International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS International Journal of Fauna And Biological Studies

E-ISSN: 2347-2677
P-ISSN: 2394-0522
IJFBS 2018; 5(1): 34-36
Received: 20-11-2017
Accepted: 25-12-2017

Ekta Singh
PG Research Scholar, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

VP Saini
Dr. V. P. Saini, Professor, Aquaculture Research Unit Directorate of Research, MPUAT, Udaipur, Rajasthan, India

OP Sharma
Dr. O. P. Sharma, Professor, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

Correspondence
Ekta Singh
PG Research Scholar, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur, Rajasthan, India

Sex reversal in red tilapia (*Oreochromis* spp) fry by immersion technique

Ekta Singh, VP Saini and OP Sharma

Abstract
In immersion technique, three doses of 17α-methyltestosterone (MT) (100, 300 and 400 µg/l) were applied for four different exposure durations (i.e 3, 6, 9 and 12 hrs). It is clear from the obtained data that the immersion of *O. niloticus* fry in 17α-methyltestosterone (MT) at 300µg/liter for 12 hours resulted in the highest percentage of male population (90.00%) showing that exposure time has significant impact (P<0.01). Similarly, other doses were also significant at 5% level of probability. However, the interaction of hormone doses and immersion time were statistically non significant.

Keywords: Red tilapia, 17α methyl testosterone, Hormone, Sex ratio

Introduction
The importance, economics and problems of tilapia culture, considerable work has been done in respect to sex reversal of tilapia, especially Nile tilapia (*Oreochromis niloticus*) in order to produce all male populations in different regions of the world. The production of a single sex population, especially of male, offers several advantages in tilapia aquaculture including enhanced growth and prevention of unwanted reproduction. The various techniques that have been developed to provide male tilapia for culture are manual sexing, hybridization, genetic manipulation and hormonal sex reversal [1]. Immersion of tilapia fry in steroid solutions may be one way to achieve masculinization and avoid these inefficiencies. This technique is well developed in salmonid aquaculture, however, it remains largely experimental in tilapia culture [2, 3]. Most of the reported studies immersed tilapia fry in androgens for periods of over one week to five weeks [4, 5]. Recently, demonstrated that immersion for just three hours in 17a-methyldihydrotestosterone (MDHT) on two days resulted in masculinization of Nile tilapia [6]. The androgen, 17α-Methyl testosterone (MT), an anabolic steroid, is being widely used in the production of all male population in aquaculture, especially the *Oreochromis* spp due to their precocious sexual maturity and a high reproductive efficacy, resulting in overpopulation in ponds. The general practice involves oral administration of MT through feed for the first twenty one days of life immediately after yolk absorption.

Materials and Methods
Experimental Fish: For conducting this study, the fish "Red Tilapia" was choosen as experiment fish. The fish belongs to family Cichlidae, which is basically originated from Africa. The original red tilapia is genetic mutants. The first red tilapia was produced in Taiwan in the late 1960s, by a cross between a mutant reddish-orange female Mozambique tilapia and a normal male Nile tilapia.

Breeding of Tilapia: The brood stock of red tilapia was collected from a cemented tank of Aquaculture Research and Seed Farm, MPUAT, Udaipur. Healthy fish were selected and kept for conditioning for two days in indoor tanks (3x3x1m). After two days the brood fish were transferred in breeding hapa fixed in outdoor cemented tank. In a hapa of 1.5x1.5x1.0 m size a pairs of brood stock comprising one male and three females were stocked. Two days after pairing, daily checking of eggs in the mouth of the female was done. Once eggs were observed, it was recorded as the first day of mouth brooding. After five to seven days post-hatching when the yolk sac was absorbed, the fry was collected for conducting final experiment on hormonal sex reversal.

Experimental Setup: The tilapia fry (yolk sac just absorbed) were stocked in 1 liter glass jars

at stocking density of 25-30 fry/liter. The fry were immersed in 17α-methyltestosterone hormone at 100, 200 and 300 µg/l for 3, 6, 9 and 12 h immersion period. Control group included immersion of fry in water only. After immersion for a particular period, the fry were collected and stocked in glass aquaria having freshwater. The immersion was repeated once again after 3 days for all groups. During hormone treatment period, fry were fed on live feed. After the immersion period, 100 randomly selected fry per group were transferred from the jars to 3 m^3 cemented cisterns. At the end of grow-out period, which lasted for 150 days, 10% fish from each group were individually examined for sex. The sex of hormone treated tilapia was determined by examining the genital papilla located immediately behind the anus. In males the genital papilla has only one opening (the urinary pore of the ureter) through which both milt and urine pass. In females the eggs exit through a separate oviduct and only urine passes through the urinary pore. Placing a drop of dye (methylene blue or food coloring) on the genital region helps to highlight the papilla and its openings.

Experimental Results

In this experiment yolk sac absorbed fry were treated with three doses (i.e., 100, 200 and 300 µg/l) of 17α-methyl testosterone were tested separately for different periods (i.e. 3, 6, 9 and 12 hrs). The desired quantity of hormone was dissolved in water and fry was directly immersed immediately for the desired periods. After immersion treatments, the fry was stocked in cemented cisterns (3x3x1m) for further rearing. During rearing period fish was fed on pelleted feed (28% Protein, Growell) for a period of 150 days. At the termination of grow out period, 10% of the fish from each treatment were captured for observing sex.

The sexes of experimental fish in different treatments are shown in Table 1. The control had male population of 43.33, 40.00, 43.33 and 46.67% and female 56.67, 60.00, 56.67 and 53.33% for 3hr, 6hr, 9hr and 12hr treatments respectively. As compared to control, the MT treated seed had higher population of male than female. Still the highest male population of 90% was recorded in MT dose of 300 µg/l applied for 12hrs. This was followed by 86.67% population in 300 µg/l for 9 hrs and 200 µg/l for 12hrs treatments.

From these results, it is clearly depicted that none of the hormone dose and exposure period has produced 100% male population, which is one of the most desired aim of any hormonal sex reversal trial. Further, while comparing results on the basis of statistical out comes, it is evident from Table 1. That exposure times has significant impact ($P<0.01$). Similarly, hormone dose were also significant at 5% level of probability. However, the interaction of doses and immersion time were statistically non-significant.

Discussion

The study revealed significant information on the effect of hormone concentration (HC) and immersion time (IT) and their interaction (HC x IT) on masculinization percentage of red tilapia. It is clear from the obtained data that the immersion of *O. niloticus* fry in 17α-methyltestosterone (MT) at 300 µg/liter for 12 hours resulted in the highest percentage of male populations (90.00 %), followed by 86.67% males in groups T_8 and T_{11} immersed in 300 µg MT/l for 9 h and 200 µg MT/l for 12 h respectively, while using the hormone at the level of 200 µg MT/l, produced lower male percentage (76.67, 73.33, 83.33, and 86.67% males in groups T_5, T_6, T_7 and T_8, respectively) than the results obtained in fish groups which were treated at 300 µg MT/l. Thus, a significant increase in male population with increasing dose was obtained. Similarly, the increased male population with increased hormone concentration (HC) and application period was also reported [7]. In their study the main effect of HC showed that the highest per cent male of about 84% was obtained at 800 µg l^{-1}, followed by about 79% at 600µg l^{-1}, 75% at 400µg l^{-1}, 67% at 200 µg l^{-1} and lowest in the control (59%). The average per cent male of 90% obtained in our experiment compared to that reported by using the traditional sex reversal may be attributed to lower hormone concentrations (i.e. 400, 600 and 800 µg l^{-1})[8]. These hormone concentrations are equivalent to 0.4, 0.6 and 0.8 mg kg^{-1}, respectively, based on the assumption that 1 liter of water is equal to 1 kg. These concentrations may still be suitable because eggs were used instead of fry. Hence, it may also be possible that the lower per cent male obtained in this study compared to that reported by was due to the management employed during the immersion and incubation. Therefore, this may be worthwhile to look at the in future investigations [8]. The lowest male percentage was obtained in fish groups which were treated at the hormonal level of 100 µg MT/l. Similarly, the manipulated the sex in *H. fossilis* through immersion in androgenic and gynogenic steroid solutions at doses ranging from 100 to 400 µg/l of water for 1 to 4 h [9]. Induced production of males and females with regard to MT, both 100 to 200 µg/l treatments for 3h significantly ($P<0.001$) altered the sex ratio from the control (2F:1M) and the highest percentage conversion to males (82%) occurred at treatment dose of 100 µg/l. Immersion of *O. niloticus* fry for 3h at the hormonal level of 400 µg MT/l produced 86±5% males; this result is lower than that obtained [6]. In the present investigation the highest female population of 60.0, 56.67, 56.67 and 53.33% respectively was in control (0 µg/l) for 6, 3, 9 and 12 hr. The immersion of sac fry for one hr in 400 µg/l Ethynylestradiol-17α EE2 resulted in 73.4% females and redoubling the time of treatment to 2 hours produced 94.5% females (Razmi *et al*., 2011) [10].

While comparing the results obtained in section "A" of this thesis, it is evident that oral administration of MT is more effective than immersion technique. Because none of the treatment doses and exposure period produced 100% male population in the later technique. Similar results were also obtained [8, 7].

Table 1: Interaction effect of HC x IT on per cent male

HC µg/l	Male HC (%) at different IT (hrs)			
	3 hrs	6 hrs	9 hrs	12 hrs
Control 0 µg/l MT	43.33	40.00	43.33	46.67
100 µg/l MT	73.33	70.00	80.00	83.33
200 µg/l MT	76.67	73.33	83.33	86.67
300 µg/l MT	80.00	76.67	86.67	90.00

Table 2: Analysis variance on Male population

SN.	Source	d.f.	SS	MS	F	SE(m)	CD(5)	CD(1)
1.	IT	3	12366.7	4122.22	86.029**	2	5.756	7.743
2.	HC	3	966.667	322.222	6.725*	1.998	5.756	7.743
3.	IT x HC	9	100	11.1111	0.232	3.997	11.51	15.49
4.	Error	32	1533.33	47.9167				

IT= Immersion Time (3, 6, 9 and 12 hrs) HC= Hormone Concentration (100, 200, 300 µg/l MT)

Table 3: Interaction effect of HC x IT on per cent female

HC µg/l	Female HC (%) at different IT (hrs)			
	3 hrs	6 hrs	9 hrs	12 hrs
Control 0 µg/l MT	56.67	60.00	56.67	53.33
100 µg/l MT	26.67	30.00	20.00	16.67
200 µg/l MT	23.33	26.67	16.67	13.33
300 µg/l MT	20.00	23.33	13.33	10.00

Table 4: Analysis variance on Female population

SN.	Source	d.f.	SS	MS	F	SE(m)	CD(5)	CD(1)
1.	IT	3	11822.9	3940.97	75.667**	2.08	6.001	8.073
2.	HC	3	1289.58	429.861	8.253**	2.083	6.001	8.073
3.	IT x HC	9	68.75	7.63889	0.147	4.167	12	16.15
4.	Error	32	1666.67	52.0833				

IT= Immersion Time (3, 6, 9 and 12 hrs) HC= Hormone Concentration (100, 200, 300 µg/l MT)

References

1. Hickling. The Malacca tilapia hybrids. Journal of Genetics. 1960; 57:1-110.
2. Piferrer F, Donaldson EM. Gonadal differentiation in coho salmon, *Oncorhynchus kisutch*, after a single treatment with androgen or estrogen at different stages during ontogenesis. Aquaculture. 1960-1989; 77:251-262.
3. Feist G, Yeoh CG, Fitzpatrick MS, Schreck CB. The production of functional sex-reversed male rainbow trout with 17a-methyl testosterone and 11b-hydroxyandrostenedione. Aquaculture. 1995; 131:145-152.
4. Varadaraj K, Pandian TJ. Masculinisation of *Oreochromis mossambicus* by administration of 17 a-Methyl-5-Androsten-3P-17 P-diol through rearing water. Current Science. 1987; 56:412-413.
5. Torrans L, Meriwether F, Lowell F, Wyatt B, Gwinup PD. Sex-reversal of *Oreochromis aureus* by immersion in mibolerone, a synthetic steroid. Journal World Aquaculture Society. 1988; 19(3):97-102.
6. Gale WL, Fitzpatrick MS, Schreck CB. Immersion of Nile tilapia (*Oreochromis niloticus*) in 17-methyl testosterone and mestanolone for the production of all-male populations. *In*: F. W. Goetz (ed.). Proc. Fifth International Symposium on the Reproductive Physiology of Fish. Fish Symposium 95. The University of Texas at Austin, Texas, U.S.A, 1995, 117.
7. Cagauan AG, Baleta FN, Abucay JS. Sex reversal of Nile tilapia, *oreochromis niloticus* L. by egg immersion technique: the effect of hormone concentration and immersion time. College of Fisheries and Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines. carp (*Cyprinus carpio*) in relation to growth rate. Aquaculture. 2004; 137:203-211.
8. Mair GC, Abucay JS, Skibinski DOF, Abella TA, Beardmore JA. Genetic manipulation of sex ratio for the large scale production of all-male tilapia, *Oreochromis niloticus* (L). Canadian Journal of Fisheries and Aquatic Sciences. 1997; 54:396-404.
9. Haniffa MA, Sridhar S, Nagarajan M. Hormonal manipulation of sex in stinging catfish, *Heteropneustes fossilis* (Bloch). Current science. 2004; 86(7):1012-1013.
10. Razmi K, Naji T, Alizadeh M, Hoseinzadeh Sahafi H. Hormonal sex reversal of rainbow trout *(Oncorhynchus mykiss)* by ethynylestradiol-17α (EE2). Iranian Journal of Fisheries Sciences. 2011; 10(2):304-315.