

Effect of oral treatment of synthetic androgens on sex ratio, survival and growth rates, in three strains of tilapia

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ABSTRACT

The effect of dietary administration of the synthetic androgens 17 α -methyltestosterone MT (40 mg), fluoxymesterone FM (1 mg) and propionate testosterone PT (50 mg) per kg of feed, on Stirling strain (*Oreochromis niloticus*, Linnaeus, 1757), Red strain (*Oreochromis niloticus*, Linnaeus, 1757) and the hybrid Rocky Mountain (*Oreochromis niloticus*, Linnaeus, 1757 x *Oreochromis aureus*, Steindachner, 1864) fry, was evaluated during a 30 day trial to induce sex reversal. The most efficient androgen was MT for male ratio (92%), mean percentage survival and specific growth rate. TP was the least efficient (67 % male). The percentage of males from FM treatment was 80.6%, but this can be improved when time period or hormone-treated dosage are increased. Thus, the FM androgen could be recommended for a successful sex reversal, instead of MT. The Stirling strain (*O. niloticus*) had the best results when compared to the other two strains of tilapia.

Key words: sex reversal, tilapias, steroid hormones, synthetic androgens.

RESUMEN

Se probó la efectividad de las hormonas 17 α metilttestosterona MT (40 mg), fluoximesterona FM (1 mg) y propionato de testosterona PT (50 mg) por kilogramo de alimento para inducir la reversión sexual en alevines de tilapia *Oreochromis niloticus* variedad Stirling (Linnaeus, 1757), *Oreochromis niloticus* variedad roja (Linnaeus, 1757) y el híbrido Rocky Mountain (*Oreochromis niloticus*, Linnaeus, 1757 x *Oreochromis aureus*, Steindachner, 1864), aplicadas a través del alimento, durante 30 días. La evaluación de la proporción de machos revertidos, el porcentaje de supervivencia y la tasa de crecimiento específico, indicaron que la hormona MT produjo los mejores resultados (92 % de machos), seguida por FM (80.6 % de machos) y PT cuya efectividad fue muy baja (67 % de machos). El efecto de la hormona FM, que se propone como virtual sustituto de la MT, mejora notablemente al incrementar el tiempo de tratamiento o la dosis. La línea *Oreochromis niloticus* variedad Stirling presentó el mayor éxito en la reversión sexual.

Palabras clave: reversión sexual, tilapias, hormonas esteroides, andrógenos sintéticos.

INTRODUCTION

A major problem in commercial farms of tilapia species is their tendency to overpopulate ponds. For this reason all-male populations of tilapia are desirable in grow-out ponds to eliminate or reduce reproduction. Among the

methods developed to overcome the problem, hormonal sex reversal had been suggested as a possible solution and research in this area has received increased attention. Populations with very high percentages of males can be obtained by administering androgens to sexually undifferentiated tilapia fry (Guerrero, 1975; Tayamen and

Shelton, 1978; Owusu-Frimpong and Nijhar, 1981; Phelps *et al.*, 1992).

In the last twenty years different experiments have been conducted to obtain successful sex reversal of *Oreochromis mossambicus* and *Oreochromis aureus*, utilising different kinds of oral synthetic androgens. These include: 17 α -methyltestosterone (MT), 17 α -ethynyltestosterone (ET) and 1-dehydrotestosterone acetate (DHT) (Clemens and Inslee, 1968; Nakamura, 1975; Guerrero, 1975, 1979). These experiments showed that ET and MT are the most potent androgens in inducing males in these species (up to 100% of phenotypic males). Macintosh *et al.* (1985) demonstrated that the anabolic and sex reversal effects of MT on tilapia are proportional to the length of the treatment and the quantity of hormone administered.

Torrans *et al.* (1988) compared the application of Mibolerone (MI), a synthetic anabolic steroid, for sex-reversal in *O. aureus*, under two conditions: dissolved in water and included in the feeding diet. The results indicated that immersion in either 1.0 or 0.6 mg/l for five weeks, resulted in an average 82% males and 18% inter-sex, ova-testicular fish, and that the feeding diet containing 1.0 mg/kg of MI gave 85% males, 11% ova-testicular fish and 4% gonadal females. Growth and survival of fry were negatively correlated with MI concentration of the immersion treatments.

O. niloticus phenotypic, all-male, populations were produced when fluoxymesterone (FM) was given at 1, 5 and 25 mg/kg of feed. Fry fed MT at 60 mg/kg of feed and 0.2 mg of FM per kg of feed had a sex ratio of 97.7% and 87.3% phenotypic males, respectively, and no anabolic effect was evident (Phelps *et al.*, 1992).

Kuwaye *et al.* (1993) suggested that continuous treatment with MT offers considerable advantages over sex reversal in the commercial culture of tilapia *O. mossambicus*. Overall, the greatest growth was observed in seawater-reared tilapia, which were continuously treated with MT and these fish were 5-7 fold larger than freshwater controls. In polyculture conditions the administration of MT consistently and significantly increased the growth of tilapia (by 22%) and carp species.

Ladu and Madara (1994) studied the effects of synthetic (MT) and natural androgens (goat testis) for the production of monosex male *O. niloticus* and found that the growth rate of fish was higher in experimental treatments than in the control. The best results were obtained with MT-treatment (sex ratio 88% male) in contrast to goat testis-treatment (60 % males).

Vera and Mair (1994) evaluated the effect of dietary administration of the androgen MT on the growth and survival

of *O. niloticus* fry. They found that the androgen had no significant effect on growth and survival of fry during the treatment period and produced a mean sex ratio of 98.4% male in ponds and 95.4% in hapas. In a second study at higher stocking densities (3000-5000 fry/m²), hormone-treated in hapas increased efficacy of sex-reversal up to 99.4% male, but resulted in decreased growth and survival rates.

Carvalho and Foresti (1996) indicated that the treatment of 30 mg of MT/kg of feed during 40 days demonstrated the most effective in the production of all male *O. niloticus*. Furthermore, Abucay and Mair (1997) determined the relevance of feeding duration and feed quality in the optimisation of masculinization and feminization treatments of *O. niloticus* fry by oral application of MT and diethylstilboestrol (DES), respectively.

According to the information described above, the synthetic androgens that have been the most consistent for sex reversing tilapia fry are MT and ET. However in some countries, local legislation prohibits the use of these substances for fish production and it is difficult to obtain these androgens in the national market.

Recently, Desprez and Melard (1998) used 17 α -ethynylestradiol treatment on males of *O. aureus* and produced pseudofemales with a male genotype (ZZ). Crossing these fish with normal males (ZZ), produced male offspring. According to Melard (1995), the multiplication of pseudofemale stock, has the advantage of producing male progenies without the use of steroid treatment, although it would be difficult to obtain 100 % male offsprings. Moreover, Mair *et al.* (1997), generated male genotypes (YY) incorporating estrogens on males of *O. niloticus*. These proved to be as viable and fertile as normal males (XY) and to sire a mean progeny sex ratio of 95.6 % male.

In order to find new alternatives, we evaluated the effect of dietary administration of three synthetic androgens: 17 α -methyltestosterone (MT), fluoxymesterone (FM) and testosterone propionate (PT) on the sex ratio, growth and survival rates of three strains of tilapia fry, on which these hormones have not been previously tested.

FM hormone has the following advantages: it is a product commonly used in human medicine, readily available, it is cheaper than MT and without importation restrictions.

MATERIALS AND METHODS

The study was conducted in a recirculating-water system with tanks and aquarium facilities at the Planta Experimental de Producción Acuícola, Universidad

Autónoma Metropolitana Iztapalapa, Distrito Federal, México. For the experiments we use locally available strains of *Oreochromis niloticus* (one strain Stirling from Temascal, Oaxaca and a Red strain produced in El Clarín, Martínez de la Torre, Veracruz) and a hybrid named Rocky Mountain (*O. niloticus* x *O. aureus*) cultured in Zacatepec, Morelos.

The best breeders of each strain (4 females: 1 male) were selected and introduced in a recirculating-water system containing four fiberglass tanks of 0.65 m³ each one, with control of temperature (28 °C) and aeration. Fry were removed from the mouth of the females. Once the yolk-sac was absorbed, the fry were separated into groups of 50 individuals that were measured and weighed before stocked into 50 litres aquaria, integrated in a recirculating-water system, without contact between control and treatment groups.

The correspondent treatments were orally administered during 30 days to each group of fry and each strain with two replicates. We used commercial food (El Pedregal Silver Cup, for tilapia initiation, containing 52% of protein) mixed with each of the three synthetic androgens: 40 mg of 17 α -methyltestosterone (MT), 1 mg of fluoxymesterone (FM) or 50 mg of testosterone propionate (PT) per kg of feed, according to references. A control group for each strain of fish was fed untreated feed. Hormone-treated food was prepared by adding 40 mg of MT or 1 mg of FM to 500 ml of 95% ethanol per kg of feed, following the procedures described by Popma and Green (1990). In the case of PT (liquid presentation with oil as c.b.p.), we used 1 ml (equivalent to 50 mg) in 250 ml vegetable oil, instead of ethanol, prior to be incorporated in the food.

Food was administrated five times a day, as a proportion of 20% biomass, from 10:00 to 18:00, every 2 hrs. Total length and total wet weight per treatment were determined every five days for each group. Specific growth rate was calculated according to Ricker (1975). Survival rate was calculated considering the surviving organisms after 30 days of experimentation.

Water temperature, dissolved oxygen, pH, total alkalinity, total hardness, total ammonia nitrogen, un-ionized ammonia, nitrite, nitrate, total phosphorus and orthophosphates were monitored at the beginning and at the end of the hormone treatment period using a YSI Model 51 and a Hach kit DR/2000.

After 30 days of the hormone treatments, fry were kept in the aquarium, during two or three months more and fed with the same food as the control group (five times a day) until they reached a total length of 7 cm, in order to make the sexed easily. When fishes reaching this size, their gonads were removed with fine forceps, mounted on a glass slide, stained with aceto-carmine in agree with Guerrero

and Shelton (1974) and examined under the microscope to verify sex reversal. At this size the oocytes were readily differentiated from spermatogonia.

Statistical analysis with two way ANOVA was conducted and a least significant difference (LSD) test applied according to Zar (1974), using the Statgraphics Program Software version 6.0.

RESULTS AND DISCUSSION

Initial lengths and weights of experimental fry treated with different hormones, were not significantly different ($p > 0.05$), suggesting that the initial experimental conditions were similar in all treatments. However, among the different strains of tilapias, differences were significant ($p < 0.05$). The hybrid Rocky Mountain fry had the highest average length and weight at birth (Table 1).

The two way ANOVA (Table 2), showed no significant differences ($p > 0.05$) in the response of the different strains of tilapia treated with different hormones. However significant differences ($p < 0.05$) were observed in the mean percentage survival and specific growth rates. Moreover, Between androgen and control treatments, the mean percentage males and survival rate were significant ($p < 0.05$), but not the specific growth rate.

Hormone-treated and control food were both well accepted by the three strains of tilapia fry, except for TP, which was scarcely consumed due perhaps to the greasy appearance produced by the hormone's solvent (vegetable oil).

Significant differences ($p < 0.05$) were observed between androgen-treatments, where MT produced the highest male ratio (92%), followed by FM (80.6%) and PT (67%). The control group (54%) also presented significant differences ($p < 0.05$) with respect to the hormone-treatments, which indicates that sex reversal was successful, except for PT (Table 3).

This study demonstrates the effectiveness of the MT treatment at a dosage of 40 mg/kg of feed supply during 30 days to produce phenotypic males. Our results agree with those of Guerrero (1975), Hanson *et al.* (1983), Macintosh *et al.* (1985), Kuwaye *et al.* (1993), Ladu and Madara (1994), who demonstrated the effectiveness of MT in other species. However the results obtained with FM also suggest that its performance to induce sex reversal in these strains of tilapia, is highly effective.

The mean percentage survival rate of fry treated with MT, FM and control after the 30 days trial, was not significantly different ($p > 0.05$), which indicates that the

Table 1. Mean lengths and wet weights of three strains of tilapias at initial and final experimental periods, and treated with PT, FM and MT hormones.

Treatment	Initial experiment		Final experiment	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Control	0.95 ^a ± 0.020	0.01 ^a ± 0.0008	3.07 ^a ± 0.16	0.82 ^a ± 0.11
PT (50 mg/kg)	0.96 ^a ± 0.026	0.01 ^a ± 0.001	2.94 ^a ± 0.19	0.78 ^a ± 0.14
FM (1 mg/kg)	0.94 ^a ± 0.026	0.01 ^a ± 0.001	2.80 ^a ± 0.19	0.64 ^a ± 0.14
MT (40 mg/kg)	0.96 ^a ± 0.026	0.01 ^a ± 0.001	3.16 ^a ± 0.25	1.04 ^a ± 0.18
Strain				
Stirling strain	0.87 ^a ± 0.029	0.005 ^a ± 0.001	2.88 ^a ± 0.21	0.72 ^{a,b} ± 0.15
Red strain	0.94 ^a ± 0.018	0.012 ^b ± 0.0008	2.83 ^a ± 0.17	0.65 ^a ± 0.12
Rocky Mountain	1.06 ^b ± 0.018	0.015 ^c ± 0.0008	3.26 ^a ± 0.14	1.09 ^b ± 0.09

Values correspond to mean ± standard error.

Treatments having the same suffix letters are not significantly different ($p > 0.05$).

PT = Propionate Testosterone; FM = Fluoxymesterone; MT = 17 α -methyltestosterone.

Table 2. Two-way ANOVA results between treatments and strains as source of variation.

Source of variation	Male ratio (%)	Mean % survival	Specific growth rate
Treatments	0.0036*	0.0232*	0.4025
Strain	0.9539	0.00001*	0.0007*

The values correspond to levels of significance.

* Significant differences ($p < 0.05$).

hormones do not have a negative effect on the fry. Only PT presented significant difference ($p < 0.05$), which we assumed is due to low consumption of food (Table 3).

The specific growth rates of fry were similar in all treatments with non-significative differences between them ($p > 0.05$) (Table 3), therefore no anabolic effect was detected. There is a controversy with respect to the induction of an anabolic effect on the fish by androgens, as mentioned by Tayamen and Shelton (1978), Macintosh *et al.* (1985), Kuwaye *et al.* (1993), Ladu and Madara (1994). However, our results agreed with Owusu-Frimpong and Nijjar (1981), Torrans *et al.* (1988), Phelps *et al.* (1992) and Vera and Mair (1994) who reported non-significant differences in growth rate between hormone-treated and untreated tilapia fry.

In the three strains of tilapia, we observed that the response to androgens-treatments were similar but the best results were obtained with MT, followed by FM and finally PT. The Stirling strain showed the highest male ratio with the three hormones (Table 4a).

The hybrid Rocky Mountain showed the highest survival rate, followed by the Stirling strain (*O. niloticus*), and non-significant differences ($p > 0.05$) between them (Table 4b). In contrast, the Red strain (*O. niloticus*) showed

high mortality and presented significant differences ($p < 0.05$) with respect to the other strains. Regardless of their high commercial value, the Red strain is delicate and requires special treatment.

Significant differences ($p < 0.05$) were also observed between the three strains with respect to specific growth rate. The Stirling strain exhibited the best specific growth rate (Table 4b), in the spite of their smallest initial size at birth.

The results of the physicochemical parameters are given in Table 5. The average values show that they are adequate for a good growth and high survival of tilapia fry, and they did not have adverse effects on the populations under experimental conditions.

Subsequent experiments using different dosages of hormones demonstrated that when we increased FM from 1 to 5 mg/kg of feed, we obtained 100 % phenotypic males of Rocky Mountain and Red strains. In the other hand, when only time was increased, up to 35 days, a 100 % of phenotypic males of Stirling strain was obtained (Jiménez and Arredondo, 2000).

The satisfactory results obtained with Fluoxymesterone, especially for Stirling and Rocky Mountain strains, confirmed

Table 3. Male ratio, mean percentage survival and specific growth rates of control and treated tilapia fry reared in aquarium for 30 days.

Treatments	Male ratio (%)	Mean % survival	Specific growth rate
Control	54.0 ^a	88.4 ^b	0.15 ^a
PT (50 mg)	67.0 ^{a,b}	67.0 ^a	0.14 ^a
FM (1 mg)	80.6 ^{b,c}	82.3 ^b	0.15 ^a
MT (40 mg)	92.0 ^c	83.6 ^b	0.13 ^a

Treatments having the same suffix letters are not significantly different ($p > 0.05$)

that this synthetic androgen could be easily substitute the 17 α -methyltestosterone, besides the advantage of be readily available.

Furthermore, the dosage of PT was not enough to induce sex-reversal in all the strains of tilapia thus we recommend to do more experiments with dosages ranging from 60 to 100 mg PT/kg of feed and with different trial periods (35-60 days). The low proportion of males obtained in PT treatment can be attributed to a scarce consumption of food. Under these circumstances, it is recommended to carry out tests to include PT in food, without losing their organoleptic properties.

Goudie, *et al.* (1986) evaluated MT in fish tissue distribution and time of elimination, when this androgen is used for sex reversal. They concluded that no potential health hazard exists for people who eat fish that have been fed MT as juveniles. Also, Rothbard *et al.* (1990), agreed to indicate that hormonal residues do not exist on fish feed with normal food, several previous months to be consumed by humans.

This study demonstrated that MT was the most effective androgen in inducing sex reversal in the three strains of tilapia analysed. The Stirling strain presented higher male ratio, survival and specific growth rate, thus is

the most viable strain to implement with sex reversal in grow-out ponds of commercial farms. Fluoxymesterone hormone is a good alternative to substitute 17 α -methyltestosterone hormone.

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Table 4. a) Male ratio (%), b) Survival (%) and specific growth rates of three strains of tilapias submitted to steroid hormones.

a)				
Tilapia strains	Control	PT (50 mg)	FM (1 mg)	MT (40 mg)
Stirling strain (<i>Oreochromis niloticus</i>)	45 ^{a,a}	77.7 ^{b,b}	89.5 ^{c,b}	100 ^{d,b}
Red strain (<i>Oreochromis niloticus</i>)	57 ^{a,b}	70.7 ^{b,b}	66.0 ^{b,a}	80 ^{c,a}
Rocky Mountain (<i>O. niloticus</i> x <i>O. aureus</i>)	58 ^{a,b}	52.6 ^{a,a}	86.2 ^{b,b}	96 ^{c,b}
b)				
Tilapia strains	Survival rates		Specific growth rates	
	Control	Treatment	Control	Treatment
Stirling strain (<i>Oreochromis niloticus</i>)	96 ^{b,b}	87 ^{a,b}	0.18 ^{a,c}	0.17 ^{a,c}
Red strain (<i>Oreochromis niloticus</i>)	72 ^{b,a}	61 ^{a,a}	0.13 ^{a,a}	0.12 ^{a,a}
Rocky Mountain (<i>O. niloticus</i> x <i>O. aureus</i>)	98 ^{a,b}	94 ^{a,b}	0.14 ^{a,b}	0.14 ^{a,b}

Treatments having the same suffix letters are not significantly different ($p > 0.05$).

Left suffix, comparison between columns.

Right suffix, comparison between rows.

Table 5. Average values of the physicochemical parameters registered in the experiment.

Parameters	Treatments	Control
Water temperature °C	27.98	27.38
Dissolved oxygen mg/l	4.65	4.64
pH	8.29	8.29
Total alkalinity mg/l	352	380
Total hardness mg/l	302	252
Nitrite (NO ₂ ⁻) mg/l	0.34	0.39
Nitrate (NO ₃ ⁻) mg/l	0.23	0.16
Un-ionized ammonia (NH ₃) mg/l	0.057	0.042
Total nitrogen ammonia (NH ₄ ⁺) mg/l	0.062	0.051
Total phosphorus (mg/l)	0.84	0.91
Orthophosphates PO ₄ mg/l	0.53	0.75

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