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Effect of Testosterone-induced Sex Reversal on the Sex Ratio, Growth Enhancement and Survival of Nile Tilapia (*Oreochromis niloticus*) Fed Coppens and Farm Produced Feed in a Semi Flow-through Culture System

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Abstract

The study aimed at determining the effect of testosterone-induced sex reversal on the sex ratio, growth enhancement and survival of Nile tilapia, *Oreochromis niloticus* fed two feed type (coppens and farm produced feed of 56% and 25% crude protein respectively) and reared intensively under controlled conditions in a semi flow-through culture system in twelve fibre glass tanks. The present study also examine the effectiveness of water flow-through culture system in controlling unwanted reproduction during culture of mixed sex *O. niloticus*. The experiment was designed as 4 treatments × 24 weeks factorial replicated thrice. The fry was treated with a steroid hormone, 17α-methyltestosterone and cultured for a period of 21 days to reverse the sex to male fishes. After completion of the trial period of 21 days, nursing of the experimental fry (mixed sex and sex reversed) was continued for further 24 weeks with the two feed type. The results of the study showed that the highest sex occurrence of 95% and 90% males was recorded in the sex reversed fishes fed Coppens (Treatment II) and Farm Produced Feed (Treatment IV) respectively. Survival rate was 100% among all the treatments. Treatment II had the highest/best mean body weight gain (19.97 g), daily weight gain (0.12 g) and highest final weight (50.11 g). The study clearly indicated that the inclusion of the steroid hormone, 17α-methyltestosterone in the diets significantly altered the sex ratio towards male and enhanced the growth performance and survival rate in the sex reversed group in Treatments II and IV ($P<0.05$) irrespective of the feed type and thus recommended for tilapia culture. In spite of the overall best performance recorded in 56% CP of the commercial diet (Coppens) fed the tilapias in Treatments I and II, which is outside the recommended levels for the growth of tilapia, a significant growth rate ($P<0.05$) was also observed in the tilapias fed Farm Produced Feed with 25% CP content and is also recommended. The inability of *O. niloticus* to reproduce during the 24 weeks of the culture period in this study is an indication that the semi flow-through culture system using fibre glass tanks is a successful reproduction control method for *O. niloticus* and thus should be adopted in the aquaculture industries.

Keywords: Testosterone; Sex ratio; Sex reversal; Growth; Nile tilapia; Coppens; Flow through culture system

Introduction

Sexual dimorphism is significant in *Oreochromis niloticus* where males grow significantly faster, larger and more uniform in size than females [1-3]. Although tilapias in general are known for their aquaculture potential but their growth and other production traits are largely influenced by genetics [4]. Early sexual maturity of this species is a well-recognized problem. The problem of precocious sexual maturity and unwanted reproduction has long been accepted as a major constraint to further development and expansion of Tilapia culture. Use of monosex (all-male) juveniles has been identified as the answer to the problem and has been widely, if inconsistently, promoted and adopted [5]. The issue is more complex, however, as much of the rapid growth in global tilapia production in recent decades has been based on mixed sex fish [6]. The development of strategies for generation of monosex populations remains a critical objective for tilapia culture industry.

Interest in the production of monosex tilapias, as a means of overcoming precocious maturation and reproduction in certain culture systems, has led to intensive research on sex determination in this species. It has an XX/XY sex determination system, which appears to be at an early stage of chromosomal differentiation [7], and all combinations of XX, XY, or YY genotypes as phenotypic males or females can be produced through sex reversal, gynogenesis, and androgenesis [8]. Techniques for the production of genetically monosex male (XY) *O. niloticus* have been developed and used commercially [9].

Synthetic androgens are used in fish culture as sex controlling agents and as growth promoters if energy is shut away from developing ovaries towards growth of somatic tissues [10]. The more common method of generating mostly male populations is through the use of steroids fed to sexually undifferentiated fry. Newly hatched tilapias are still developing their gonads. Even though they are determined genotypically, their phenotype or morphological characteristics can still be altered. By exposing the fish to forms of testosterone or estrogen, the gonad can switch [11]. Typically the desire is to produce all males, so methyltestosterone (MT) is included in the diet for several weeks when the fish starts eating. Utilizing MT at 60 mg/ kg has consistently produced 95% male sex ratio [12,13]. Studies involving sex reversal of Nile tilapia species using 17α-methyltestosterone is well documented. Numerous papers have reported that 60 mg/ kg was found to induce monosex male populations [12-15]. This classical breeding approach

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coupled with the culture of sex reversed and mixed sex *O. niloticus* in a flow-through culture system throughout the fingerling phase of production is yet to be well tested in tilapias. Thus, the study is aimed at determining the effect of sex reversal on the sex ratio, growth and survival of Nile tilapia (*O. niloticus*) fed Coppens and Farm Produced Feed and reared intensively under controlled conditions in a semi flow-through culture system. The present study also examine the effectiveness of water flow-through culture system in controlling unwanted reproduction during culture of mixed sex *O. niloticus*.

Materials and Methods

Location

The study was conducted at the Nigerian Institute for Oceanography and Marine Research (NIOMR), Sapele Out-station (N05054´03.5´ E005039´56.4´) Nigeria.

Selection, maintenance of broodfish and mating: Nile tilapia, *O. niloticus* broodstock of average weight of 300 g were procured from the Nigerian Institute for Oceanography and Marine Research (NIOMR) farm and acclimatized for a week. The male and female tilapia broodstock were paired up in the fiber glass tanks. Broodstock was fed a commercially available floating feed Coppens (45% crude protein) twice daily to satiation. Natural spawning of fish was allowed (i.e. fish will be allowed to spawn of their own volition while the eggs laid by the female tilapia will be fertilized by the male, who discharges sperm over the eggs). The female will collect the fertilized eggs in her mouth, and mouthbroods the eggs for around 6-10 days. After hatching, the newly hatched fry will continue to shelter in her mouth for another 4-7 days before the fry begin to swim freely in schools in the tanks. The tanks were subsequently partially drained and samples of fry from each tank were collected and moved to an indoor flow-through culture system with twelve (12) circular fibre glass tanks, each with a capacity of 3.08 m^3 of water at a nominal flow rate of 2 L/min. Fish were acclimatized to flow-through culture system for 24 hours before the beginning of each trial, at which time, any initial mortality should be recorded and removed.

Preparation of feed with hormone and feeding regime

The 17α-methyltestosterone (MT) was the hormone used in this present study. A stock solution was made by dissolving 0.06 g of hormone in 750 cm^3 of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from stock solution and brought up to 100 ml by addition of 95% ethanol. This solution was evenly sprayed over 1 kg of Coppens (0.5 mm-0.8 mm) with 56% crude protein and mixed. The mixture was mixed again and this was repeated to ensure an equal distribution of the MT throughout the feed. Treated diets were fan dried in shade at 25°C for 24 h then kept in air-tight containers. After natural spawning, free swimming fry were separated into two groups. One group was fed a fine fry Coppens® feed treated with 17α-methyltestosterone dissolved in food grade ethanol using standard sex reversal methods (Macintosh and Little, 1995) for a 21-day period in two identical fibre glass tanks separated within the same flow through culture system. The second group was fed with Coppens but not treated with the hormone. The fry was fed at 20%, 15% and 10% biomass day^{-1} during days 0-10, 11-20 and 21-30, respectively.

Experimental tanks, fish and feeding

Twelve (12) circular fibre glass tanks each with capacity of 3.08 m^3 of water at a nominal flow rate of 2 L/min was mounted indoor in a flow-through and arranged in a row. The experimental fish (mixed sex and the sex reversed tilapia) were randomly allocated at a stocking density of 53 fish m^{-3} to the twelve fibre glass tanks receiving two feed types: Coppens and Farm Produced Feed of 56% and 25% crude protein respectively and fed twice daily at 0800 hour and 1600 hour. Four experimental treatments were used and each treatment replicated thrice as represented in (Table 1). The experiment was designed as 4 treatments × 24 weeks factorial replicated thrice.

Fish sampling and measurement

Sampling of the cultured fish was carried out bi-weekly for a period of 24 weeks for the collection of data to determine the variation among the treatments. Parameters measured included growth parameters i.e. bi-weekly weight gain, specific growth rate, absolute growth rate, daily weight gain. At the end of the study, sex ratios were also determined. Secondary sexual characteristics (especially genital papilla) were used to distinguish males from females.

Chemical analysis

Analysis of crude protein, crude fibre, ether extracts, ash and moisture content were done in triplicate, generally following [16] procedures for the feed and the results were presented in (Table 2).

Experimental design and data analyses

The experiment was designed as 4 treatments × 24 weeks factorial replicated thrice. The Genstat Statistical Package (version 8.1) was used for the analysis of data.

Results

Sex ratios

Sex occurrence of mixed sex and sex reversed *O. niloticus* fed two feed types are shown in Figure 1. The highest male population (95%) was recorded in Treatment II (Sex reversed tilapia fed Coppens). This was followed by 90% male population in Treatment IV (Sex reversed tilapia fed Farm Produced Feed) while the least male population (53.3%) was recorded in Treatment III (mixed sex tilapia fed Farm Produced Feed). The male *O. niloticus* was significantly higher in the sex reversed fish than the mixed sex fish ($P<0.05$). The distribution is shown in Figure 1. Based on overall sex performance, sex occurrence of 75% males was revealed which is significantly higher ($P<0.05$) than the females with 25% sex occurrence among all the treatments. In the present study, the highest sex occurrence in the male tilapias (exhibited in the sex reversed group) compared to the other treatments is an indication that the steroid hormone has a significant effect ($P<0.05$) on their male population. However, the two feed type (Coppens and Farm Produced Feed) did not influence their male population.

Growth

The growth performance of sex reversed *O. niloticus* fed two types of diets over the 24 weeks culture period is shown in Figure 2

Sex type	Treatments	Feed type	No. Fish/m^3
Mixed sex	I	Coppens	53
	III	Farm produced	53
Sex reversed	II	Coppens	53
	IV	Farm produced	53

I: Mixed sex Coppens @ 53 fish/m^3; II: Sex reversed Coppens @ 53 fish/m^3; III: Mixed sex Farm Produced Feed @ 53 fish/m^3; IV: Sex reversed Farm Produced Feed @ 53 fish/m^3V

Table 1: Experimental design based on Sex type/Feed type.

Farm produced feed		**Coppens**	
Ingredients	**Diet (%)**	**Ingredients**	**Diet (%)**
Fish meal	14.50	-	-
Soybean meal	14.50	-	-
Maize	71.00	-	-
Palm oil	10.00	-	-
Min. Premix	1.00	-	-
Vit. Premix	1.00	-	-
Binding agent (Starch)	2.00	--	-
Total	114	-	-
Chemical analysis (%)			
Dry matter	90.00	Crude protein	56.00
Crude protein	25.21	Crude fat	15.00
Ether extract	9.00	Crude fiber	0.60
Crude fiber	1.00	Crude ash	8.90
Ash	7.00	Phosphorus	1.40
NFE	57.79	Calcium	2.30
		Sodium	0.70
		Vitamin A	13,300 IU/kg
		Vitamin C	285 mg/kg
		Vitamin E	266 mg/kg
		Vitamin D_3	2,500 IU/kg

Table 2: Ingredients and chemical composition of the experimental diets (on dry matter basis).

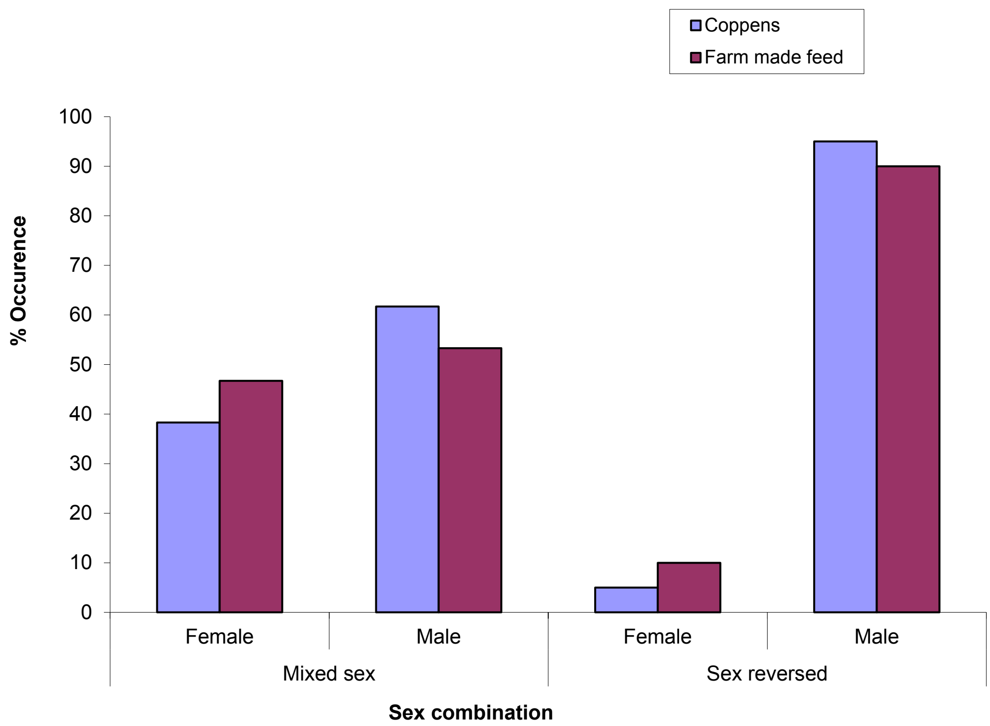

Figure 1: Sex occurrence of mixed sex and sex reversed *O. niloticus* fed two feed types.

and Table 3. Growth performance of *O. niloticus* in terms of mean weight gain and final weight was significantly affected by the androgen, 17α-methyltestosterone ($P<0.05$). Fish in all treatments gradually grew with time, and the highest individual weight was obtained in the 24th week (Figure 2), and there was also a steady increase in the average mean weight gain from week zero to the 24th week. The highest mean body weight gain of 19.97 g was recorded in Treatment II (sex reversed tilapias fed Coppens), followed by Treatment I (mixed sex tilapia fed Coppens) with mean value of 18.72 g while the least mean body weight gain of 10.16 g was recorded in Treatment III (mixed sex tilapia fed Farm Produced Feed) as shown in Table 3. The daily weight gain followed the same trend with the mean body weight gain. Based on sex type (pooled mixed sex and sex reversed), the sex reversed *O. niloticus* fed coppens (Treatment II) had the highest/best mean body weight gain (19.97

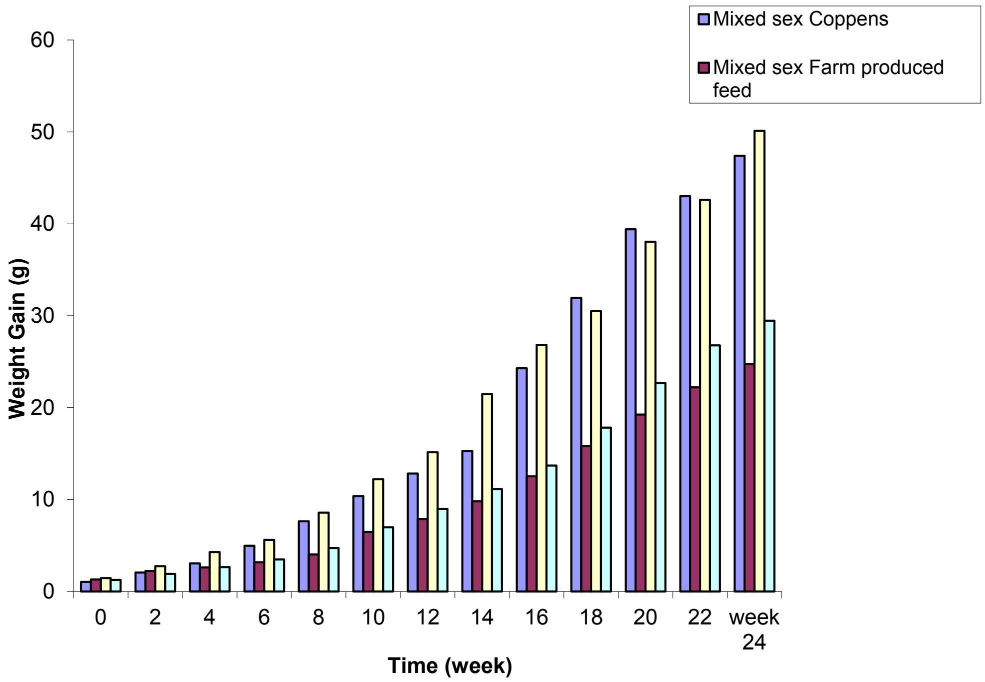

Figure 2: Body weight gain of *O. niloticus* over time.

Parameters	Treatments			
	I	II	III	IV
Initial weight (g)	1.05	1.46	1.31	1.27
Final weight (g)	47.39[b]	50.11[a]	24.73[d]	29.47[c]
Mean weight gain (g)	18.72[b]	19.97[a]	10.16[d]	11.67[c]
Daily weight gain (g)	0.11[b]	0.12[a]	0.06[d]	0.07[c]
Specific growth rate (%/day)	1.3910[a]	1.2869[b]	1.0056[d]	1.0710[c]
Survival rate (%)	100[a]	100[a]	100[a]	100[a]

Means in same row with different superscripts are significantly different (P<0.05).
I: Mixed sex Coppens @ 53 fish/m^3; II: Sex reversed Coppens @ 53 fish/m^3; III: Mixed sex Farm Produced Feed @ 53 fish/m^3; IV: Sex reversed Farm Produced Feed @ 53 fish/m^3

Table 3: Effect of sex reversal on the growth performance parameters of *O. niloticus*.

g) and highest final weight (50.11 g). The specific growth rate (SGR) showed a significant difference among all the treatments (P<0.05) as indicated in Table 3. The mixed sex tilapia fed Coppens (Treatment I) had the highest SGR of 1.3910% day^{-1}, followed by Treatment II (sex reversed tilapia fed Coppens) with SGR of 1.2869% day^{-1} while Treatment III (mixed sex tilapia fed Farm Produced Feed) had the least SGR of 1.0056% day^{-1}. In general, fish fed Coppens (Treatments I and II) had a significantly higher (P<0.05) SGR compared to those fed Farm Produced Feed.

Survival rate

The result of the survival in Table 3 was not significantly different among all treatments (P>0.05). Hundred percentage survivals were recorded in all. No spawning was observed in mixed sex fish throughout the 24 weeks culture period under the semi flow-through culture system in the present study.

Discussion

The percentage of male fish observed in the sex reversed fish receiving MT showed a greater and significantly higher (P<0.05) male proportion as compared to the mixed sex fish without MT. In view of this, the result of the sex ratio is an indication that feeding of 17α-methyltestosterone (MT) to the fry significantly altered the sex ratio towards male which exhibits faster growth and arrested reproduction of the cultured fish. This is very obvious in the highest sex occurrence of male population of 95% recorded in Treatment II (sex reversed tilapia fed Coppens). A similar higher sex occurrence of 90% male was also recorded in Treatment IV (sex reversed tilapia fed Farm Produced Feed) which were significantly higher (P<0.05) than the mixed sex group in Treatments I and III with sex occurrence of 61.7% and 53.3% males respectively as shown in Figure 1. This agreed with the findings of AOAC [17] which recorded about 95% success with the use of 17α-methyltestosterone. Similarly, it was reported by Omitogun OG, Barry [18,19] that over 95% of the population was masculinized in 21-28 days when 30-60 mg of 17α-MT/Kg feed was applied orally to the tilapia larvae (7-12 days of age, 9-11 mm in total length and 10-15 mg of total weight). Utilizing MT at 60 mg/kg has consistently produced 95% male sex ratio [12-13]. Mair et al., Green et al., [9,20] both recorded 98%-100%. Lai and Yang [21] obtained 97% success rate, Jae-Yoon [22] obtained 98% males at the dose rate of 60 mg kg^{-1} MT while Romerio [23] also obtained 71.9% males at the dose rate of 120 mg kg^{-1} MT of feed. Macintosh [14] reported a sex conversion of 95.7% and 92.8% in their experimental units stocked with methyltestosterone-treated fry. The highest proportion of males (90.3%) was obtained by Okoko [24] when O. spilurus fry was orally administrated with 70 mg/kg of 17α-MT. The highest percentage of male (93-100%) was recorded when *O. niloticus* was treated at the hormone dose of 70 mg MT/kg of feed with 30 and 40% protein levels [25]. Oral administration of 17α-MT to newly hatched tilapia fry (3-12 d old) for ~28 consecutive days results in populations composed of >90% males [5,26]. The highest percentage occurrence of male in the sex reversed group in this present study is an indication that the androgen had a significant effect ($P<0.05$) on their male population among all the treatments. Previous studies have shown that the methodology used to produce monosex stocks in the current trial consistently produces sex ratios consistently in excess of 99% male [27]. However, in this present study, diets containing 17α-methyltestosterone had significant effects ($P<0.05$) on sex ratios of *O. niloticus* as presented in Figure 1. On the other hand, dietary protein levels have no influence on the sex reversing activity in Nile tilapia. This is an indication that oral administration of 17α-MT was effective in producing masculinized tilapia as presented in Figure 1. *O. niloticus* has an XX/XY chromosome sex determination system [28], but the process of sex differentiation is labile rendering sex reversal possible in the species [29]. Several techniques have been adopted for production of monosex (all-male) tilapia [30], and hormonal sex reversal of tilapia has been an active area of research for the past three decades [31]. Oral administration of exogenous male sex steroid hormones before the differentiation of primal gonadal cells can cause reversal of phenotypic sex [32] as observed in this present study. In an earlier study, almost 100% all-male monosex tilapia population was produced by treating 3 days old fry with a synthetic male hormone 17α-methyltestosterone (MT) at a treatment regime of 10 mg kg^{-1} feed for 30 days [33].

All-male tilapia production by hormonal sex reversal generally focuses on one specific aspect, namely hormone dose or treatment duration [34]. Such a single minded strategy is important for achieving initial success with a particular technique. The time has now been reached to study other modifying factors necessary to achieve 100% success with this technology. It is imperative to consider genetic as well as other factors - hormone dose, treatment duration [31], solubility of hormone in the solvent [35], water temperature, salinity, feeding regime, photoperiod, stocking density, effect of growth promoters like thyroxine, storage conditions of steroid hormone and hormone-treated feed, and others - if successful culture of all-male tilapias and other commercially important fish species is to be realized. However, in this present study, it is apparent that a small proportion of females are still observed, emphasizing the need for optimizing sex-reversal to produce very high sex conversion rate, preferably in excess of 98% male.

The results of the present study showed an excellent survival rates of 100% among all the treatments as observed in both mixed sex and sex reversed fish and was not also influenced by the feed type. The androgen, 17α-methyltestosterone was reported to have no deleterious effect on survival of tilapia [36,37]. In trials with farmers, Chakraborty [38] observed that survival of monosex Nile tilapia nursed in hapas over a 4-6-week period was close to 100%. Sodsook [39] found that survival of monosex fries stocked at high densities in hapas (>2000 fries m^{-2} hapa) exceeded 80% across a range of feeding management over 90 days. This is in agreement with the findings of Wangpen [40] who indicated that higher (100%) survival rates could be linked to favorable ecological conditions. Sherif [41] also reported that Nile tilapia have high adaptation to stress, which, in turn, contributed to their high survival rate and stable conditioning factor. To support these notions, the flow through culture system adopted in the present study may have attributed to the high survival rates of the fish among all the treatments. Similarly, VeraCruz [37] reported that continuous exchange of water in flow-through system might have sustained a better general environment for fish growth than in cistern culture system [37]. In the present study, no mortality was recorded throughout the experimental period. However, further research on impact of water flow rate during flow-through culture system is to be carried out since it has been observed that at a very high water flow rate, fish spend a substantial amount of dietary energy for continuous swimming, leading to reduced growth and increased mortality [42]. Surprisingly, no spawning was observed in mixed sex fish under semi flow-through culture system in the present study. This could be attributed to the constant water flow in all the treatments. This is in agreement with El-Sayed, DeLong El-Sayed, DeLong [43,44] who reported that intensive tank culture offers several advantages over the use of pond in the sense that it disrupts breeding behavior and allows male and female tilapia to be grown together to marketable size as observed in this present study. In addition, the inability of the tilapias to spawn in the experimental fibre glass tanks could also be attributed to the sex reversal method adopted in this study, to alter the sex of the genotypic female tilapias to phenotypic males. This is in agreement with research findings of Ajiboye [4] who reported that mono-sex culture method has been widely used to control the precocious maturity and uncontrolled reproduction in tilapias.

The different growth rate parameters (final body weight, weight gain and specific growth rate (SGR) of *O. niloticus* fed Coppens and Farm Produced Feed of 56 and 25% protein diets respectively are shown in Table 3. The obtained results in the present study revealed that administration of 17α-methyltestosterone (MT) induced significant increase in fish growth of treated Nile tilapia (Table 3). The increase in body weight gain (Figure 2) may attribute to that androgenic steroids enhance the release of growth hormone from the pituitary somatotrops of fish and/or induce the feed digestion and absorption rate causing increase in body weight [45-46]. Statistical analysis on mean body weight gain and mean final weight showed a highly significant ($P<0.05$) difference among the treatments. In this study, the sex reversed *O. niloticus* fed Coppens (Treatment II) had the highest/best mean body weight gain (19.97 g) and highest final weight (50.11 g) compared to the mixed sex fish. The same trend was observed with the daily gain in weight where the sex reversed *O. niloticus* fed Coppens (Treatment II) recorded the highest ($P < 0.05$) values compared to the other treatments. These results show that the different growth parameters were significantly affected by protein level and hormone (MT) ($P<0.05$). The results also indicate that the inclusion of protein and hormone in fish diet is beneficial for fish growth. These results are in agreement with El-Greisy [47] who found that the hormone significantly ($P<0.05$) increased the final weight of fish (Nile tilapia) as compare to untreated fish. The interaction of both factors was affected on the growth parameters. Khouraiba [48] reported that 10-60 ppm MT-treatment showed the best growth than control. Hanson [49] observed faster growth in O. mossambicus when fed 17α-methyltestosterone. These results are also in line with Varadaraj [50] who compared the culture performance of different strains of *O. niloticus* and found that considering all strains, MT treatment resulted in a final size of fish larger than mixed sex fish. The increase in fish growth may be because MT administration increased the proteolytic activity of the gut as the case in mirror carp leading to increase in growth rate [51]. Furthermore, MT treatment may stimulate thyroid and internal functions as well as insulin secretion from the pancreatic

B cells of fish [52]. Also, androgenic steroids may promote the release of growth hormone from the pituitary somatotrops fish [52]. Anabolic steroids are potentially useful compounds in aquaculture due to their ability to increase weight gains and muscle deposition of treated fish [53].

In this study, fish fed Farm Produced Feed of 25% CP compares favourably with those fed foreign feed (Coppens) of 56% CP irrespective of the protein level which also shows that the locally made feed meets up with the nutritional requirements of *O. niloticus*. Similarly, Ajiboye and Yakubu (2010) reported that most nutritionists or fish farmers based the nutritional value of a feed or feedstuff solely on its chemical composition instead of the amount of the nutrients or energy the fish can absorb and use. The crude protein of 25% (Farm Produced Feed) used in the present experimental diets for *O. niloticus* fingerlings (Table 2) falls within the recommended range of 25% - 35% crude protein requirement for tilapia species [54] and 30%-35% recommended by Santiago, National Research Council [55-56] and satisfied the nutrient requirement for tilapias [57] while 56% CP of the commercial diet (Coppens) used in this study is well outside the recommended levels but with satisfactory results. In this study, there was significant increase in growth parameters ($P<0.05$) with increasing protein levels. These results are in agreement with Jauncy [58] who showed that the final body weight, weight gain and specific growth rate (SGR) were positively enhanced by protein level. Also, Khattab [59] found that the better growth rate of Nile tilapia was obtained at high dietary protein levels (40-45%) than at 25- 35% protein level.

The study clearly indicated that the inclusion of the steroid hormone, 17α-methyltestosterone in the diets significantly altered the sex ratio towards male and enhanced the growth performance and survival rate in the sex reversed group ($P<0.05$) irrespective of the feed type and thus recommended for tilapia culture. Further research on impact of water flow rate during flow-through culture system is to be carried out since it has been observed that at a very high water flow rate, fish spend a substantial amount of dietary energy for continuous swimming, leading to reduced growth and increased mortality. In this present study, it is apparent that a small proportion of females are still observed, emphasizing the need for optimizing sex-reversal to produce very high sex conversion rate, preferably in excess of 98% male. It is therefore imperative to consider genetic as well as other factors - if successful culture of all-male tilapias is to be realized [60]. The inability of *O. niloticus* to reproduce during the 24 weeks of the culture period in this study is an indication that the semi flow-through culture system using fibre glass tanks is a successful reproduction control method for *O. niloticus* and thus should be adopted in the aquaculture industries.

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