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Sex Reversal of Black-Chinned Tilapia (*Sarotherodon melanotheron*) Fry Using 17α-Methyltestosterone

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Abstract: This study was conducted to determine the effective dose of 17α-methyltestosterone (MT) that produces all-male fry of black-chinned tilapia, *Sarotherodon melanotheron*. Five concentrations: 0, 30, 60, 90 and 120 mg 17α-methyltestosterone (17α-MT) per kilogram feed were prepared using the ethanol evaporation method, and administered orally to black-chinned tilapia fry for 28 days in triplicate aquarium glass tanks. After the treatment period, triplicates of 60 fry from each treatment were stocked in hapas and further cultured in freshwater ponds. They were fed with commercial fish feed *ad libitum*. The sex ratio, growth performance and survival were evaluated after 60 and 180 days. At the end of the 60-day period, the sex ratio of 30 fish from each treatment was determined by using the gonadal squash method. The remaining 30 fish from each treatment were grown further for 180 days and their sex ratio determined using the genital papillae. The results demonstrated that 17α-methyltestosterone was effective in producing phenotypic male in *S. melanotheron*. However, in both 60-day and 180-day experiments, no treatment gave 100% male population. The 90 mg/kg MT yielded the highest all-male fish of 84.13%, highest survival of 70% and highest final mean body weight of 103.99 ± 7.92 g.

Key words: 17α-methyltestosterone, sex reversal, tilapia, growth performance, masculinization.

1. Introduction

Sex reversal is a technique of changing sexes of fish from one sex to another (e.g. female to male or vice versa) by administering synthetic steroid hormones (e.g. 17α-methyltestosterone and 1-dehydrotestosterone) before or during the period of sexual differentiation [1]. According to Phelps and Popma [2] hormone treatment does not modify the genetic make-up of the fish but directs the expression of the phenotype, and therefore defined sex reversal as the alteration of the phenotype (sex) by administration of synthetic sex hormones. According to Gale et al. [3], the hormones act as sex-inversion agents by functionally masculinizing or feminizing individual fish in the population. Andersen et al. [4] observed that at the time of hatching Zebra fish (*Danio rerio*) fry were sexually undeveloped and during the early period of gonadal differentiation, changes in sex hormone concentration affect the phenotypic sex irrespective of the genetic sex.

Ferdous and Ali [1] evaluated the optimum dose rate of 17α-methyltestosterone (MT) treatment for sex reversal and its effect on growth performance of Nile tilapia (*Oreochromis niloticus*). They treated the fry with MT dosages of 0 mg/kg (control), 40 mg/kg, 50 mg/kg, 60 mg/kg and 70 mg/kg *ad libitum* for 28 days. Their results showed that each hormone-treated group gave a significantly higher male to female ratio, while the control group showed normal male to female ratio. The results established that 17α-MT had substantial effect in directing gonadal sex differentiation of *O. niloticus* towards males depending on the dosage involved in the treatment. The researchers observed that the maximum male population (94.28%) of *O. niloticus* was obtained at the dose of 60 mg MT/kg of feed for 28 days and concluded that 60 mg/kg MT was the optimum effective dose for sex reversal in *O. niloticus*. However, in a similar assessment carried out on *O. niloticus*, Okoko [5] reported the optimum dosage

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of 30 mg/kg (99.3% males), while 60 mg/kg recorded 97% males. In another comparative study on *Oreochromis niloticus*, Shepperd [6] reported an equal performance of 98% males for both 30 and 60 mg/kg MT for the 28 days treatment. In the same studies, Shepperd [6] reported 94% and 91% males respectively for 30 and 60 mg/kg MT for Red tilapia (Hybrid, *O. mossambicus* × *O. hornorum*). Green and Teichert-Coddington [7] worked on *O. niloticus* using 60 mg/kg of 17α-MT and had 96.8% males. Phelps et al. [8] reported similar results (97.8% males) when 60 mg/kg 17α-MT was administered to *O. niloticus*. Guerrero III [9] evaluated the effect of the hormone, 17α-MT on the treatment of *Oreochromis aureus* for sex reversal. He administered doses of 15, 30 and 60 mg/kg for 18 days, and the 30 mg/kg had the highest percentage conversion of 98% males. Nakamura [10] demonstrated that at high doses the efficacy of 17α-MT in reversing the genotypic female fish to a phenotypic male was lowered (and vice versa) as demonstrated in the study with *Oreochromis mossambicus.* Nakamura [10] reported that 50 mg/kg MT treatment of *O. mossambicus* gave 100% males conversion, whereas 100 mg/kg gave 61% males in the same species. The results from McGeachin et al. [11] on the same *O. mossambicus* species when treated with 60, 90 and 120 mg/kg MT for 22 days gave 99%, 98%, and 96% males respectively. On the other hand, Varadaraj and Pandian [12] reported that doses of 5, 10, 20, 30 and 40 mg/kg MT when administered *ad libitum* to *O. mossambicus* for 19 days gave 100% male conversion in each case.

There are few reports on the use of other androgen-base hormones for the treatment of tilapia species for sex reversal. Guerrero III [9] examined the effect of the hormone, 1-dehydrotestosterone in the treatment of *Oreochromis aureus* at 15, 30 and 60 mg/kg concentrations for sex inversion, and the results were 69%, 59% and 44% males respectively. Guerrero III [9] again in the same experiment tested the hormone, ethynyltestosterone on *O. aureus* at 15, 30 and 60 mg/kg doses for sex reversal, the results were 85%, 98% and 100% male conversion respectively. Phelps et al. [8] evaluated fluoxymesterone at doses of 1, 5 and 25 mg/kg on *O. niloticus* for sex inversion, and obtained 87.3%, 100% and 100% males respectively for that particular androgen. The hormone, mestanolone was also tested on *O. niloticus* at 5, 10 and 20 mg/kg for sex reversal; the results were 99.5%, 97.0% and 99.0% males [13]. Guerrero III and Guerrero [14] worked on *O. mossambicus* using the hormone, mibolerone at levels of 1.5, 1.75 and 2.0 mg/kg, and the results were 84%, 88% and 94% males respectively. Varadaraj [15] also tested the androgen-based hormone, 19-norethisterone acetate for sex reversal in *O. mossambicus* at a concentration of 1 mg/kg and the outcome was 52% males. Galvez et al. [16] studied sex reversal in *Oreochromis aureus* using 25, 50 and 100 mg/kg of trenbolone acetate, and the outcome was 98.3%, 99.3% and 99.0% males respectively.

Studies on the use of synthetic androgens in sex reversal of tilapia [2, 17-19] showed that the steroids usually have both androgenic and anabolic effect on fish. El-Greisy and El-Gamal [20] worked on *Oreochromis niloticus* using 17α-methyltestosterone at 40, 60 and 80 mg/kg to produce sex reversed population and evaluated them for growth performance, survival, sex ratio and sex stability after 15, 35, 75 and 365 days of culture in freshwater. The results indicated that the maximum sex ratios of males (97%) and highest mean harvest body weight (215.0 ± 12.8 g) were recorded at 60 mg of 17α-MT/kg of diet after 365 days. Generally, the values of growth parameters recorded were always higher for those fish treated with the 17α-MT/kg diet compared to the control. They attributed the high growth performance of the hormone-treated fish to anabolic effect of the steroid.

However, according to Phelps and Popma [2] any enhanced growth of sex reversed tilapia, as a result of androgen treatment, is more related to the superior growth of males (naturally metabolic energy is channelled towards growth) than the usual anabolic effect related to enhanced protein synthesis and

increase in muscle mass. Hanson et al. [21] worked on *O. niloticus* and evaluated the growth performance of sex-reversed males, hybridized males and hand-sexed males (using the genital papilla). Both sets of sex-reversed and hybridized male fish (99.4% and 100% male respectively) grew larger than the hand-sexed fish (93.8% male), but the hand-sexed male population had 6.2% females which could have lowered the mean final body weight. Green and Teichert-Coddington [7] assessed growth and survival performance of 17α-MT treated and untreated Nile tilapia (*Oreochromis niloticus*) in the fry treatment, nursery and grow-out stages under semi-intensive conditions in earthen ponds. They found no significant difference in growth in any of the stages. Tuan et al. [22] also found no difference in growth between all hybridized male population (96.2% male) and 17α-MT sex reversed male (99.3% male) populations. However, in a second experiment, Tuan et al. [22] found out that the sex-reversed males (100% males) grew larger compared to the all hybridized males, which were made up of only 82.6% males.

There is paucity of information on sex reversal of *S. melanotheron* regarding the optimum dosage requirement. The objective of this study therefore, was to determine the effective concentration of 17α-methyltestosterone for production of all-male individuals of black-chinned tilapia.

2. Materials and Methods

2.1 Sources of Broodstock

Brood fish of *S. melanotheron* were obtained from the Weija reservoir (5 °34′11″ N; 0 °20′39″ W near Cape Coast, Ghana). Males and females were held separately in 6 m^3 hapas mounted in a 30 m^2 earthen pond.

2.2 Preparation of Hormone-Feed

Five concentrations of 17α-methyltestosterone (17α-MT) (0, 30, 60, 90 and 120 mg per kilogram feed) were prepared using the ethanol evaporation method [23]. A commercial feed (Ranaan) with 40% crude protein was ground and sieved to obtain fine particles. One kilogram (1 kg) of the feed was mixed with the different concentrations of 17α-MT. The control diet (0 mg of 17α-MT) was a mixture of 200 mL of alcohol and 1 kg feed. The hormone feeds were then air-dried in the laboratory and stored in a refrigerator until use.

2.3 Fry Production for Sex Reversal Assessment

Fry were produced by crossing 6 male and 6 female brood fish in a 6 m^3 hapa. After 2 weeks, fry of length less than 11 mm were stocked in fifteen 72-litre aquarium glass tanks (Fig. 1).

Fig. 1 72-litre capacity aquaria for sex reversal treatment.

Fig. 2 The 6 m^3 experimental hapas.

Triplicate tanks for each feed treatment were stocked with 100 fry and fed at 20% biomass, four times daily at 09:00, 11:00, 13:00 and 15:00 local time for 28 days. Unconsumed feed and excreta were siphoned from the tanks daily, and the water was partially changed to reduce the production of algae. The tanks were constantly aerated during the treatment period and pH, DO and temperature of the water were monitored daily using a multi-parametric water quality checker (Hanna Instrument HI 9829).

2.4 Growth Performance Evaluation of 17α-MT

Following the 28-day hormone-treatment, 60 fry were stocked in fifteen 6 m^3 hapas mounted in a 1,200 m^2 earthen pond (Fig. 2) comprising three replicates for each treatment. Fish were fed 30% crude protein feed at 5% biomass divided into three times daily at 09:00, 12:00 and 15:00 local time.

Thirty fish from each treatment were sampled fortnightly to record weight and length to the nearest 0.01 g and 0.1 cm respectively, and returned to their hapas. The total feed administered and fish mortalities were recorded during each sampling period. The following formulae were used to estimate the growth parameters:

(1) Absolute Growth Rate (AGR) [24]

$$AGR = \frac{W_f - W_i}{T}$$

where, AGR is the absolute growth rate, W_f, W_i, and T are the final weight, initial weight, and culture period, respectively.

(2) Specific Growth Rate (SGR) [25]

$$SGR = \frac{lnW_2 - lnW_1}{T} \times 100$$

where, SGR is the specific growth rate, W_2 and W_1 are final and initial weights of the fish, and T is the culture period.

(3) Feed Conversion Ratio (FCR) [26]

$$FCR = \frac{weight\ of\ feed\ given}{weight\ gain\ by\ fish}$$

(4) Condition Factor [27]

$$K = \frac{W}{L^3} \times 100$$

where, K is the condition factor, W is the final weight and L is the standard body length in cm.

2.5 Sex Determination Using the Gonadal Squash Method

Thirty fish from each treatment were harvested after 60 days; each specimen was weighed and standard body lengths measured and preserved in 10% formalin for sex determination using the gonadal squash method with a slight modification to the method described by Guerrero III and Shelton [28].

The female ovarian tissue was identified by the presence of pre-vitellogenic and vitellogenic oocytes (Fig. 3a) and the male testicular tissues were identified by their characteristic lobular structures (Fig. 3b). The numbers of males and females were recorded, and the percentage of sex reversal for each dosage of 17α-MT was calculated. A Chi-squared analysis was used to test for significant deviation of the sexes from the expected 1:1 ratio at the 5% level of significance.

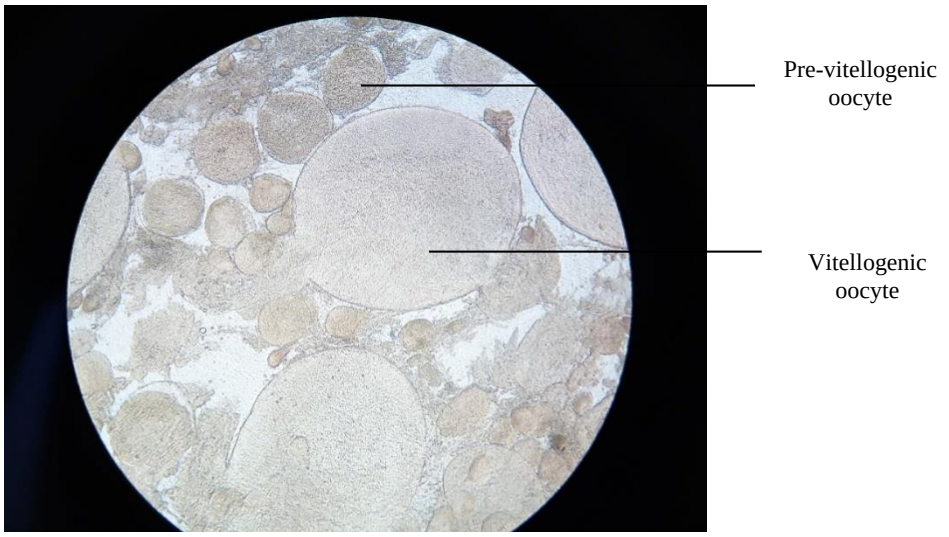

Fig. 3a Squashed ovarian tissue of black-chinned tilapia under light microscope (× 100).

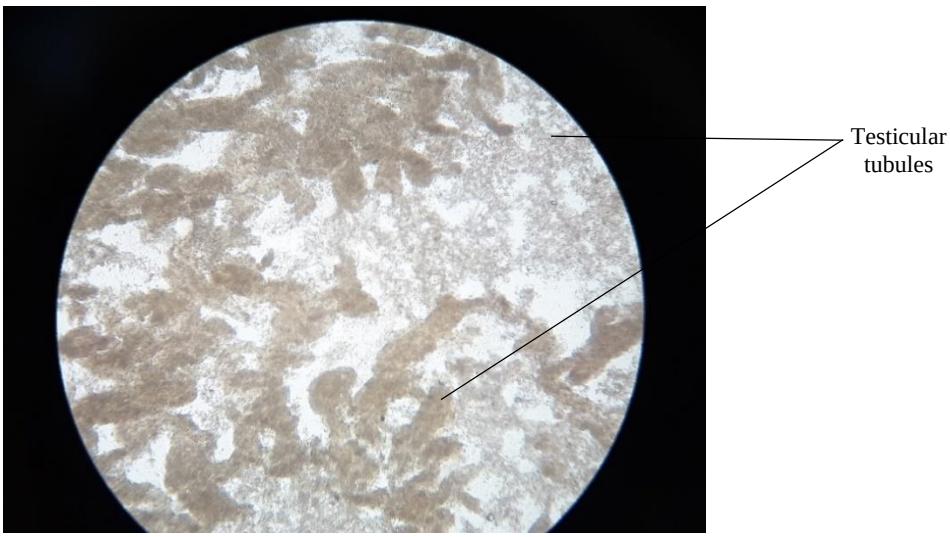

Fig. 3b Squashed testicular tissue of black-chinned tilapia under light microscope (× 100).

(a) (b)

Fig. 4 Female (a) and male (b) black-chinned tilapia.

2.6 Sex Determination Using the Genital Papillae

The remaining treated fingerlings in the hapas were further grown for 180 days and their sexes determined using the structure of the genital papillae by applying methylene blue to the genital area and rinsing it with water. The blue stain was either absorbed by the papillae (making the oviduct opening more conspicuous) if the fish was a female or not absorbed by the papilla (leaving no stain on the papilla) if the fish was a male (Fig. 4a).

The female black-chinned tilapia has three openings: urethra, oviduct and anus, whereas the male counterpart has only two openings: urethra and anus. It is the oviduct opening that absorbs the blue stain (Fig. 4a), making it easier to identify the female from the male. Fig. 4b shows the colour differences between the male and female black-chinned tilapia. The female was identified by its transparent operculum, which appears deep mauve because of the blood that flows through the gills beneath it. The male black-chinned tilapia on the other hand, has a bright golden yellow colour on the operculum (Fig. 4b).

3. Results

3.1 Percentage of Sex Conversion of Treated S. melanotheron after the 60-Day Culture

Table 1 presents results of percentage of sex conversion determined by gonadal squash method. The fish fed 90 mg/kg 17α-MT feed exhibited the highest male percentage conversion of 80.0% ± 3.9% which was significantly higher than those fed 0, 30 and 120 mg/kg but not significantly different from the fish fed 60 mg/kg.

3.2 Percentage of Sex Conversion of Hormone-Treated S. melanotheron after 180-Day Culture

Table 2 gives the percentage of male conversion of hormone-treated fish cultured for 180 days. At this stage, the fish had developed the sex coloration in the opercula and the urogenital papilla of males and females was recognizable. Using the hand sexing technique and opercular coloration, 84.0% ± 1.0% males were produced by the 90 mg/kg 17α-MT feed, which was significantly higher ($p < 0.05$) than the 53.3% ±3.3% males obtained from the 0 mg/kg feed (control).

Table 1 Percentage of sex conversion of hormone-treated *S. melanotheron* after 60-day culture.

	Percentage males			
Conc. of 17α-MT (mg/kg)	1	2	3	Mean ± SE (%)
0	36.0	53.3	56.7	48.7 ± 6.4^{b}
30	57.1	56.7	-	56.9
60	73.3	60.0	-	66.7
90	73.3	86.7	80.0	80.0 ± 3.9^{a}
120	43.3	63.3	60.0	55.6 ± 6.2^{b}

Means that do not share a letter in the superscripts are significantly different at 5% level of significance

Table 2 Percentage of sex conversion of hormone-treated *S. melanotheron* after 180-day culture.

	Percentage males			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE (%)
0	50.0	60.0	50.0	53.3 ± 3.3^{b}
30	61.1	52.0	76.9	63.3 ± 7.3^{ab}
60	82.4	72.0	60.0	71.45 ± 6.5^{ab}
90	84.0	82.4	85.7	84.02 ± 1.0^{a}
120	87.5	28.0	-	57.8

Means that do not share the same superscripts are significantly different at 5% level of significance

3.3 Mean Body Weight of S. melanotheron Fry after the 28-Day Treatment with 17α-MT

Fig. 5 shows mean body weights attained by treated fry after 28 days of hormone treatment. The highest mean body weight (0.62 ± 0.03 g) was attained by the fry fed 30 mg/kg feed, and those fed 120 mg/kg had the lowest mean body weight (0.41 ± 0.09 g). The mean body weight of fish fed 30 mg/kg 17α-MT feed was significantly higher ($p < 0.05$) than those fed 60, 90 and 120 mg/kg feed.

3.4 Mean Body Weight of Hormone-Treated S. melanotheron after the 60-Day Culture

Fig. 6 shows the mean body weights of treated *S. melanotheron* cultured for 60 days. The highest mean body weight of 12.85 ± 1.64 g was attained by the fish fed 30 mg/kg 17α-MT feed; those fed 120 mg MT/kg feed had the lowest mean body weight (9.70 ± 0.37 g). The body weights of the fish fed 30, 60 and 90 mg MT/kg feed were similar but were significantly higher ($p < 0.05$) than those fed at 0 and 120 mg MT/kg feed.

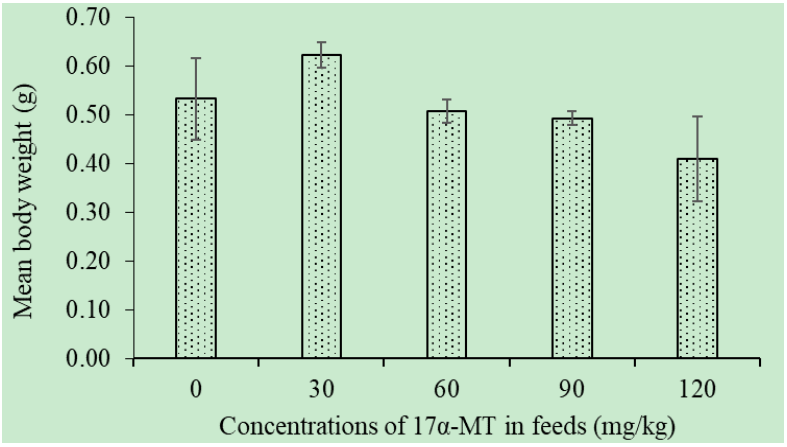

Fig. 5 Mean body weight of *S. melanotheron* fry after the 28-day treatment 17α-MT.
Vertical bars represent standard errors.

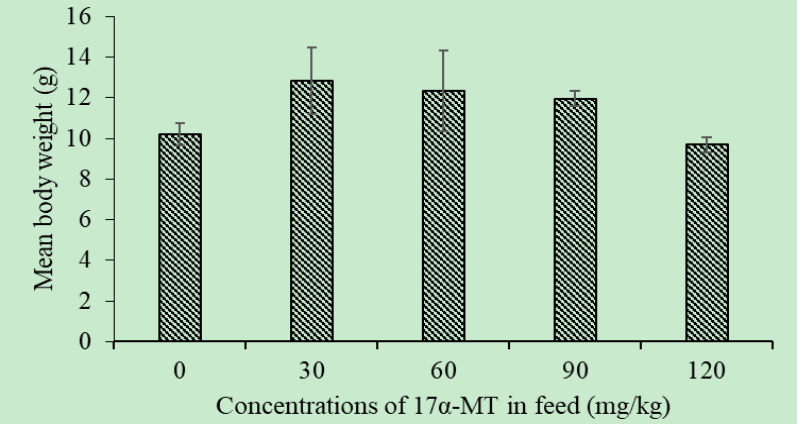

Fig. 6 Mean body weight of treated *S. melanotheron* fry cultured in freshwater for 60 days.
Vertical bars represent standard errors.

Table 3 Absolute growth rates of hormone-treated *S. melanotheron* cultured for 60 days.

	AGR (g/day)			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE (g/day)
0	0.17	0.14	0.17	0.16 ± 0.01
30	0.18	0.17	0.26	0.20 ± 0.03
60	0.26	0.18	0.15	0.20 ± 0.03
90	0.20	0.18	0.19	0.19 ± 0.01
120	0.14	0.16	0.16	0.15 ± 0.01

Table 4 Specific growth rates of hormone-treated *S. melanotheron* cultured in freshwater for 60 days.

	SGR (%/day)			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE (%/day)
0	4.80	4.52	5.57	4.96 ± 0.31
30	4.82	4.90	5.35	5.02 ± 0.17
60	5.63	5.24	4.97	5.28 ± 0.19
90	5.30	5.23	5.42	5.32 ± 0.06
120	5.54	4.78	5.71	5.34 ± 0.29

3.5 Absolute Growth Rates of Hormone-Treated S. melanotheron Cultured for 60 Days

Table 3 shows the absolute growth rates (AGR) of hormone-treated *S. melanotheron* cultured for 60 days. The highest AGR occurred in fish fed 30 and 60 mg/kg 17α-MT with the lowest in fish given the 120 mg/kg 17α-MT feed. There was no significant difference among the mean AGR when the entire treatment was subjected to ANOVA test ($F = 1.24$; $p = 0.355$).

3.6 Specific Growth Rates of Hormone-Treated S. melanotheron Cultured for 60 Days

Results of the specific growth rate (SGR) analysis are presented in Table 4. SGR was highest in fish fed 120 mg/kg 17α-MT feed and lowest in fish with no MT treatment. There was no significant difference in SGR among the treatments, according to results of the ANOVA test ($F = 0.63$; $P = 0.652$).

3.7 Mean Body Weight of Hormone-Treated S. melanotheron Cultured for 180 Days

Fig. 7 shows the final mean weight of hormone-treated fish cultured for 180 days. The fish fed 0 mg MT/kg feed had the lowest mean body weight (70.45 ± 4.67 g), and those fed 90 mg/kg exhibited the highest mean body weight (103.99 ± 7.92 g), and the latter was significantly higher than the mean body weight of fish treated with 0 mg MT and 120 mg MT (Fig. 7).

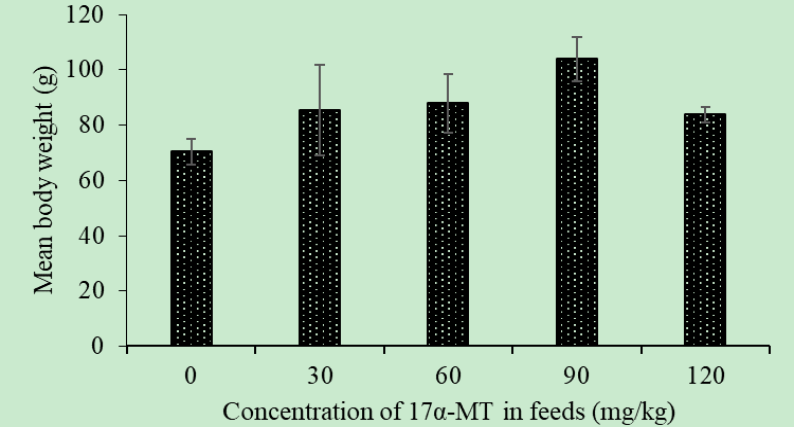

Fig. 7 Mean body weight of hormone-treated *S. melanotheron* cultured in freshwater for 180 days.
Vertical bars represent standard errors.

Table 5 Absolute growth rate of hormone-treated *S. melanotheron* cultured for 180 days.

	Replicates (g/day)			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE (g/day)
0	0.35	0.44	0.37	0.39 ± 0.03^{b}
30	0.38	0.38	0.65	0.47 ± 0.09^{ab}
60	0.60	0.44	0.41	0.49 ± 0.06^{ab}
90	0.65	0.58	0.50	0.58 ± 0.04^{a}
120	0.49	0.44	0.46	0.46 ± 0.02^{ab}

Means that do not share the same superscript are significantly different at 5% level of significance.

Table 6 Specific growth rate of hormone-treated *S. melanotheron* cultured in freshwater for 180 days.

	SGR (%/day)			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE (%/day)
0	2.58	2.71	2.90	2.73 ± 0.09
30	2.59	2.66	2.89	2.72 ± 0.09
60	2.94	2.83	2.81	2.86 ± 0.04
90	3.01	2.98	2.92	2.97 ± 0.03
120	3.11	2.73	3.09	2.98 ± 0.13

3.8 Absolute Growth Rates of Treated S. melanotheron Cultured for 180 Days

Table 5 presents results of the absolute growth rates of *S. melanotheron* given different treatments of 17α-MT and cultured for 180 days. The mean AGR of fish fed 90 mg MT was significantly higher ($p < 0.05$) compared to those fed 0 mg/kg.

3.9 Specific Growth Rate of Hormone-Treated S. melanotheron Cultured for 180 Days

The SGRs of the fish under the different hormone treatments are presented in Table 6. There was no significant difference among the specific growth rates when subjected to ANOVA test ($F = 2.29$; $p = 0.132$).

3.10 Feed Conversion Ratio of Hormone-Treated S. melanotheron Cultured for 180 Days

Fig. 8 illustrates the feed conversion ratio (FCR) of hormone-treated fish after 180 days of culture. The FCRs of fish in the control set-up, the 30 mg MT and 60 mg MT treatments were significantly higher than fish fed the 90 mg MT and 120 mg MT ($p < 0.05$). This suggests that a feed conversion in *S. melanotheron* was at higher concentrations of 17α-MT.

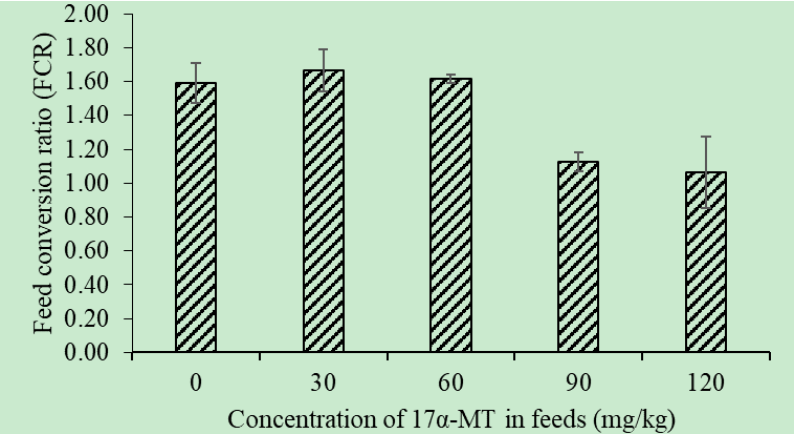

Fig. 8 Feed conversion ratio of *S. melanotheron* treated with 17α-MT feeds and cultured in freshwater for 180 days.
Vertical bars represent standard errors.

Table 7 Mean condition factor of hormone-treated *S. melanotheron* cultured in freshwater for 180 days.

	Condition factor (K)			
Conc. of 17α-MT in feeds (mg/kg)	1	2	3	Mean ± SE
0	3.40	3.45	3.54	3.46 ± 0.04^{a}
30	3.29	3.36	3.32	3.32 ± 0.02^{b}
60	3.04	3.20	3.19	3.14 ± 0.05^{c}
90	3.43	3.38	3.33	3.38 ± 0.03^{ab}
120	3.28	3.36	-	3.32

Means that do not share the same superscript are significantly different at 5% level of significance.

Table 8 Mean water quality estimates within hapas containing sex-reversed *S. melanotheron* cultured for 180 days.

Conc. of 17α-MT in feeds (mg/kg)	pH	Mean DO (mg/L)	Mean temp (°C)
0	8.64 ± 0.08	5.02 ± 0.95	28.62 ± 0.45
30	8.69 ± 0.07	4.99 ± 0.90	28.69 ± 0.55
60	8.67 ± 0.07	5.16 ± 1.11	28.65 ± 0.37
90	8.70 ± 0.09	4.89 ± 0.86	28.05 ± 0.83
120	8.64 ± 0.07	4.94 ± 0.92	28.56 ± 0.55

3.11 Condition Factor of Hormone-Treated S. melanotheron Cultured for 180 days

The condition factors (K) calculated for fish treated with the experimental feeds are recorded in Table 7. The fish treated with the control feed had the highest condition factor with 60 mg MT generating the lowest fish condition. The condition factor of fish fed 60 mg/kg 17α-MT feed was significantly lower ($p < 0.05$) compared to all the other experimental fish.

3.12 Water Quality Parameters Recorded during the 180-Day Culture Period

Table 8 shows the water quality parameters recorded within the hapas containing the experimental fish. The minimum mean pH recorded over the period was recorded in the hapas containing fish fed 120 mg/kg 17α-MT whereas the maximum was recorded for those fed 90 mg/kg 17α-MT. The mean DO recordings ranged from 4.89 ± 0.86 mg/L to 5.16 ± 1.11 mg/L for those fed 90 mg/kg 17α-MT and 60 mg/kg 17α-MT respectively. The water surface temperatures in the various hapas did not vary significantly during the culture period; they ranged between 28.05 ± 0.83 ℃ and 28.69 ± 0.55 ℃.

4. Discussion

The maximum male population (80.0% and 84.13%

male) of *S. melanotheron* was obtained at the dose of 90 mg/kg 17α-MT feed, whereas the minimum male proportion was recorded for the dose of 120 mg/kg 17α-MT feed in both 90- and 180-day experiments. These results are in line with reports on other tilapia species. Okoko [5] worked on *O. niloticus*, and observed higher male populations at lower doses of 17α-MT than at higher doses. Okoko [5] reported 99.3%, 97% and 71.9% males at doses of 30, 60 and 120 mg/kg respectively. The low male to female ratio recorded for 120 mg/kg was in line with the observation of Goudie et al. [29], who reported that excessive doses of hormone leading to sterility or paradoxical feminization in channel catfish, due to aromatization of androgens to estrogens. According to El-Greisy and El-Gamal [20], 17α-methyltestosterone functions by suppressing the process of oogenesis, and this inhibitory effect on the development of oocytes is dependent on the dose of methyltestosterone administered to the fish. They reported that the highest sex reversal occurred at 60 mg/kg feed when *O. niloticus* was fed 40, 60 and 80 mg/kg of 17α-MT for 21 days post-hatching to produce all male tilapia population. The current study recorded highest sex reversal of 80%-84% male at 90 mg/kg 17α-MT fed for 28 days.

In a related study, Apenuvor [30] studied the effect of different levels (30, 60 and 120 mg/kg) of 17α-MT on *S. melanotheron* but did not consider 90 mg/kg. The current results were not in agreement with that of Apenuvor [30], who reported that *S. melanotheron* fed 120 mg/kg 17α-MT exhibited the highest sex reversal of 92.7% male as compared to 89% male for those fed 60 mg/kg 17α-MT. The deviation could be due to the differences in the strains (Fosu lagoon, brackish water versus Weija reservoir, freshwater) used. Nevertheless, the purity of the *S. melanotheron* fry and the methodology used by Apenuvor [30] left more questions unanswered. For example, how pure were the fry of *S. melanotheron* harvested from the midst of several other species (e.g. *Pellonula leonensis*, *Liza falcipinnis*) in that Lagoon where they were harvested for sex reversal experiment? Apenuvor [30] collected the swim-up fry for conversion directly from the Fosu Lagoon with the aid of a scoop net and stocked them in hapas mounted in concrete fish ponds. He started feeding the fry after five days of acclimatization process. This method of acquiring fry for such experiment cannot guarantee that fry (with unknown parents) harvested were made up of only *S. melanotheron* species, even if it was established that only *S. melanotheron* existed in the lagoon. Again, feeding the fry with different doses of 17α-MT in hapas mounted in the same fish pond cannot also ensure that the right doses were available for the targeted samples due to leaching of the 17α-MT feed from one hapa to the other. Even delaying the feeding of the swim-up fry with the hormone feed for five days during the acclimatization period could also affect the efficacy of the hormone on the sex reversal since the fry could have grown past the recommended length of 9-11 mm [29], when the sex of tilapia is not yet developed. Again, the researcher did not indicate how the sex of the treated fish was determined. Therefore, the results from Apenuvor [30] left many questions unanswered. According to Mair and Little [31], the rate of masculinization in tilapia can be influenced by the hormone concentration, treatment duration, age and size of fry, availability of natural feed, stocking density and feeding frequency.

In an experiment with *Oreochromis aureus*, McGeachin et al. [11] reported lowest sex reversal (96% male) for administering 120 mg/kg 17α-MT as compared to 98% and 99% male for 90 mg/kg and 60 mg/kg 17α-MT respectively. Although, the species were different from that of Okoko [5] and the current study (i.e. *O. aureus*, *O. niloticus*, and *S. melanotheron*), the trend and performance of fish fed 120 mg/kg 17α-MT were all in agreement, recorded lower percentage of male conversion, indicating a possible paradoxical feminization.

Hormone-treated tilapia usually exhibits higher growth performance compared to the control group.

According to Jo et al. [32], the higher growth performance in the treated tilapia compared to the control group can be attributed to anabolic effect of 17α-MT. However, the results of the current study showed that certain doses of 17α-MT exhibited higher anabolic effect on the fry of *S. melanotheron* compared to other doses. The 90 mg 17α-MT/kg feed, which recorded the highest male population also exhibited the highest mean final weight of 103.99 ± 7.92 g, whereas those fed 120 mg/kg 17α-MT attained the lowest weight of 83.78 ± 2.62 g. In another experiment, although 90 mg/kg attained the highest male sex reversal, it did not give the highest mean final weight; there were no significant differences among the mean body weights of the treated fish. It could also mean that the anabolic effect of the hormone has not fully taken effect at that early age of the fish. The increased growth performances of the 17α-MT treated tilapia compared to the control group in the current study are in agreement with published reports on other tilapia species [17, 19, 31]. It could be seen that in all these reports, the optimum dose usually exhibits highest growth performance as well as the highest percentage masculinization. These may suggest that the higher body weights recorded for the optimum dosage groups could be because of the high percentage of males present as compared to the control and other doses, which might contain more females with lower weights because of reallocation of metabolic energy towards reproduction. In males, the metabolic energy is directed towards growth, and they may benefit from anabolism-enhancing androgens [33, 34]. Ahmad et al. [35] studied different doses of 17α-methyltestosterone as a growth promoter in Nile tilapia, *Oreochromis niloticus*, and reported that the 5 mg/kg was the optimum and effective dose in promoting significant weight gain and specific growth rate when doses of 0.5, 1.0, 2.5, 5, 10, 20 and 40 mg/kg MT were administered to the fish for 90 days. This could explain why the highest dose of 120 mg/kg 17α-MT could not attain the highest final weight, because only a small quantity of 17α-MT was required to promote growth in fish.

The percentage of male conversion of 84% was lower compared to findings in other tilapia species. Vera-Cruz and Mair [36] obtained 98% and 99% males with 40 mg 17α-MT/kg and 60 mg 17α-MT/kg diets respectively for *O. niloticus* fed at 20% body weight for 25 days. Smith and Phelps [37] also worked on *O. niloticus* and reported 99%-100% male when given 17α-MT at 60 mg/kg of feed. The results of this study exhibited a lower male proportion for optimum dose rate of androgen of 90 mg 17α-MT/kg of feed. The lower conversion rate in the current study may be due to difference in species, poor handling of the hormone by the chemical sellers and the shelf life of the steroid. According to Phelps and Popma [2], androgens breakdown when exposed to sunlight or high temperatures. Varadaraj et al. [38] compared storage conditions of 17α-MT stock solutions and treated feed and the impact on efficacy of sex reversal of *O. mossambicus*. They report that when either the stock solution or feed of 17α-MT was exposed to light the efficacy of the treatment was significantly reduced.

5. Conclusions

The results demonstrated that the synthetic androgen, 17α-methyltestosterone was effective in producing phenotypic male in *S. melanotheron*. The optimum dosage of 17α-methyltestosterone for effective sex reversal in *S. melanotheron* was 90 mg/kg with a feeding duration of 28 days. At a higher dose of 120 mg 17α-MT/kg of feed, the sex ratio of *S. melanotheron* was not influenced in favour of males. The treatment of *S. melanotheron* with 90 mg/kg of 17α-methyltestosterone for 28 days yielded the highest all-male conversion of 84.13% male, highest survival of 70% and highest final mean body weight of 103.99 ± 7.92 g as compared to the other doses of 0, 30, 60 and 120 mg/kg 17α-MT.

Recommendations

The optimum dose of 90 mg/kg of 17α-MT for sex reversal of *S. melanotheron* should be tested on other

strains of black-chinned tilapia to evaluate its effectiveness.

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