



Effect of Different Doses of 17 α -Methyltestosterone on Masculinization, Pre-Post Treatment Growth Parameters and Condition Factor of Sarotherodon Melanotheron in Pond System

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Abstract

Over-population and stunted growth had been major challenges in the culture of tilapia. The use of synthetic androgen 17- α Methyl Testosterone (MT) was a breakthrough. However, its optimum level towards effective masculinization and growth is a concern. The aim of this research was to ascertain the optimum level of MT towards effective all-male population production and growth of Black-Chinned tilapia. In the present study, the effect of different dose rates of synthetic androgen 17- α Methyl Testosterone (MT) i.e., 0, 30, 60, and 120 mg of the hormone per kg of feed on sex, growth, and condition of Black-Chinned tilapia was evaluated. MT was administered orally by using powdered dry starter feed (Crude Protein 40 %) and Ethanol. The fry was fed for 30 days in the experimental tanks. At the end of the experiment, the sex ratios were determined by examining the operculum coloration as a means of sex identification. Growth performance was monitored by measuring and recording the morphometric characteristics. Bodyweight and total length of the fish on the start of feeding, end of feeding (one month sex reversal period), and two months after feeding were measured. The results of the present study showed that all MT receiving treatment showed a significantly higher male proportion than the control (0 mg MT/kg feed individuals). In all MT treatments groups, the control expects the 30 mg MT/kg in feed individuals' deviate significantly from the normal 1:1 sex ratio (Chi-square analysis). The dose rate of 120 mg MT /kg feed resulted in the maximum male population (92.7%). Hence, for an effective high percentage of all-male population production in Black-Chinned tilapia, 120 mg MT /kg in feed is recommended. In terms of growth and condition factor, all the individual treatments, as well as the control, showed no significant difference. All the treated individuals showed similar condition factors during the pre and post-treatment, however, the individuals treated with 30 mg MT /kg feed exhibited better condition during the pre-treatment than the post-treatment period. Temperature, pH and dissolved oxygen recorded in this study were within the desirable limit for tilapia.

Keywords: Sex-reversal; Sarotherodon melanotheron; 17 α -methyltestosterone; hormonal treatment; Different doses of hormone; Growth performance.

1. Introduction

Sarotherodom melanotheron (Black-Chinned tilapia) is one of the most important fish species identified for culture both in fresh and brackish water environment, particularly for the developing and under-developed countries in West Africa [1]. It's candidature is characterized by stress-free spawning, good growth, ability to feed at the lower portion of the food chain, [Ofori-Danson and Kumi \[2\]](#), feeding on cheap artificial feed, inhabitation of wild range of aquatic habitat (both fresh and brackish water, [Gueye, et al. \[3\]](#), high tolerance to environmental changes. Good taste, flavor, flesh, texture and marketable request are also among the necessitating characteristics [4]. Few of the setbacks in culturing tilapia is early maturation and uncontrolled reproduction, which often lead to overpopulation of the production system and stunted growth [5]. In our quest to culture Black-Chinned tilapia, their tendency to overpopulate culture systems may be high. As a consequence of the afore-mentioned challenges most uncontrolled cultured populations of Black-Chinned tilapia lose their market value in weight and size.

Ensuring high profitability for cultured Black-Chinned tilapia in the face of dwindling capture fisheries will require efficient method for controlling the prolific breeding in the species. Among these methods are; sex reversal, cage culture, use of predators, sterilization and selective harvesting [5-7]. Hormonal sex-reversal of fry has proven to be effective attaining in monosex culture in *Oreochromis niloticus* in Ghana. Hormonal treatment has the potential to also increase weight and muscle of cultured fish species [8], However its efficacy depends on the hormonal concentration [5]. Different dosages of the hormone (17 α -methyltestosterone (MT) have been used in Nile tilapia to achieve efficiency. For example: [9, 10] achieve good masculinization and reversed sex with 60 mg MT/kg of

feed and [Abucay and Mair \[11\]](#) produced 100 % male sex population of Nile tilapia with 40 mg MT/kg of feed. [Marjani, et al. \[8\]](#) found that the dose rate 75 mgMT/kg feed gave the maximum gain in body weight.

This study therefore sought to ascertain the optimum level of MT treatment for sex reversal in *S. melanotheron* towards its adoption as a viable finfish candidate for coastal aquaculture in Ghana.

2. Materials and Methods

2.1. Study Area

The research was conducted in the University of Cape Coast research tank. Experiment for sex reversal were set up in concrete tanks each measuring $3.7 \times 3.7 \times 0.77$ m and filled with dechlorinated water to a depth of 0.58 m. The ponds were filled a day before the trial began to ensure absence of plankton in the pond before stocking. Twelve cages, each measuring $50 \times 50 \times 0.6$ cm were suspended in the ponds such that the cages floated 0.1 m above the water surface. Occasionally, the outlet pipes of the ponds were opened to allow some of the pond water to drain out while the inlets were opened for inflow of fresh water to replenish the drained water. Temperature, dissolved oxygen and pH in the pond were measured three times daily at 08:00 GMT, 12:00 GMT and 16:00 GMT during the experimental duration a water quality checker (WQC Model 22A) and the weekly and monthly means of these physico-chemical parameters were calculated.

2.2. Hormonal Feed Preparation

The various concentrations of the hormonal feed for the study were prepared as described by [Killian and Kohler \[12\]](#). Thirty milligrams, 60 mg, 120 mg of the hormone, 17 α – methyltestosterone (MT) were weighed with an electronic balance and dissolved in 250 ml of 95 % ethyl alcohol. Each of the hormone solutions was mixed with 1 kg of commercial fish feed (Crude protein 40%), until a homogenous feed-hormone mixture was obtained. Raanan, which is commonly used for fish farming in Ghana, was used. The control (0 mg MT/kg feed) was mixed with only alcohol without the hormone. The treated feeds were air-dried at room temperature for 4 hours. The dried hormonal feeds, designated 0 mg MT /kg feed (control), 30 mg MT /kg feed, 60 mg MT /kg feed and 120 mg MT /kg feed were packed in air-tight polyethylene bags and stored in a refrigerator.

2.3. Sex Reversal

Swim up fry of *S. melanotheron* for the study were collected from Fosu lagoon (Cape Coast) using a scoop net and sent to the experimental site and conditioned for five days. Prior to sex reversal, they were sorted to eliminate weak fry and individuals longer than 11mm. A total of fifty fry each measuring between 9 and 11 mm were counted and stocked in each of 12 experimental cages. Fry were fed daily at 20 % body weight, divided into four portions and administered at 08:00 GMT, 12:00 GMT, 15:00 GMT and 17:00 GMT, for the first fourteen days. The feeding rate was then reduced to 10 % body weight from day 15 to day 30.

2.4. Growth of Performance Sex-Reversed Fry

The parameters chosen for the evaluation of growth of the sex – reversed fish were according to [De Silva and Anderson \[13\]](#).

Absolute growth (AG) was estimated by the relation

$$AG = W_1 - W_0,$$

where W_1 = final wet weight and W_0 = initial wet weight.

Specific growth rate [(SGR) % /day] was calculated using the formula,

$$SGR = (\ln W_1 - \ln W_0) \times 100 / t,$$

where, W_1 = final wet weight, W_0 = initial wet weight, t = time interval in days.

Condition factor (K) which refers to the well-being of fish was calculated by using the relation $K = 100 \times BW / SL^3$, [14, 15],

where K = condition factor, BW = body weight of fish (g), SL = standard length of fish (cm).

2.5. Post Hormonal Treatment Sex Examination

Percentage sex reversal was achieved by identifying and sorting of the individual into male and female category. Male *S. melanotheron* was noted by the golden yellow colouration of operculum and female by the purple colouration of operculum.

2.6. Statistical Analysis

The data were analyzed using Minitab (version 16). *t*- test and One-way analysis of variance (ANOVA) was to compare the various concentrations of the hormonal treatment. The critical *p*- value was taken to be 0.05. Whenever a significant difference was found in any set of data, the Turkey post hoc test was used to determine which pairs of means were significantly different.

3. Results

Table-1. Monthly mean pond physico-chemical conditions of the sex reversal pond

Month, 2013	Mean \pm s. e		
	Temperature ($^{\circ}$ C)	pH	DO (mg/l)
July	27.55 \pm 0.10	7.16 \pm 0.08	3.70 \pm 0.29
August	27.32 \pm 0.12	7.64 \pm 0.03	3.16 \pm 0.16
September	27.50 \pm 0.11	7.61 \pm 0.04	3.12 \pm 0.17

3.1. Response of Black-Chinned Tilapia Fry to 17 α Methyltestosterone

The maximum male population of 92.70 % was obtained in fish fed 120 mg MT/kg feed while the minimum male proportion of 39.3 % was recorded for fish fed 0 mg MT/kg feed (control). The hormone concentrations of 30 mg MT/kg feed and 60 mg MT/kg feed resulted in 84 % and 89 % male respectively as presented in Figure 1.

3.2. Sex Ratio of Fingerlings

The highest mean percentage male of 92.7 % with a corresponding mean percentage female of 3.77 % and a sex ratio (M: F) of 2.45: 1 was obtained in 120 mgMT/kg Feed (Table 2). This was followed by 60mgMT/kg Feed with mean percentage male of 89.3 % and mean percentage females of 7.99 % in the ratio of (M: F) as 1.9: 1. 30mgMT/kg Feed had mean percentage males of 84 % and mean percentage females as 9.05 % with the ratio of (M : F) as 11 : 1 and the lowest mean percentage males of 39.3 % and the mean percentage females as 59.79 % with the ratio of (M : F) 1: 1.5 was recorded by 0 mgMT/kg Feed (control).

Figure-1. Percentage males of *Sarotherodon melanotheron* produced by different concentrations of 17 α - methyltestosterone (MT) (Vertical bars represent males \pm standard error)

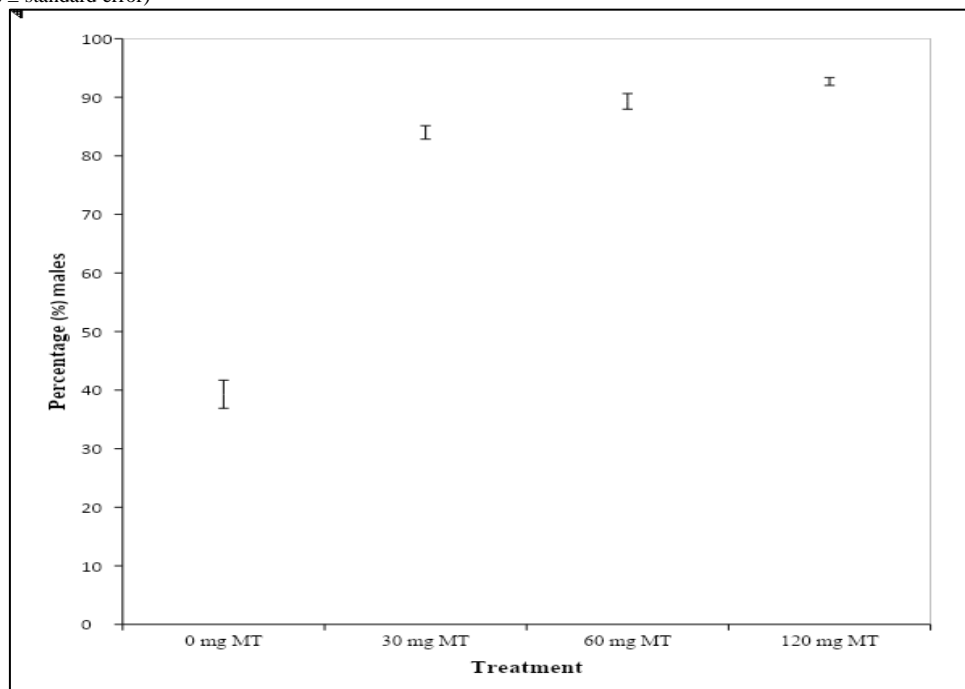


Table-2. Different concentrations of 17 α - methyltestosterone and the percentage males produced from 30- day sex reversal trial.

Treatments	NO. OF MALES	% MALES	NO. OF FEMALES	% FEMALES	SUCCESS REVERSAL RATE (%)	SEX RATIO (F : M)
0mgMT/kg (Control) Feed	19	38.0	30	61.66	39.3%	1:1.5
	22	44.0	26	54.16		
	18	36.0	32	64		
30mgMT/kg Feed	43	86.0	5	10.41	84.0%	1:11
	41	82.0	6	12.78		
	42	84.0	7	14.28		
60mgMT/kg Feed	44	88.0	4	8.33	89.3%	1:1.9
	46	92.0	3	7.69		
	44	88.0	4	8.33		
120mgMT/kg Feed	46	92.0	3	6.12	92.7%	1:2.45
	47	94.0	2	4.08		
	46	92.0	3	6.12		

Stocking rate = 50 fry per cage Female (F) Male (M)

3.3. Growth Performance of *S. Melanotheron* During and After Hormonal Treatment

Fish fed 120 mg MT/kg feed grew from 0.02 g to 0.57 ± 0.38 g while fry fed 30 mg MT/kg feed grew from 0.02 g to 0.41 ± 0.17 g by the end of the 30- day hormone treatment period (Figure 2). Growth from 0.02 g to 0.51 ± 0.26 g was observed by fish fed 60 mg MT/kg feed while the fry fed 0 mg MT/kg feed grew from the initial 0.02 g to 0.43 ± 0.49 g at the end of the treatment period. By the end of the twelve - week trial period, the heaviest weight of 3.14 ± 2.05 g was attained by the fish fed 120 mg MT/kg feed whilst the least body weight of 2.82 ± 1.78 g was attained by fish fed 0 mg MT/kg feed. Weights of 3.07 ± 2.11 g and 3.01 ± 1.43 g were attained by fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively.

In terms of length, fry fed 120 mg MT/kg feed increased from 1.1 cm to 3.04 ± 0.53 cm while the fish fed 0 mg MT/kg feed grew from 1.1 cm to 2.65 ± 0.39 cm by the end of the hormone treatment period (Figure 3). The fish group that were fed 30 mg MT/kg feed and 60 mg MT/kg feed grew from 1.1 cm to 2.73 ± 0.41 cm and 2.94 ± 0.44 cm respectively. At the end of the trial, fish fed 120 mg MT grew to 5.40 ± 1.27 cm while fish group fed 0 mg MT/kg feed attained the length of 5.03 ± 1.15 cm. Equal lengths of 5.41 ± 1.27 cm and 5.41 ± 0.99 cm were attained by fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. ANOVA test indicated no significant difference ($F = 0.02$, $p > 0.05$) in length of fish among the treatments at the end of the trial.

Absolute growth rate of hormone - treated fry ranged from 0.34 ± 0.10 g /day to 0.41 ± 0.11 g /day (Table 3). The highest absolute growth of 0.41 ± 0.11 g/day was recorded in the fish fed 120 mg MT/kg feed while the fry fed 0 mg MT/kg feed were observed to have the least absolute growth of 0.34 ± 0.10 g /day. Absolute growth values of 0.35 ± 0.09 g/day and 0.37 ± 0.14 g/day were obtained from fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. ANOVA test indicated no significant difference ($F = 0.063$, $p > 0.05$) in absolute growth of fry fed different concentrations of 17 α - methyltestosterone.

Figure-2. Changes in body length of *Sarotherodon melanotheron* fry fed different concentrations of 17 α - methyltestosterone during sex reversal treatment (Vertical bars \pm standard error)

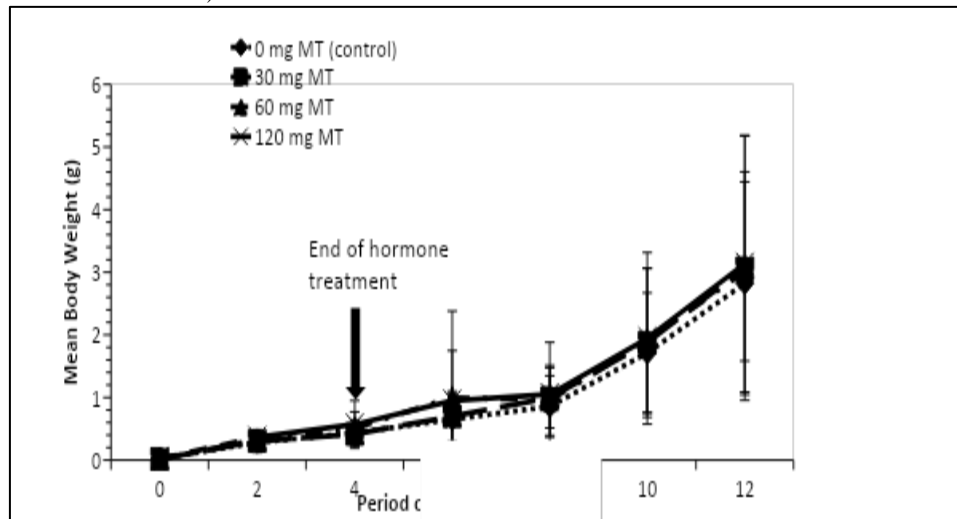
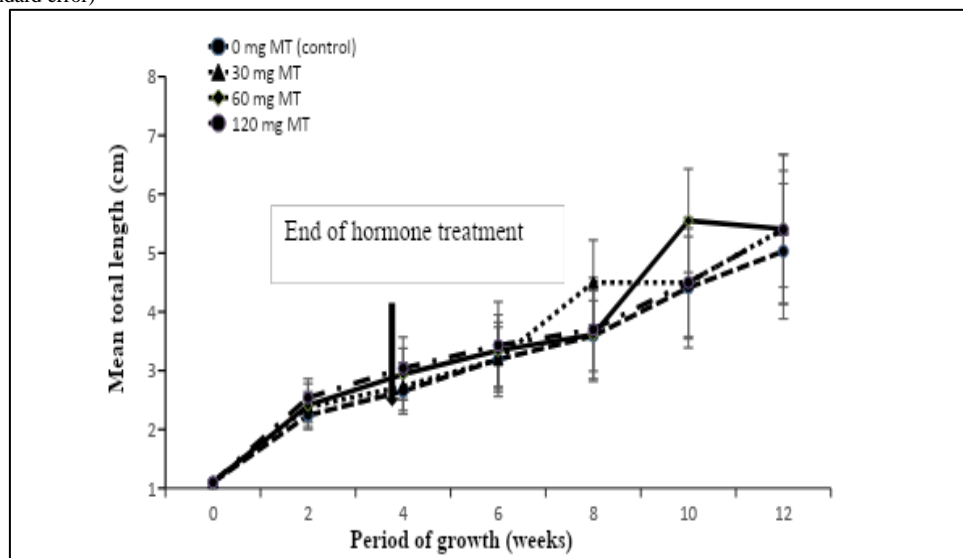


Figure-3. Changes in body length of *Sarotherodon melanotheron* fry fed different concentrations of 17 α - methyltestosterone (Vertical bars represent \pm standard error)



Fish fed 0 mg MT/kg feed showed the highest specific growth rate (9.70 ± 4.31 % BW per day) while the lowest specific growth (9.01 ± 4.16 % BW per day) was recorded in fish fed 60 mg MT/kg feed (Table 3). Specific growth

rates of 9.45 ± 3.81 % BW per day and 9.29 ± 4.13 % BW per day were recorded in 30 mg MT/kg feed and 120 mg MT/kg feed respectively. ANOVA test indicated no significant difference ($F = 0.005$, $p > 0.05$) in the specific growth rate of fry under the different concentrations of 17 α -methyltestosterone.

Fingerlings fed 120 mg MT/kg feed grew better and had the highest absolute growth of 1.57 ± 0.04 g/d while the fish fed 0 mg MT/kg feed (control) were observed to have the lowest absolute growth of 1.43 ± 0.10 g/d at the end of the twelfth week (Table 4). Absolute growth values of 1.54 ± 0.09 g/d and 1.51 ± 0.25 g/d were obtained for fish fed 30 mg MT/kg feed and 60 mg MT/kg feed respectively. Differences in absolute growth of fish under the various treatments was not significant ($F = 0.206$, $p > 0.05$).

Specific growth rate of hormone – treated fingerlings ranged between 7.95 ± 2.59 % per day and 10.79 ± 6.92 % per day after 84 days (Table 4). The fish fed 60 mg MT/kg feed had a specific growth rate of 10.79 ± 6.92 % per day whilst 7.95 ± 2.59 % per day was recorded in fish fed 30 mg MT/kg feed. Specific growth rates of 8.71 ± 3.94 % per day and 8.36 ± 3.46 % per day were recorded in fingerlings fed 120 mg MT/kg feed and 0 mg MT/kg feed respectively. The ANOVA test conducted indicated that the specific growth rate of fingerlings raised under different treatments was not significantly different ($F = 0.078$, $p > 0.05$).

Table-3. Absolute growth rates [(AGR (g day⁻¹)] and specific growth rate [(SGR) % day⁻¹] of fry of *Sarotherodon melanotheron* treated with 17 α -methyltestosterone for 30 days

Treatment	AGR (g day ⁻¹ \pm SD)	SGR (% day ⁻¹ \pm SD)
0 mg MT/kg	0.34 ± 0.10	9.70 ± 4.31
30 mg MT/kg	0.35 ± 0.09	9.45 ± 3.81
60 mg MT/kg	0.37 ± 0.14	9.01 ± 4.16
120 mg MT/kg	0.41 ± 0.11	9.29 ± 4.13

Table-4. Absolute growth rate (g day⁻¹) and specific growth rate (% day⁻¹) of hormone - treated fingerlings of *Sarotherodon melanotheron* from day 31 to day 84

Treatment	AGR (g day ⁻¹ \pm SD)	SGR (% day ⁻¹ \pm SD)
0 mg MT/kg	1.43 ± 0.10	8.36 ± 3.46
30 mg MT/kg	1.54 ± 0.09	7.95 ± 2.59
60 mg MT/kg	1.51 ± 0.25	10.79 ± 6.92
120mg MT/kg	1.57 ± 0.04	8.71 ± 3.94

3.4. Condition Factor of Hormone-Treated Fish

Fish were fed different concentrations of hormone – treated feeds for 30 days after which the condition of the fish was assessed (Table 5). The fish fed control feed attained the highest condition factor (2.10 ± 0.34) at the end of the hormonal feed administration while fish fed 120 mg MT/kg feed had the lowest condition factor (2.04 ± 0.44). Condition factor values of 2.07 ± 0.58 and 2.09 ± 0.35 were recorded for fish fed 60 mg MT/kg feed and 30 mg MT/kg feed respectively.

The results show that the condition factor of the fish during the treatment period was slightly higher for fish fed 30 mg MT/kg feed, 120 mg MT/kg feed and 0 mg MT/kg feed than the period after the treatment (Table 5). Condition factor for fish fed 60 mg MT/kg feed was however higher at post treatment period than the treatment period. Using paired- sample *t*- test, the difference between the condition factor values for treatment and post treatment periods for fish fed 0 mg MT/kg feed was significant ($t = 6.362$, $p < 0.05$). Significant difference ($t = 3.593$, $p < 0.05$) was observed in the condition factors between treatment and post treatment for fish fed 30 mg MT/kg feed.

Table-5. Comparison of mean condition factor of *Sarotherodon melanotheron* after 30-day hormone treatment and post treatment period

Treatment	Condition	Factor \pm (S. D)	t- statistic	p- value
	Treatment	Post treatment		
0 mg MT/kg	2.10 ± 0.34	1.86 ± 0.13	6.362	0.000*
30 mg MT/kg	2.09 ± 0.35	1.90 ± 0.53	3.593	0.000*
60 mg MT/kg	2.07 ± 0.58	2.15 ± 2.29	- 0.378	0.706
120 mg MT/kg	2.04 ± 0.44	2.00 ± 1.70	0.229	0.819

Significant level at $p < 0.05$

4. Discussion

4.1. Physico-chemical Parameters

Temperature, pH and dissolved oxygen recorded in this study as presented in table 1 were within the desirable limit for tilapia as reported by Gupta and Gupta [16] and Xie, *et al.* [17] that is: DO should be above 3 mg/L, pH within of 6.0- 9.5, temperature ($28 \pm 1^\circ\text{C}$). It also in line with the report of Abdel-Tawwab and Ahmad [18], who found that dissolved oxygen concentrations ranged from 6.6 to 7.4 mg L⁻¹, the water temperature range was 24.5- 26.8 $^\circ\text{C}$, the pH range was 7.8-8.1 Hence, any change in the morphology of the fish species could be attributed to the hormone administered.

Result of the present study showed that each hormone treated groups exhibited male/female ratio that deviate significantly from the normal 1:1 ratio (Table 2) except fish fed on 30mgMT/kg that exhibited normal 1:1 sex ratio.

None of the treatment groups exhibited 100% male population of the *Sarotherodon melanotheron*. The results of the present study showed that almost all MT receiving treatment showed a significantly higher male proportion than the control. There was a significant difference ($F = 266.22$, $p < 0.05$) between the MT treated groups and the control. Proportion of male in the group of fish fed 30 mg MT/kg feed (84 %) was significantly lower ($p < 0.05$) than the percentage males produced in the group of fish fed 120 mg MT/kg feed. There was however, no significant difference ($p > 0.05$) between the proportion of male produced in fish fed 60 mg MT/kg feed and those fed 120 mg MT/kg feed. The maximum male population of 92.70 % obtained in fish fed 120 mg MT/kg feed is higher than the figure reported by Okoko [19] when the same dosage was used. The result obtained using 60 mg MT/kg in feed produced male population of 89.3%, was lower than 95% recorded by Marjani, *et al.* [8], 98% by Lai and Yang [20]; 100% by Mair, *et al.* [21]; 97% by Jae-Yoon, *et al.* [22] and 98 % by Hanson, *et al.* [23]. Reduction in the percentage male produced in the current studies can be attributed to the different feeding strategies.

Different dose rates of MT in feed did not significantly affect the growth of *Sarotherodon melanotheron* in this study. All the treatments which received MT as well as the control showed a similar trend with regards to increment in body weight and length (Figure 2 and 3). The result of the current studies is in contrast with the finding of Ayoola and Akapo [7]; Hanson, *et al.* [23] and Dan and Little [24], who reported gain in body weight of individuals treated with MT.

This research showed that androgen 17- α Methyl Testosterone in feed can be used to produce all male population in *S. melanotheron* but higher dosage will produce high percentage male population. Hence the recommendation of 120 mg MT/kg in feed. High or low dosage of the MT had no effect on growth and condition factor of *S. melanotheron*.

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