EFFECTS OF DIETARY 17α -METHYL TESTOSTERONE ON SEX REVERSAL AND GROWTH PERFORMANCE OF AFRICAN MUD CATFISH (C*larias gariepinus)*

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Abstract
*The search for faster growing fish with higher weight at harvest and higher yield is crucial to profitable fish culture. Male fishes are known to grow bigger than female fishes of same age. This study determined the effects of 17alpha-methyltestosterone (MT), an androgen on the sex reversal and growth performance of the African mudcatfish (Clarias gariepinus). The research was conducted at the faculty of Agriculture laboratory and the experimental fish tanks of Department of Aquaculture and Fisheries management, University of Benin, Edo Nigeria. T*he hormone 17 alpha – methyl testosterone was administered orally at constant rates of 30 mg/kg of feed and 60 mg/kg of feed for two time durations (28 and 35 days respectively) *The MT treated fish were transferred outdoor and cultured for another 24 weeks where the effect of MT on percentage male to female, weight gain, survival rate, absolute specific and relative growth rate and net production were recorded. The fishes were evaluated to determine their sex by physical observation of the genital papilla. Data collected for the fish growth performance were analyzed and subjected to analysis of variance and significant means were separated using Duncan Multiple Range Test (DMRT). All treatment groups that received androgen 17 alpha methyltestosterone (MT) showed sex reversal in C. gariepinus with significantly (P<0.05) higher male population than female population. There was also significant difference (P<0.05) in growth performance in terms of weight gain compared with the control. The 60 mg (35 days) treatment resulted in 80.86 % male populations. 17-alpha methyltestosterone (MT) treatment of C. gariepinus resulted in mean yield of 3950 g /m^2 /180 days culture period and 4226 g /m^2 /180 days culture period respectively for treatment duration of 28 and 35 days of 30 mg dose while 60 mg dose for respective 28 and 35 days treatment duration gave 4474 g, 5531 g as against 3295 g /2m /180 days culture period recorded from the control group. Thus, higher fish yield was recorded for higher MT dosage and higher duration of application.*

Keywords: 17α -methyl testosterone (MT), African mudcatfish (Clarias gariepinus), sex reversal, growth performance,

INTRODUCTION

The sex of fish can be significant in aquaculture because of differences between male and female fish in terms of growth rate, size, behavioral patterns and breeding time. In the African mud catfish (*Clarias gariepinus),* males grow faster and reach a larger final size than females (Dekimpe and Micha, 1974). Therefore, it would be commercially advantageous to produce all male catfish populations. Administration of exogenous steroids can be effective in controlling sexual development (Al-albani and Phelps, 2002). 17α -methyltestosterone is a synthetic specific hormone commonly used to induce sex reversal in teleost fish. Steroid treatment sometimes generates some problems in marketing and public health concern (Tave, 1992). It should be noted however, that administered synthetic steroids are eliminated from the fish in short term period and thus pose no harmful effect to the fish or consumers (Tave, 1992).

Global expansion of aquaculture requires species or stocks more adapted to localized conditions and less likely to affect genetic diversity of natural populations in the event of escape. Sex control technologies (including direct endocrine and genetic manipulations (gynogenesis, androgenesis, induced polyploidization) to prevent uncontrolled reproduction as well as to capitalize on differential culture performances of the sexes, have been reviewed by Piferrer (2001) and Beardmore *et al.* (2001). In the present study the amenability of the African mud catfish (*Clarias gariepinus)* to sex reversal during embryogonesis and the feasibility of producing a monosex male population by hormonal diet treatment was investigated. Specifically, the objective of this study was to examine: impact of various dosages and duration of 17α -methyl testosterone on sex reversal and growth performance of *C. gariepinus*.

MATERIALS AND METHOD

This study was conducted in two phases; (i.e Phase I, Sex reversal and Phase II, growth study phase), in order to determine the optimal duration and dosage of oral administration of androgen, 17 alpha–methyl testosterone (MT), for sex reversal of catfish *Clarias gariepinus*. In Phase 1 of the experiment, the hormone 17 alpha – methyl testosterone was administered at constant rates of 30 mg/kg and 60 mg/kg of feed for two time durations

(28 and 35 days respectively). In Phase 2 of the experiment, the growth performance in terms of yield and growth rates were examined by culturing the hormone treated fish seed for 180 days.

Fry Collection and Sex Reversal

Forty-eight (48) hours after hatching, the fry of *C. gariepinus* were collected, transferred and stocked at the rate of 60 fry per 40 litre capacity plastic container. A total of 15 plastic rearing Units were used.

Feed containing 40 % crude protein and 17alpha-methyltestosterone was used in the experiment. The hormone was mixed with finely grounded feed using the alcohol evaporation method (Guerroro, 1975). A control treatment, which was feeding of fish with feed without hormone made up the third treatment.

Feeding

Fry in each treatment group along with its replicates were fed formulated feed to satiation. Ration was fed 3 times daily i.e. at 7.00hr, 13.00 hr and 17.00hr. After 40 minutes of application of feed, the remaining feed and excreta were usually removed from the container by siphoning.

Stocking of Fish and Feeding of Fish during Grow - out

The fishes were transferred into a partitioned concrete tank. The tank was partitioned using "mosquito net" of a dimension of 1 m x 1.2 m 1.5 m. The tank was filled with borehole water up to the level of 1.0 m and this level was maintained throughout the experimental period by the changing of borehole water at frequent intervals as influenced by environmental factors (i.e when secchi disc reading is less than 30 cm).

Fishes were fed 40 % crude protein formulated feed to satiation twice daily at 8:00 hr and 16:00 hr

Parameters measured

The bi-weekly weight gain was measured and the following parameters were computed;

a) Net production (g) = $W_2 - W_1$
b) Increase in total length (cm) = $L_2 - L_1$
c) Specific growth rate (SGR)

$$SGR = \frac{\ln (W_2 - (W_1)}{t(days)}$$

d) Standing crop = W_2
e) Absolute growth rate = $\frac{W_2 - W_1}{t(days)}$
f) Relative growth rate (% RGR) = $\frac{W_2 - W_1}{W_1} \times 100$ (Ahmed *et al*., 2002)

Where:

W_2 = Final weight, W_1 = Initial weight, L_2 = Final length, L_2 = Initial length, t = Time (days)/duration.

The sex of fish was established through physical examination of the genital papilla.

Experimental Design and Statistical Analysis

The experiment was laid out in a complete randomized design with one control and 4 treatments (i.e. 30 mg/28 days, 30 mg/35 days, 60 mg/28 days and 60 mg/35 days MT) replicated thrice.

Data collected were subjected to analysis of variance. The separation of means was carried out using the Duncans multiple range tests (Steel and Torrie, 1980) at 5 % level of probability.

RESULT

Sex Distribution

The result of the evaluated sex of *Clarias gariepinus* under all the sex reversal treatment and the control is shown in Figure 1. The control treatment showed an observed sex ratio of *Clarias gariepinus* with a 53.35 % male and to 46.65 % female (Figure 1), while all other treatments with MT showed a significant difference ($P<0.05$) in sex ratio with higher male proportion as compared to the control treatment. The female population was higher in control than all treatment with MT. The male population increased with increase MT dosage and increase in period of administration. The treatment with 60 mg MT administered for 35 days show the highest number of male compared to the female followed by the treatment with 60 mg MT administered for 28 days. Also treatment with 30 mg MT administered for 35 days had higher male population than treatment with 30 mg MT administered for 28 days. Thus male population increased with hormonal dosage and increasing duration of administration.

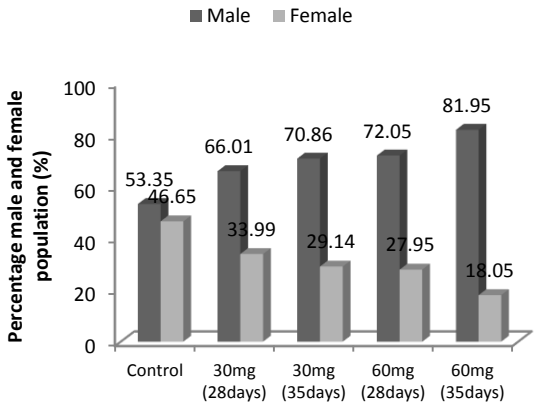

Figure 1: Number of surviving male and female at harvest treated with MT.

Effect of varying dosage and length of treatment of 17ȁ-Methyl Testosterone (MT) on bi weekly weight change of *Clarias gariepinus* of overtime

The mean growth rate of *Clarias gariepinus* over time is shown in Table 1. The treatments had significant ($P<0.05$) effect on the mean weight gain of the fish over time. Outstanding rise in weight was observed with groups treated with 60 mg/kg feed

dose of MT with mean total weight gain of 4474 g and 5531 for 60mg/kg feed administered for 28 and 35 days respectively. g. The mean bi weekly weight change increased significantly with increase in dosage and duration of MT overtime ($P < 0.05$). The 60mg/MT administered for 28 and 35 days were higher than that of 30 mg/MT and control.

Effect of MT dosage and duration on fish yield based on sex

The results on the effect of MT dosage and duration of fish yield is shown in Table 2. Fish yield also increased significantly with increase in dosage and duration of MT ($P < 0.05$) and the male fish significantly recorded higher yield than female fishes.

Absolute, Specific, Relative Growth Rate and Net Yield

The results of absolute, specific and relative growth rates are shown in Table 3. The result shows significant increase in these parameters with increasing dosage and duration of administration of MT to *C. gariepinus* ($P<0.05$). The net fish yield was also higher with increased dosage and duration ($P<0.05$).

Table 1: Effect of MT dosage and duration on mean bi-weekly weight change (g) of *Clarias gariepinus*

Treatment	Time (Weeks)													
	0	2	4	6	8	10	12	14	16	18	20	22	24	Mean Yield
Control	17	91^{ab}	298	760	1457	2350	3317^{b}	4783	3205^{b}	6067^{b}	6183	6903	7400^{b}	3295^{a}
30mg (28days)	21	113^{a}	277	1124	1800	2900	4383^{ab}	5450	6473^{ab}	5900^{b}	6550	7875	8483^{b}	3950^{ab}
30mg (35days)	23	74^{b}	353	992	1450	2849	3883^{ab}	5317	6250^{ab}	6967^{ab}	7817	9500	9467^{ab}	4226^{ab}
60mg (28days)	20	103^{a}	381	1045	1917	3333	4533^{ab}	5583	6477^{ab}	7000^{ab}	7967	9300	10500^{ab}	4474^{ab}
60mg (35days)	22	89^{ab}	370	1029	1867	3217	4917^{a}	6762	8450^{a}	9767^{a}	10517	11467	13433^{a}	5531^{b}
SEM±	1.746	7.38	62.5	266.5	171.8	346.3	421.6	616.1	997.7	892.4	1928.6	1590.6	1266.4	591.7

Values are least square means (± sem), n = 5

^{abc} Means with different superscripts within the same parametric row differ significantly ($P<0.05$)

Table 2: Effect of Various Dosage and Duration of 17$\dot{\alpha}$-Methyl Testosterone treatment on Fish yield

Sex	Treatment					Mean Sex
	30mg 28days	30mg 35days	60mg 28days	60mg 35days	Control	
Female	2883	2646	2935	2425	3452	2866.2^{b}±356
Male	5600	6821	7565	11008	3948	6988.4^{a}±2436
Mean Treatment (X)	4242.5^{d}±1488	4733.5^{c}±2287	5250^{b}±2536	6716.5^{a}±4701	3700^{e}±272	

Values are least square means (± sem), n = 5

^{abc} Means with different superscripts within the same parametric row and column differ significantly ($P<0.05$)

Table 3: Absolute, Relative and Specific Growth Rates

Treatments	Absolute Growth Rate (g/day)	Net Yield (g)	Relative Growth Rate (%)	Specific Growth Rate
Control	41.02^{b}	7383^{a}	45846	0.03300
30mg (28days)	47.68^{b}	8463^{a}	40547	0.03333
30mg (35days)	54.13^{ab}	9444^{ab}	43684	0.03300
60mg (28days)	58.22^{ab}	10480^{ab}	54117	0.03467
60mg (35days)	74.51^{a}	13411^{b}	60815	0.03533
SEM±	7.30	1742	8765.3	0.001633

Values are least square means (± sem), n = 5

^{abc}Means with different superscripts within the same parametric column differ significantly ($P<0.05$)

DISCUSSION

The result of this study revealed that each hormone treated group showed a mean male/female ratio that deviated significantly ($P<0.05$) from the usual 1:1 ratio, with the male ratio significantly higher than the females. The control group showed a normal 1:1 ratio. However, none of the dose or duration of MT used in this study gave a 100 % male population of *C. gariepinus*. This is in contrast with findings of Popma and Green (1990) who reported over 95 % males of *O. niloticus* when MT was used for sex reversal for 28 days. These results may indicate that *Clarias gariepinus* requires more dosage and time to achieve higher sex reversal rates.

In this study, the maximum male population (81.95 %) was obtained at a dose of 60 mg treated for 35 days, while the minimum male proportion (53.35 %) was recorded for the control. The dose rates of 60mg MT/kg feed for 28 days recorded (72.05 %) of male and 30 mg MT/Kg of feed for 35 days recorded (70.86 %) and 30 mg 28 days was 66.01 %. Thus, the optimum dosage and duration is yet to be achieved.

Turan and Cek (2007), reported similar result of less than 90 % proportion of male with the use of Gokshura (*Tribulus terrestis*) extract as a masculinization treatment for *C. gariepinus*. It was observed that at higher dosages of the treatment, masculinisation increased. The minimum of 3 g/30 L and 9 g/30 L of treatment in that study revealed 74.54 % and 80.42 % of males respectively. In this study, the hormone mixed feed was fed to the fish to satiation daily which resulted in less than 90 % male population.

Haniffa *et al.* (2004) observed the highest percentage conversion to males (82 %) of MT treatment at dose rate of 100 µg/L for *Heteropnneustes fossilis* (stinging catfish). They also observed the highest percentage conversion to males (80 %) of 17alpha – ethynyltestosterone (ET) treatment at dose rate of 100 µg/l for the stinging catfish. Eding *et al.* (1999) recorded 46% of sex reversed male with the use of MT treatment on *C. gariepinus* at 50 ppm dose rate and also conversion to males (75 %) of 11-Keto-androstenedione treatment on the fish. Thus, the dosage has significant effect on sex reversal rate.

Jay-Yoon *et al.* (1988) obtained 87 % *Oreochromis niloticus* males at dose rate of 10mg/kg of diet. Other authors have used the higher dose rates to sex reverse *O. niloticus*. Tayamen and Shelton (1978) obtained 99% to 100% males after treating *O. niloticus* for 25 days at 30mg and 60 mg/MT. Guerrero and Guerrero (1988) obtained 99 % males with 30 mg of MT fed at 21 days. Vera - Cruz and Mair (1994) reported 95% to 98% males with 40mg MT/kg of diet and 99 % with 60 mg MT/kg of diet fed at 20 % body weight for 25 days.

Obi and Shelton (1983) reported that androgen treatments yielded sex ratios that were significantly different ($P<0.05$) from 1:1. Percentages of males for MT treatments ranged from 94 % to 100 %; MT – 30mg was the most successful treatment yielding 100 % males at both 21 – and – 28 days duration on *Tilapia hornorum* formerly *T. mossambica* (Hickling, 1960).

Contrary to the results obtained in this study, Orn *et al.* (2003), Bhanderi *et al.* (2006) reported that androgen (including MT and ethynyltestosterone) treated southern catfish (*Silurus meridionalis*) at different dosages (10, 25 and 50 mg/kg) failed to induce sex reversal, both morphologically and histologically. More so, morphological abnormality and behavioral changes were observed where the fish became more aggressive and the continuous caudal fin became forked after treatment. Also, Davis *et al.* (1990) reported that, neither aromatisable nor non-aromatisable androgen could induce female to male sex reversal in channel catfish (*Ictalurus punctatus*). These reports were attributed to a possibility of partial inhibition of male gonad development and associated diets in agreement with observed feminizing action of MT and other testosterone derivatives on gonad developments in the catfish (Hurk *et al.*, 1989).

On the whole, varied reasons are presented to justify each observation as there is no general reason to explain this contradiction in the level of success rates between the species of fish (Tilapia and catfish). Notwithstanding, previous studies have shown that the amenability to hormonal induction of sex manipulation varied not only from species to species, but also from family to family (Haniffa *et al.*, 2004).

Generally, it has been observed that androgen can induce sex reversal in some species, such as the African mudcatfish (*Clarias gariepinus*; Raghuveer *et al*., 2006), the Nile tilapia (*O. niloticus*), (Bhandari *et al.*, 2006) and rainbow trout (Baron *et al.*, 2007), but at different success rates and dosages.

Improved growth rate was recorded in this study. This can be attributed to more male population. According to Dekimpe and Micha (1974), males of African mud catfish grow larger than females, which can be attributed to the anabolic effect of androgen. The exogenous androgen (MT) also enhanced the growth of *Clarias gariepinus* by its stimulation of protein biosynthesis with increased dosage and duration of MT treatments. The potential of improving fish growth by natural and synthetic steroid treatments have been examined in several fish species such as *Oncorhynchus mykiss*, *Cyprinus carpio*, *Oreochromis niloticus*, *O. aureus* and *Perca flavescens* (Pandian and Sheela, 1995). Park *et al.* (2003), reported that tamoxifen-incorporated feed to access the relative growth promoting efficiency on bagrid catfish (*Pseudobagrus fulvidraco*) through pellets diets, and a promoted growth rate to a significant level compared to control and the 50 ppm concentration recorded a maximum growth rate. Furthermore, Turan and Akyurk (2005) recorded significant improved growth of African mud catfish (*Clarias gariepinus*) fingerlings receiving the 75 mg red clover (a phytoestrogen) kg^{-1} diet compared to control group. Moreover, growth-promoting effects of *Triubulus terrestris* extract (a phytoandrogen) on convict cichlid (*Cichlasoma nigrofasciatum*) (Cek *et al.*, 2007a) and guppy (*Poecilia reticulate*) (Cek *et al*., 2007b) have been recorded.

However, the primary indicator of growth performance in fish is fresh body weight (Mateen, 2007). In this study, the final body weight and net production of *C. gariepinus* after a period of 180 days of grow-out phase, showed a marked increase for 60 mg at 28 and 35 days dose rates and time durations of treatments of 17α-methyletestosterone (MT) than the control, clearly indicating that MT treatment enhances the growth rate and biomass production of *C. gariepinus* very efficiently. The treatment group of 60mg MT/kg of feed for 35 days showed the highest total fish production of 13411 g.

This fish production was 1.4 times greater than the control (received no hormone treatment) and the results are consistent with the findings regarding the anabolic effects of MT in fish (Mateen, 2007). Similar observation was also reported by Tayamen and Shelton (1978) who observed faster growth of hormone treated *O. niloticus.* Howerton *et al*. (1992) and Varadaraj *et al.* (1994) as well observed faster growth in *O. mossambicus* when fed MT.

The results in the present study indicated an increased trend towards total production of fish with an increase of MT dose of up to 60 mg/kg of feed. However, there could still be increase in fish production if the dose of MT is increased as shown in Figure 1. According to Kamler (1992), in a male or sterile population of fish, feed conversion is more efficient since feed is used for somatic growth only, and not for reproduction. Androgens promote both muscle growth and the development of male sexual characters. Growth is thereby improved by directing energy used for reproduction into the production of flesh. This fish flesh translates to the increased weight gained and yield recorded in this study as hormone dosage and duration increased. Survival of *C. gariepinus* ranged from 75.91 % to 86.87 % during the grow-out phase of this study. Data indicated no significant relationship between survival and MT treatment concentration or duration of treatments. Soto (1992), Vera-Cruiz and Mair (1994) also concluded similar results by observing that MT administration has no significant effect on survival in *O. niloticus*. Guerrero (1975), also observed that hormone treatment has no effect on survival in *O. aureues.* Dan and Little (2000) compared the culture performance of different strains of *O. niloticus* and found survival rate in all treatments ranged from 70.1 to 82.5 % and this was not significantly different with MT treatment. The findings of Pechsiri and Yakupitiyage (2005) are also similar in regard to survival rate (73.3-76.0 %) of *O. niloticus.*

The specific growth rate (SGR) of *C. gariepinus* ranged between 3.3 % (control) to 3.5 % (60mg MT/kg of feed for 35 days) during this study. Statistical analysis on SGR data revealed that all the MT treatments showed a non-significant effect on the (SGR) of fish. These findings are in accordance with the findings of Pechsiri and Yakupitiyage (2005) that recorded specific growth rate that ranged between 2.13 – 2.23 at 3 % of body weight feeding level for Nile tilapia and also reported no significant difference in (SGR) of this species.

Hormonal manipulation and breeding studies are capable of producing superior fish seeds of the desired genotype–phenotype characteristics to aquaculture production. However, these efforts require many years and abundant facilities to separately maintaining large numbers of offspring from various mating, and the ability to uniquely identify individuals which produce offspring of the selected genotype-phenotype combination. Any procedure which reduces generation time evaluation or eliminates a step in progeny sex ratio evaluation would have a significant impact on the success of these long-term experiments. Sex reversal is a viable breeding programme that has improved growth and quality of fish produced.

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