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Research Article

Optimization of Hormone Induced All Male Production of *Oreochromis niloticus* Under Laboratory Condition at Ambo, Ethiopia

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Abstract

All male- culture is preferred by farmers for several species of fish considering the fast growth. The sex reversal by using hormone has been successful in Nile tilapia (*Oreochromis niloticus* L.). In the present experiment, the tilapia fry were collected from the mouth of brooding female fishes and fed with 17α Methyltestosterone (17 α MT) mixed feed at the dosage of 40 mg kg^{-1}, 50 mg kg^{-1} and 60 mg kg^{-1} to produce all-male progeny. 17 α-MT was incorporated into a diet containing 30% protein and the treatment lasted for 50 days. The mean total length of the fry used was 1.59 cm and weight 0.148g. After 21 days of hormonal treatment, 86.66% of the fishes in the 60 mg/kg treatment group turned into males with 3.33% mortality. Among the 50 mg/kg treatment group 75% and in 40 mg/kg 53.3% fishes turned into males. The specific growth was significant in 40 mg kg^{-1} of MT treatment. The length-weight relationship is not positively correlated even though it was linear in the treatments.

Keywords: 17 α Methyl Testosterone; Oreochromis niloticus; Monosex; Masculinization

Introduction

In aquaculture male tilapia are preferred because of their higher growth performance compared to female [1,2]. In most culture systems male tilapia produces greater harvested yields than mixed-sex populations. This is because the metabolic energy in males is mainly transferred towards growth unlike in the case of female fishes, where a greater reallocation of metabolic energy towards reproduction and benefit from anabolism enhancing androgens [3,4]. There are several methods of producing monosex fishes under controlled conditions such as hormonal sex reversal, manual sexing, hybridization or super male production. Sex reversal method is a valuable tool consisting in the administration of steroids to undifferentiated fish through diets as common mechanisms in aquaculture [5,6]. The direct masculinization of tilapias using hormone is a common method for monosex male production [2,7]. The male population can be obtained by direct oral administration of 17α-methyltestosterone (17α-MT) through feed to the tilapia fry (start to feed) so that the undifferentiated gonadal tissue of genetic females develops into testicular tissue, producing individuals that grow and function reproductively as males [8,9]. It has been reported that sex reversed fish may grow up to two to three times faster, when treated at optimal dose for sex reversal [10]. High rate of masculinization in tilapia can be influenced by some important factors like hormone concentration, treatment duration, age and size of fry, availability of natural feed, stocking density and feeding frequency [11]. Previous studies were conducted with hatchery produced fry stages of fish which is not always possible for farmers having limited facilities. Hence an attempt was made to produce all-male Nile tilapia population by rearing fry collected from the fish ponds using 17α-methyltestosterone under laboratory conditions.

Materials and Methods

The Nile tilapia hatchlings were collected from the farmer's pond located at Bako, West Shoa zone. The hatchlings were sorted based on their length and weight. The experimental diet having 30% protein was prepared using pea, wheat bran and yellow corn. Fish meal was added as a source of minerals and fatty acid. The crude protein level was estimated following Pearson's square method [12,13]. The prepared feed was dried at room temperature and then sealed in air tight container and stored in refrigerator [9].

Feeding protocol

For the experiment 40, 50 and 60 mg/kg of 17α-MT were added to the diet just before feeding every day. For the control fishes the feed was given without the addition of hormone. The diet containing 17α-MT was fed to the swim up fry (≈ 0.12 g) from the 5^{th} to the 26^{th} day after stocking them in glass aquaria. The feeding was continued up to 50 days with the control diet alone. The feed was given two times daily during the day light hours. At the beginning of feeding, the diet was given as fine powder. The daily ration of feed ranged from 15% of fish weight initially and decreased to 10% during the rest of the rearing period. For each experimental and control diet, triplicate treatments were maintained. The unfed feed and faecal pellets were collected daily and water level was maintained (20L) every day. Temperature and pH of the water was maintained at optimum level. The number of fishes dead in each treatment during the experiment was also recorded for calculating the survival rate.

Confirmation test

After the completion of hormonal treatment with test diets (Hormone mixed feed) and further growing with control diet for

50 days the fishes were subjected to histological examination for the identification of the sex. The gonads, the fingerlings were dissected to prepare the smear under microscope after staining with Aceto carmine for sex confirmation [14].

Growth Analysis

Growth performance of the fry and fingerlings was analyzed during the experiment after every 10 days of treatment.

The following calculations were made to evaluate fish growth performance:

a) Weight gain = Mean final fish weight- Mean initial fish weight (mg)

b) Specific Growth Rate (%/ day) = (Log W2 - Log W1 ×100)/T2-T1) [15])

Where;

W1=the initial live body weight (g) at time T1 (day)

W2= the final live body weight (g) at time T2 (day)

c) Survival (%) = Number of fry present on completion of experiment ×100/No. of fishes stocked

d) Length-weight relationship:

The length-weight relationship of the experimented fish was worked out as per cube law

$W=aL^b$ [16]

Where, W=Weight of fish (g), L is observed total length (cm), 'a' is the regression intercept and 'b' is the regression slope.

Results

The experiment was conducted during November 2016 to February 2017 in the biology department laboratory at Ambo University. The mean initial total length of the fry of the treatments varied from 1.46 to 1.64 cm and the control was 1.7 cm (Table 1 and 2). Plastic pools capacity of 50L were used for treatment of fishes with 40 mg/kg MT, 50 mg/kg MT and 60 mg/kg MT in triplicates. In each treatment and control 15 individuals were stocked.

Treatment	Trial	IBL (cm)	BL@10 days	BL @20 days	BL @30 days	BL @40 days	BL @50 days
Control		**1.7**	**3.35**	**3.72**	**4.03**	**4.1**	**5.19**
40 mg	TR1	1.53	2.95	2.97	3.12	3.58	4.5
	TR2	1.76	2.86	2.96	2.98	3.41	4.44
	TR3	1.63	3.3	3.71	3.71	3.77	4.62
Avg		**1.64**	**3.03**	**3.21**	**3.27**	**3.59**	**4.52**
50 mg	TR1	1.43	3.1	3.1	3.41	3.51	4.53
	TR2	1.6	3.1	3.16	3.5	3.83	4.81
	TR3	1.66	3.13	3.14	3.51	3.5	4.5
Avg		**1.56**	**3.11**	**3.13**	**3.47**	**3.61**	**4.61**
60 mg	TR1	1.63	3.06	3.27	3.36	3.53	4.53
	TR2	1.36	2.8	3.03	3.08	3.38	4.51
	TR3	1.4	2.8	2.925	3.166	3.45	4.65
Avg		**1.46**	**2.88**	**3.07**	**3.20**	**3.45**	**4.56**

Table 1: Body length of fishes in different dose of 17α methyl testosterone.

Treatment	Trial	IBW	BW @10	BW @20	BW @30	BW @40	BW@50
Control		0.16	0.67	0.93	1.24	1.72	2.64
40 mg	TR1	0.12	0.37	0.39	1.22	1.74	2.74
	TR2	0.17	0.35	0.38	0.82	1.57	2.54
	TR3	0.16	0.72	0.80	1.39	1.75	3.18
Avg.		**0.150**	**0.479**	**0.522**	**1.143**	**1.687**	**2.819**
50 mg	TR1	0.14	0.59	0.63	0.90	1.28	2.57
	TR2	0.17	0.49	0.50	0.60	1.10	2.40
	TR3	0.16	0.51	0.58	0.68	0.97	1.77
Avg.		**0.157**	**0.533**	**0.568**	**0.725**	**1.116**	**2.246**
60 mg	TR1	0.13	0.42	0.46	0.58	0.93	1.80
	TR2	0.12	0.42	0.56	0.60	1.08	2.45
	TR3	0.12	0.36	0.39	0.43	1.07	2.38
Avg.		**0.125**	**0.399**	**0.468**	**0.534**	**1.028**	**2.210**

Table 2: Body weight of fishes in different dose of 17α methyl testosterone.

Male fingerling production

The different doses of methyltestosterone mixed diet showed variation in its effect on production of male tilapia fingerlings. The highest (86.66%) male production was noticed in the 60 mg/kg of 17α MT after 26 days of oral treatment as feed additive with 3.33% mortality. The second highest (75%) of male fingerlings was observed with 50 mg/kg of MT treatment with 10% mortality. The least percentage of males (53.33%) was noticed in 40 mg of MT which was slightly higher than the control fishes (45%) (Table 3). The results revealed that as the amount of the hormone in the diet increases the percentage of male fishes also increases.

Hatchlings	Swim up Fry	Dose of MT and mode of treatment	Total fishes	Days	Male	Female	% Mortality	% of Male
240	220	Control	40	26	18	22	12.5	45
		40 mg MT as feed additive	60	26	32	24	6.67	53.33
		50 mg MT as feed additive	60	26	45	14	10	75
		60 mg MT as feed additive	60	26	52	6	3.33	86.66

Table 3: Dose of 17α methyl testosterone treatment for Oreochromis niloticus.

Histomorphology of gonads

For microscopic examination the fish were cut and a few drops of Bouin's fluid was added so as the gonads to become hard. The gonads were removed and placed on a clean slide. Male gonads were thin, translucent and located behind to the swim bladder, extending caudally from the head to the genital papilla. Female gonads were thick opaque, had a round shape, and were also located on the ventral side of the swim bladder. However, the anterior part of the ovary was located more caudally than the testis and it was possible to identify the ligaments that held the tissue to the body wall.

The tissues were pressed gently and aceto carmine solution was added and examined under a compound microscope using higher magnifications. The criteria followed to define male and female gonadal tissue is the presence of cyst-like structures containing spermatogonia and spermatocytes and appearance of oocytes at different stages of development. The gonadal tissue was defined as testis by the presence of cysts with different developmental stages. The ovaries appear with oocytes at the perinucleolar stage. However, the nucleoli appeared like granules (Plate 1, Figure 1 and 2).

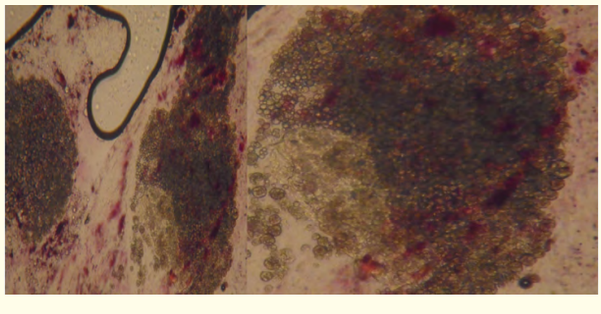


Figure 1: Appearance of female gonads of the fingerlings.

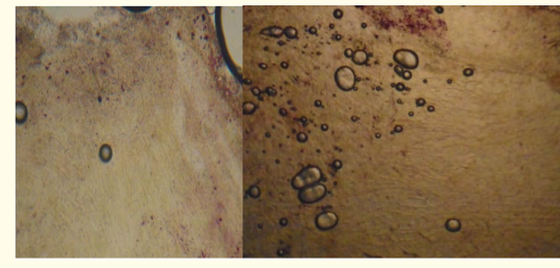


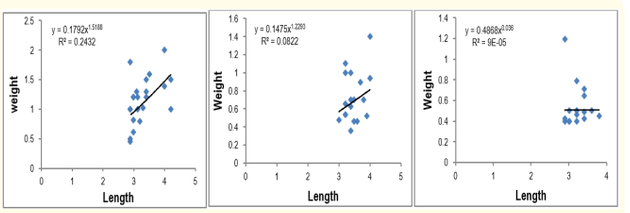
Figure 2: Appearance of male gonads of the fingerlings.

Plate 1: Section of gonads of the male and female fishes after hormonal treatment.

The experiment results showed the progressive growth in length from initial body length. The rate of growth was found to be faster during the first ten days and gradually decreased during the rest of the period (Table 1). The maximum length 4.61 cm was observed in 50 mg/kg MT treatment followed by 60 mg/kg MT (4.56 cm) and 40 mg/kg MT (4.52 cm). However, there was a slow and progressive increase in length from 10th day to 40 day. Further, the growth rate was found to be higher from 40th day to 50th day in all the three treatment doses (Table 1). The body weight of the fishes followed the same trend as that of body length. The increment in weight was high for the initial 10 days. However, the control fishes showed more body weight than the MT treated fishes except the fishes treated with 40 mg of MT. Earlier to initiate the experiment the body weight of the fishes was not the same, ranging between 0.12 and 0.17g. Compared to 40 mg/kg and 50 mg/kg of MT, the fishes treated with 60 mg MT showed less growth with respect to body weight. Finally, the body weight of the fishes in 40 mg of MT was highest (2.819g) followed by 50 mg of MT (2.246g) and 60 mg

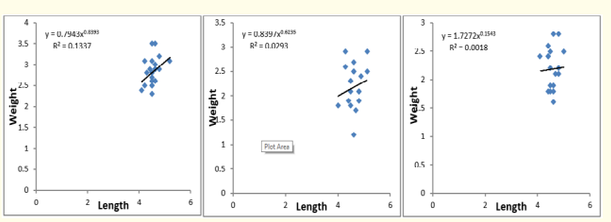
of MT (2.210g) (Table 2). However, the body weight of all fishes increased from 30th day to 50th day.

The quality of the fish fingerlings are assessed by considering the growth and condition of the fishes by measuring the length–weight proportion besides the disease and parasite infestations. According to cube law the 'b' value of the fishes in a good condition will be 3 or slightly higher. In the present experiment, the 'b' values showed an increasing trend up to 20 days of hormonal treatment and later the values declined in all the treatments (Figure 3 and 4).



40mg/kg 50mg/kg 60mg/kg

Figure 3: Length –weight relationship of fingerlings treated with 40 mg (L), 50 mg (C) and 60 mg (R) of MT for 30 days.



40mg/kg 50mg/kg 60mg/kg

Figure 4: Length –weight relationship of fingerlings treated with 40 mg (L), 50 mg (C) and 60 mg (R) of MT for 50 days.

The specific growth rate of the fingerlings throughout the experimental period is given in table 4 and illustrated in the figure 5. The results of the present experiment revealed that during the initial 10 days period the mean growth rate was highest followed by subsequent ten days. The daily growth rate of the fingerlings showed decrement from 10th day to 50th day of the experiments. It is clearly indicated that the initial growth rate of fingerlings was faster than the later stages (Figure 5). The mortality rate was high (10%) at 50 mg/kg MT treatment. The second highest mortality (6.67%) was observed in 40 mg/kg of MT treatment followed by 60 mg/kg of MT (3.33%).

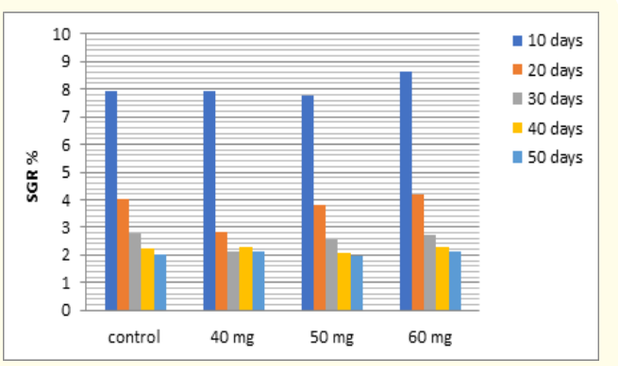


Figure 5: Daily percentage growth of fishes at different MT dose and days.

The mean values of important water quality parameters such as, temperature, pH, dissolved oxygen, conductivity, carbon dioxide, and Turbidity were measured (Table 5). The water temperature varied between 26 and 27˚C and the pH was slightly alkaline (7.2-7.6). The dissolved oxygen was maximum 5.1 mg/l. The free carbon dioxide was initially high and then declined, ranging between 5 mg/l to 13.1 mg/l. Ammonia and nitrite were low and were within the required limits.

Dose	Trial	10 days	20 days	30 days	40 days	50 days
Control		7.919168	4.018847	2.793122	2.259778	2.04094
40 mg	I	8.776202	4.195065	3.169704	2.542549	2.27984
	II	7.235954	3.425492	2.479888	2.120287	1.94442
	III	7.816332	3.881013	2.794857	2.232788	2.0935
	Avg	7.942829	3.833857	2.814816	2.298541	2.10592
50 mg	I	8.312274	4.066421	2.799755	2.243078	2.11711
	II	7.389111	3.543482	2.344713	1.964721	1.91931
	III	7.669371	3.742928	2.482304	1.974277	1.84008
	Avg	7.790252	3.784277	2.542257	2.060692	1.958833
60 mg	I	8.376827	4.038258	2.67795	2.157026	2.0054
	II	8.827824	4.348293	2.847485	2.335424	2.2308
	III	8.672276	4.150565	2.668056	2.310471	2.20091
	Avg	8.625642	4.179039	2.731164	2.26764	2.145703

Table 4: Specific growth rate of fingerlings at different interval days.

S.N	Parameter	1st 10 days	2nd 10 days	3rd 10 days	4th 10 days	5th 10 days	Mean value	Standards
1	Temp.° C	26	27	27	26	27	26.6	
2	pH	7.2	7.3	7.6	7.3	7.6	7.4	6.5 -9 Lloyd, (1992)
3	DO (mg/L)	4	4.7	3.8	5.1	4.4	4.4	>5.0 LAMEQC, 1999
4	Conductivity	300	455	387	244	298	336.8	20-1500 µ mhos/cm (Abowei, 2010)
5	CO_2 (mg/L)	11.2	13.1	9.2	7.8	4.5	9.16	
6	Ammonia (ppm)	0.01	0.012	0.017	0.011	0.02	0.014	0-2 mg L-1 Stone and Thomforde (2004)
7	Nitrite (mg/L)	0.5	0.52	0.65	0.45	0.19	0.462	< 0.5 mg/L Awann 1993

Table 5: Water quality parameters (Mean).

	L40	L50	L60	W40	W50	W60	Temp	pH	Cond.	Nitrite	DO	CO_2	Amm.
L40	1												
L50	-.612**	1											
L60	0.3	-0.1	1										
W40	0.35	-0.32	-0.36	1									
W50	-0.24	0.19	-.489*	-0.31	1								
W60	-0.06	0.15	0.06	0.15	-0.46	1							
Temp	0.8	-0.84	0.76	0	0	0	1						
pH	0.6	-0.27	0.73	-0.22	0.06	-0.33	0.73	1					
Cond.	0.37	-0.35	0.34	0.4	-0.4	-0.05	0.71	0.11	1				
Nitrite	0.12	0.28	-0.16	0.63	-.866*	-0.67	-0.11	-0.29	0.47	1			
DO	-0.2	0.11	-0.77	0.07	0.29	0.4	-0.38	-0.53	-0.16	0.02	1		
CO_2	-0.15	0.06	-0.27	0.55	-0.55	-0.05	-0.09	-0.65	0.64	0.75	0.17	1	
Ammonia	0.71	-0.46	0.63	0	0.12	-0.35	0.67	.926**	0.06	-0.28	-0.68	-0.61	1

Table 6: Pearson correlation among different parameters.
Correlation is significant at the 0.01 level (2-tailed)**
Correlation is significant at the 0.05 level (2-tailed).*

The Pearson correlation between and among different parameters shows that variation and significance at 0.01 level (2-tailed)** and 0.05 level (2-tailed)*. Nitrite is strongly correlated with weight of the fishes at 50 mg/kg of MT treatment. Ammonia is correlated with pH of the water at 0.01 levels.

Discussion

Production of all-male fish population of *O. niloticus* for aquaculture is of high priority since males have a higher growth rate compared to females. Technique used for sex reversal depends on several factors such as species, effectiveness of the practice, legality and public concerns. The period of gonadal differentiation varies significantly among species. In general, this period is much shorter in warm water fishes compared to cold water fishes. The oral hormonal treatment for sex reversal is conducted with the beginning of exogenous feeding of the fry before the gonadal differentiation. 17α-Methyltestosterone is effective in producing phenotypic male of tilapia. More than 90% male populations have been obtained at a variety of dose rates. With the administration of 10 mg MT/ kg of diet 97% males [17] *O. niloticus* have been produced. However, 95 to 98% males with 40 mg MT/kg of diet and 99% with 60 mg MT/kg of diet fed at 20% body weight for 25 days feeding trials were also obtained [18]. Romerio., *et al.* [19] obtained 98% male population at the dose of 60 mg MT/kg of feed whereas 99 - 100% male were resulted for the same dose by Smith and Phelps [20] for Nile tilapia. The results of the present study showed a significantly lower male fish (86.66%) for highest dose of androgen i.e. 60 mg MT/kg of feed. This finding is in line with the findings of Guerrero [1] who obtained 85.0% males at 60 mg MT/kg in 18 days of treatment who observed that 30 MT/kg dose resulted in increment of male percentage. Also increase in duration of 30 mg MT treatment can result 99.2% [21] to 99.3% male [22] tilapia population and still prolonged duration and exposure at moderate doses like 30 and 40 mg of MT led to 100% males [23,24].

In this study a relatively high percentage (86.66%) of male compared to the females [25,24] implying that 17-α MT hormone is an effective androgen in sex reversal in fry collected from natural environments and grown under laboratory conditions. However, male: female sex ratio in this study (45:55 in control, 53.33:40 in 40 mg of MT, 75: 23.33in 50 mg of MT and 86.66: 10 in 60 mg of MT) were less when compared to earlier studies [26, 24].

The major environmental factors influencing sex reversal are temperature and dissolved oxygen [27,28]. In the present experiment even though the feeding rate was similar, fed at 10% body weight two times a day, a low male percentage observed seems to be highly correlated to the low temperature (20 to 24°C) and the dissolved oxygen in the water which were slightly lower than recommended values (4 mg/L) for optimality [29].

Although treatment with Methyl testosterone for 21 days significantly increased the male proportion, even with the lowest dose (40 mg/kg), it was not as efficient as the treatment for higher dose and prolonged duration of treatment. In Nile tilapia ovarian differentiation occurs 23 - 26 days after hatching [30] for complete feminization using ethynil estradiol, the oral treatment should be given from 6 to 25 days of age. It may be possible that with the highest dose the conversion of androgens to estrogens was inhibited for longer periods than with the lowest one. The findings of this study are in accordance with the observation that the time of onset of treatment, the duration, the drug, and the dose used also influence the percentage of sex reversal in fishes [31].

The mortality observed during the hormonal treatment may be explained by the establishment of feeding hierarchy among fish. Dominant individuals within the population may consume more food and grow faster leaving less food for submissive individuals who have less growth and become consequently vulnerable to cannibalism and death by starvation. This might be the reason for the high rate of mortality occurred (10%) in treatments with 50 mg of MT. The size of the fry used were not uniform since they were collected from the ponds unlike the hatchery reared ones and this variation might have led to high mortality rate through the establishment of feed hierarchy since the diet was the only source of food [18,32]. Sex reversal experiments in indoor tanks encountered greater mortality than in outdoor tanks [33]. About 40% survival was reported when *Oreochromis niloticus* was subjected to sex inverse in indoor tanks [34] which is lower than the present experiment results.

The length-weight relationship of the various treatments indicated a positive and linear correlation between the two variables for about 20 days of treatment which is the early fast growing period for fishes. This is also well established through the specific growth rate analysis in this study. It has been observed that animals under stress need to spend more energy for homeostatic processes [35] leading to reduced growth rate observed in the reduced weight increment after a period of 20 days in fish with high dose of MT treatment [33].

Conflict of Interest

There is no conflict on any issues relating to the research between the authors.

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