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Journal of Biotechnology and Bioprocessing
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Research Article

Effect of 17 α methyl testosterone on sex reversal of Oreochromis niloticus fry

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Received date: March 13, 2021; Accepted date: March 28, 2021; Published date: March 30, 2021
Citation: Elsadig A. Hagar, Eitdal M. Tom and Namarig B. Ahmed (2021) Effect of 17 α methyl testosterone on sex reversal of Oreochromis niloticus fry. ***J,*** *Biotechnology and Bioprocessing.* 2(3); DOI: 10.31579/2766-2314/033

Abstract

The effect of oral application of three concentrations of 17 α methyl testosterone (17 α-MT) in sex reversal of *Oreochromis niloticus* fry was studied. About 1,200 day one *O. niloticus* larvae with an average weight of 0.002g distributed into 12 plastic container (each 14L capacity). The experiment consisted of four treatments and three replicates for 17 α-MT application in stage one, and 12 happa (1m×1m×1m) in stage two for fry rearing. The male% increased significantly ($p<0.05$) with increase of 17 α-MT concentration. T_1 (63%) yielded 84%; T_2 (78.3%) yielded 87% and T_3 (86.7%) yielded (89%). Survival rates significantly decreased ($p<0.05$) with the increase of 17 α-MT level (T_3, T_2 and T_1, respectively). The proved that oral application of 17 α-MT is useful in sex reversal and production of male of *O. niloticus*.

Keyword: oral, methyl testosterone, sex reversal, fry**,** *oreochromis niloticus*

Introduction

Oreochromis niloticus is considered one of the most important fresh-water species cultured all over the world [18]. According to [13] *O. niloticus* is characterized by fast growth, resistance to high temperature and diseases, adaptability to low water quality, and suitability to different farming systems. One of the problems associated with its culture is excessive breeding [32]. As the male tilapias grow faster than females, culture of a mono-sex reduce the fish breeding and divert less energy into reproduction [7]. However, the male tilapia can be produced by manual sexing, environmental manipulation, hybridization, triploid, pulse electric field inductions [30]; genetic manipulation or hormonal sex reversal [7]. None of these methods can achieve 100% sex reversal and thus a combination of methods is suggested [9]. According to [31] induction of sex reversal may serve as a valuable tool to produce mono-sex populations for the aquaculture industry. According to [21], sex reversal by 17 α-MT application in feed is probably the most effective and practical method for the production of all male *O. niloticus*. However, this technique require same age group of fish in early stage to ensure good reversal percentage; and easily lowering percent when natural feed is available in production environment [22]. Long time required in nursery stages results in high rate of mortality [4]. Oral application (egg and fry immersion) shows gave the best results, with a higher success rate and without the risk to employees due to contact with the 17 α-MT during the preparation of the feed [26]. Some other advantages of these techniques are: it takes less period of time, less water quality and quantity is needed and no influences from fish feeding behaviour [4]. Studies on sex reversal by oral immersion of 17α methyl testosterone are very few and focused mainly on eggs instead of fry such as in Coho salmon *Onchorynhus kisutch* [28]; *Oreochromis mossambicus* [32]; *O. niloticus* [15 and 16]. [10] and [27] reported that, the masculinization technique by oral administration of 17 α-MT is the most common method used in the Philippines for the production of all male tilapia [3] revealed that about 88% male were resulted when immersion of 2 day old eggs in 500 µg/L of methyl testosterone at 24 hours. [5] used methyl testosterone at 200-400µg/L and immersed new hatched larvae for 2h duration per week resulted in 82-100% males. 90% male were obtained by [8] when he immersed 21-30 day post-hatched tilapia fry in 5mg/L methyl testosterone for 3 days. However, [14] obtained 79.3% males when immersing the newly hatch tilapia in 200µg/L methyl testosterone in 13 days [15] showed that 3-hr exposure of *O. niloticus* fry at 10 and 13 days in methyl-testosterone at 500 mg l-1 getting more than 93% male. The objective of this research is to determine the efficiency of orally application method of 17 α-MT on sex reversal; growth and survival of *O. niloticus*.

Materials and Methods

Source of *O. niloticus* larvae

The experiment was conducted in a private hatchery facilities located at Asellait Agriculture Scheme (17 km Khartoum North). Newly hatched 1200 larva (day one) *O. niloticus* with an average body weight of 0.02g were collected from the hatchery.

Feed preparation and impregnation

Commercial feed of 44% crude protein was impregnated by 17 α methyl testosterone (17 α-MT). A stock solution of 17 α-MT was made by mixing and dissolving 0.50g of 17 α-MT in 3ml absolute alcohol, and then add 9ml of distilled water. The stock solution was used as follows: a 63% to be added to rearing water (T1); preparation of two impregnated feeds (T2

at 78.3% and T3 at 86.7%). The control with 0.0% 17 α-MT. Preparation of impregnated feed followed [2 and 8]. The prepared impregnated feeds were spread to obtain a 3-5cm flakes under shade and stored in refrigerator when dry. The amount of food offered was 0.5g/fish/day.

The experiments

17 α-MT **application stage for 28 days**

Twelve rectangle plastic containers (each 16L capacity) were used to create four treatment (T_1, T_2, T_3 and control) and three replicates R_1-R_3 (Figure 1). To each tank filled with 12L chlorine free water, 100 day one larvae (*O. niloticus*) from incubation jars were stocked. Each tank was covered with a fine mesh. T_1 larvae were subject to 17 α-MT at 63% in rearing water. After adding the 17 α-MT to the tanks, facilitate alcohol evaporation at room temperature by using air stone diffuser.–T_2 larvae were subject to feed impregnated with 17 α-MT at 78.3%. T_3 larvae were subject to feed impregnated with 17 α-MT at 86.7% and kept at the same time in rearing water treated with 17 α-MT at 63%. The control was deprived from 17 α-MT (0.0%).

Figure 1. Experimental setup for 17 α-MT application stage.

Larvae rearing stage

Twenty eight days larvae were reared in 1×1×1m plastic happa (800 micron mesh size, P), The 12 happas were fixed at earthen pond. Larvae were randomly distributed in four treatment and three replicates (Figure 2). The feeding protocol was as in 17 α-MT**.**

In both experiments, the mortality, feed quantity, water siphoning and exchange were monitored daily.

Plate 2. Experiment.

Figure 2. Experimental setup for larvae rearing stage.

Sex ratio determination

At the end of rearing stage, the sex of each fry was determined following [17].

Data analysis:

Data was analyzed using SPSS programme (version 22). Results were compared with one-way ANOVA and considered significant at $p<0.05$. Duncan's test were used to identify statistically significant differences among treatment means.

Results and discussions

Growth performance and survival rate:

Analysis of variance (Table 1) showed significant effects of 17 α-MT application methods ($p<0.05$) in final weight, weight gain and survival rate, while initial weight and FCR showed insignificant differences ($p>0.05$). The lowest survival of 53.67% occurred at the T_3 and the highest weight gain (90.3368%) was obtained at T_1.

Parameter	T_1	T_2	T_3	Control	F
Initial weight	$0.0193^{a}\pm0.002$	$0.0177^{a}\pm0.004$	$0.0173^{a}\pm0.003$	$0.0193^{a}\pm0.002$	0.539^{NS}
Final weight	$0.050^{a}\pm0.01$	$0.075^{c}\pm0.00$	$0.057^{ab}\pm0.00$	$0.062^{b}\pm0.00$	13.365*
Weight gain	$0.031^{a}\pm0.012$	$0.057^{b}\pm0.004$	$0.040^{a}\pm0.003$	$0.043^{a}\pm0.002$	9.479*
FCR	$13.290^{a}\pm2.370$	$14.080^{a}\pm2.792$	$13.639^{a}\pm1.207$	$11.728^{a}\pm0.425$	0.833^{NS}
Survival rate %	$90.33^{c}\pm2.31$	$74.67^{b}\pm4.62$	$53.67^{a}\pm2.31$	$68.00^{b}\pm10.15$	20.589*

Means with similar superscripts in a raw are statistically insignificantly different ($p>0.05$); those with different superscripts are statistically significantly different ($p<0.05$).

Table 1. Growth performance and survival rate of O. niloticus larvae under treatments of (T1, T2, T3 and control). (Means ± SD). *Significant, NS: not significant.

Larvae rearing stage

Table 2 showed no significant differences ($p>0.05$) in initial weight, final weight and weight gain of the four experimental groups in happas. There is a significant difference ($p<0.05$) in FCR and survival rate percentages and highly significant difference ($p<0.01$) in male production.

Parameter	**T_1**	**T_2**	**T_3**	control	**F**
Initial weight	$0.150^{a}\pm0.000$	$0.075^{a}\pm0.000$	$0.057^{a}\pm0.000$	$0.062^{a}\pm0.000$	0.00^{NS}
Final weight	$3.521^{b}\pm0.532$	$3.232^{b}\pm0.845$	$3.327^{b}\pm0.339$	$1.954^{a}\pm0.692$	3.83^{NS}
Weight gain	$3.421^{b}\pm0.614$	$3.157^{b}\pm0.845$	$3.270^{b}\pm0.339$	$1.892^{a}\pm0.692$	3.52^{NS}
FCR	$4.696^{ab}\pm0.803$	$6.092^{b}\pm0.963$	$3.653^{a}\pm0.771$	$8.607^{c}\pm0.695$	20.79*
Sur %	$86.667^{b}\pm5.132$	$78.333^{b}\pm10.066$	$63.000^{a}\pm9.165$	$78.667^{b}\pm4.163$	5.13*
Male %	$84.000^{b}\pm3.000$	$87.000^{b}\pm2.000$	$89.000^{b}\pm1.000$	$56.667^{a}\pm4.933$	71.76**

Means with similar superscripts in a raw are statistically insignificantly different ($p>0.05$); those with different superscripts are statistically significantly different ($p<0.05$).

Table 2. Growth performance and survival rate of treated O. niloticus larvae fed commercial feed. (Means ± SD). NS: not significant, *significant and ** highly significant differences.

Sex determination

The sex identification and determination of fish in general and *O. niloticus* in particular are based on morphological characteristic of the fish which is difficult to apply in early fry stage [1]. According to [17], [12], [16] and [1] staining squashed gonads by acetocarmine can differentiate clearly testes from ovaries. The present study used the squash technique and found intersex confirming the results obtained by [1] who observed oocytes scattered among testicular tissue.

In the present study highest *O. niloticus* male production (89.00%) occurred at T_3, followed by 87% in T_2 and 84% in T_1. All these were highly significantly ($p<0.01$) different from 56.67% in the control. At 60 mg 17α-MT dose, the maximum male *O. niloticus* was 93.3% by [19] of for 21 days; [11] reported 93.7 % males after 28 days; [8] observed 95% males after 21 days [25] found treatment with 60mg/kg of 17α-MT diet resulted in 80±3.34%% males. [4] obtained 85% *O. niloticus* males when egg and larvae were immersed in 1000µg/L of 17 α-MT. [12] found that the highest dose of 90mg 17 α-MT resulted in significantly lower ($p<0.05$) male proportion (59.3%).[28] by adding 400 ug/L17-α MT in rearing water of *Oncorhynchus kisutch* obtained 73.1% males. [29] found that in *Oreochromis spilurus* the highest proportion of males (90.3%) was given by 70 mg/kg of 17α-MT. [23] used a dose rate of 75mg 17α-MT in feed and recorded a maximum *O. mossambicus* male production of 98.09% [33]. Administration of 17α-MT effectively masculinized *Rhamdia quelen* fry; however, the lowest dose of 60 mg/kg of feed is recommended, since higher doses have inhibitory effects on gonadal development in both sexes [20] found that *Labeo rohita* testis produced more male population and can safely and effectively replace the synthetic 17 α-MT.

The minimum *O. niloticus* male proportion of 83.3% was recorded for a dose rate of 50 mg 17α-MT by [19] and by [24] for 25 days [23] who got lower male proportion (79.38%) for the same dose in *O. mossambicus* for the same duration of treatment.

According to [16] 17 α-MT is rapidly absorbed and metabolized resulting in increased protein synthesis, promoting of growth and enhancement of masculinization. The present study on sex reversal of *O. niloticus* by larvae immersion reduced the duration of the treatment and decreased the cost of 17 α-MT used. This result is in line with [31]. The result of [19] showed that no intersex was found in the treated groups and control groups which is similar to the earlier findings of [11]. But contradictory results were reported by [24] where intersex (6.2% for 50 mg MT kg-1) and (2.7% for 60 mg MT kg-1) was obtained in these dose rate when the hormone was given for a period of 25 days. [25] found treatment with 60mg/kg of 17α-MT resulted in 20.0±7.8% intersex in *O. niloticus*.

Survival rate and growth:

The present found that the mean survival rate (86%) was significantly higher in oral 17 α-MT application and lowest (63%) in mixed application. Comparison between sex reversal and weight gain showed that the FCR in a descending order as follows: control (8.6)>feed

application (6.1)> oral group (4.7) and> mixed application method (3.7). The finding is in agreement with [6] mono-sex tilapia culture.

The minimum *O. niloticus* male proportion of 83.3% was recorded for a dose rate of 50 mg 17α-MT by [19] and by [24] for 25 days [23] who got lower male proportion (79.38%) for the same dose in *O. mossambicus* for the same duration of treatment.

The survival rate and sex reversal % seems to be 17α-MT dose and duration dependent and species related [11] reported 80% survival in the control group than in 17a-MT treated groups. On the contrary, [8] and [19] reported that 17a-MT administration has no significant effect on survival of related *O. niloticus*; which is in line with [17] findings in *Oreochromis aureues.*

Conclusion

The survival rate and sex reversal % seems to be 17α-MT dose and duration dependent and species related. This study revealed significant male production rate of *O. niloticus* was achieved by immersion of larvae increasing with increase of 17 α-MT concentration. The survival rate significantly decreased with the increased of the 17 α-MT level. Further studies should be carried out to determine the optimum dose and duration to achieve 100% masculinization.

Acknowledgement

We are grateful to Prof. Zuheir N. Mahmoud, Department of Zoology, Faculty of Science, University of Khartoum, for critically reading the manuscript.

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