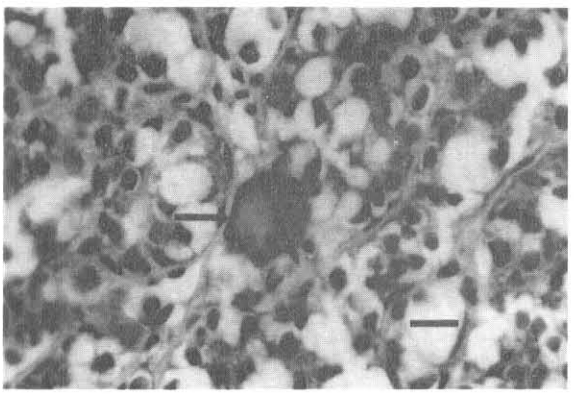

Figure 3 An ovotestes found at day 93 in *Clarias macrocephalus* treated with 30 mg of 17 alpha-methyltestosterone for 60 days. Notice regressed oocyte (arrow) and retarded spermatogenic cells. Scale = 10 microns

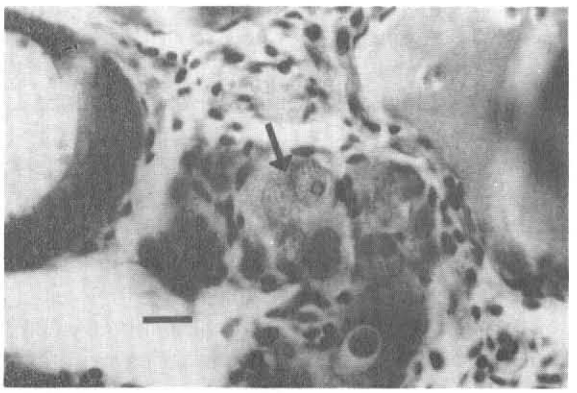

Figure 4 An ovary at day 90 in *Clarias macrocephalus* treated with 60 mg of 17 alpha-methyltestosterone for 60 days showing an area in which irregularly divided nuclei were found (arrow). Scale = 10 microns

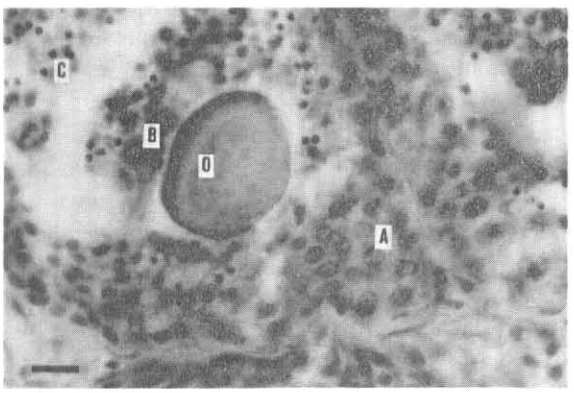

Figure 5 An ovotestes found at day 93 in *Clarias macrocephalus* treated with 60 mg of 17 alpha-methyltestosterone for 60 days showing spermatocytes (A), spermatids (B), spermatozoa (C), and oocyte (O). Scale = 10 microns

The irregular nuclear division found in one ovary of the 60 mg-60 D group could represent spermatogenesis. In coho salmon, *Oncorhynchus kisutch*, clusters of primary and secondary spermatocytes have been observed in some ovaries of MT treated fish (Higgs *et al.*, 1977).

Individuals with undifferentiated gonads may have been genetic males in which MT severely retarded testicular development making the small gonads indistinguishable from undifferentiated gonads.

Partial sex reversal may be a consequence of low dosage, early treatment or short treatment duration. Regressed gonads were only found in the groups treated for 60 days. Similarly, regressed gonads were observed in tawes, *Puntius gonionotus* treated with MT for 60 days with 30, 60 or 120 mg MT/kg diet compared to tawes treated for 30 days (Na-Nakorn and Sangsri, unpublished). Long treatment periods apparently cause the regressed gonads.

The dosage used may not be too low since retardation of gonads was observed in every treatment. MT administration may have been too early. Successful sex reversal may be achieved when effective dosage have been applied just before the onset of gonadal differentiation and continued until the time of morphological differentiation (Yamamoto, 1969). Sex differentiation of Thai walking catfish may occur late so that the treatment at day 3 may have been too early. Size may be more important than age for determining the onset of sex differentiation (Dunham, 1990). Even in the 60 days treatment, the *C. macrocephalus* fry may not have received MT for a sufficient period in relation to the timing of sex differentiation, or since MT severely retarded their growth they may have been too small to sexually differentiate leading to the unsuccessful sex - reversal.

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