Comparative effects of a natural androgen, 11β hydroxyandrostenedione, and a synthetic androgen, 17α-methyltestosterone, on the sex ratios of Oreochromis niloticus

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Comparative Effects of a Natural Androgen, 11ß-Hydroxyandrostenedione, and a Synthetic Androgen, 17α-Methyltestosterone, on the Sex Ratios of *Oreochromis niloticus*

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BAROILLER, J.F. and A. TOGUYENI. 1996. Comparative effects of a natural androgen, 11ß-hydroxyandrostenedione, and a synthetic androgen, 17α-methyltestosterone, on the sex ratios of *Oreochromis niloticus*, p. 238-245. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Synthetic androgens are often considered to be more potent than natural androgens for sex reversal treatments in fish. Preliminary experiments on the natural androgen, 11ß-hydroxyandrostenedione (11ß-OHΔ4), recently identified in the gonads of fry of *O. niloticus* in the early stages of testicular ontogenesis, showed a high masculinizing potential. Fry of 10-14 days post-fertilization (dPF) were produced from single pair matings using untreated females and either normal or sex reversed males, to assess any significant deviation in sex ratio compared to the controls. The fry were then treated during a minimum period of 21 days either with the natural or synthetic androgen added to the feed at different concentrations. Complete sex reversal was achieved with both steroids. All-male populations were produced in eight groups treated with 10-35 µg 11ß-OHΔ4·g^{-1} of feed and in two groups treated with 17α-methyltestosterone (17α-MT) at 5 and 20 µg·g^{-1} of feed, respectively. There was no significant difference in the potency of the two androgens for concentrations higher or equal to 10 µg·g^{-1}. However, at 5 µg·g^{-1}, only 17α-MT produced 100% male populations whereas 11ß-OHΔ4 produced 88.9%. In contrast, the lowest concentration of 11ß-OHΔ4 (1 µg·g^{-1}) significantly deviated sex ratios whereas 17α-MT had no effect compared to the controls.

The natural androgen 11ß-OHΔ4 can therefore constitute an alternative treatment to synthetic sterolds. Low concentration treatments can be optimized by increasing the duration of treatment.

Introduction

Today, with an annual global production of approximately 500,000 tons (Lazard 1990), tilapias (*Oreochromis*, *Sarotherodon* and *Tilapia*) constitute, with the cyprinids and salmonids, one of the three most important groups of freshwater fish for aquaculture. However, in culture conditions where competition

for food is high, the highly efficient reproduction of the species of *Oreochromis* (Baroiller and Jalabert 1989) and their early sexual maturity lead to overcrowding and stunting, resulting in limited economic yields for fish farms. A solution to this problem consists in producing monosex populations. All-male populations are preferred because their growth performance is better compared to females (Pruginin 1967; Hickling 1968; Hanson et al. 1983).

Currently, two techniques are used to produce monosex populations for fish culture (Baroiller and Jalabert 1989):

Manual sexing. Based on the sexual dimorphism observed in the urogenital papilla, this technique entails the elimination of all females, i.e., approximately half of the initial population as soon as possible (after a two to three month nursing period). Manual sexing, which is used in Africa, is time-consuming; it also requires qualified personnel, and includes 3-10% errors (Lazard 1980; Chervinski and Rothbard 1982). In addition, this technique requires rearing a population of fry for two to three months after which half (the females) will be eliminated. Although manual sexing is simple, it is expensive in terms of time and labor and implies the underutilization of farming infrastructures and lower feed productivity.

Hormonal sex reversal. This technique consists in masculinizing the entire population of fry by adding a steroid to the feed for a short period (Guerrero 1982; Hunter and Donaldson 1983; Pandian and Varadaraj 1987; Baroiller and Jalabert 1989). Hormonal sex reversal has been commonly used for several decades by a number of tilapia producing countries such as Israel, Taiwan and the Philippines. This technique requires the systematic treatment of every new population of fry. However, the use of hormones in the production of animals for human consumption is still prohibited in many countries (France and the United Kingdom, for example) which consider that the treatment and the effects of the synthetic steroid waste products are still insufficiently understood, especially their ecological impact.

In tilapias, as in all teleost fish, no definite physiological proof supports the hypothesis of Yamamoto (1969) that steroids are the natural inducers of differentiation (Adkins-Regan 1987). The modification of the natural process of sexual differentiation by exogenous steroids could be due to pharmacological effects (Reinboth 1970). In fact, few studies have investigated early steroidogenesis during the gonadal sex differentiation of gonochoristic fish (van den Hurk et al. 1982; Rothbard et al. 1987; Baroiller 1988a and b; Baroiller et al. 1988).

In *Oreochromis niloticus*, early steroidogenous potential in male and female gonads has been analyzed during the first three months of their life; this period covers the entire process of testicular and ovarian differentiation (Baroiller et al., in press). Testosterone can be synthesized by the gonads of both sexes whereas oestradiol is exclusively produced by the ovaries (Baroiller 1988b).

In contrast, certain androgens like 11ß-hydroxyandrostenedione (11ß-OHΔ4) and adrenosterone prove male sex-specific during the same period (Baroiller 1988a and b) and show masculinizing potentialities (Baroiller 1988b).

Artificial steroids are generally more potent in their masculinizing effects than natural androgens (Hunter and Donaldson 1983). The potency of 17α-methyltestosterone (17α-MT) is attributed to the presence of the 17α-methyl group which makes its elimination slower than in natural androgen like testosterone (Fagerlund and McBride 1978; Donaldson et al. 1979).

A study of the masculinizing potential of 11ß-OHΔ4 and 17α-MT was conducted in *O. niloticus* to compare the respective performances of both artificial and natural hormones; to test the hypothesis of a possible role of 11ß-OHΔ4 in the process of testicular differentiation; and also to study possible alternatives to traditional sex reversal treatments.

Materials and Methods

Animals Tested

Two types of male broodfish of *O. niloticus* of the "Bouake" strain (Baroiller 1988b) were used for the production of fry families: normal males (XY) and sex reversed males (XX). The latter came from two related families and yielded, for most of them, significant proportions of males in their progenies produced by single pair matings (Baroiller, this vol.).

Breeding

Single male broodfish were placed in 400-l aquaria with normal females at a sex ratio of 4:1. The water in the breeding aquaria was filtered and maintained at a constant temperature of 27°C. Each animal was identified by a mark inserted in the dorsal muscles. Reproduction was detected at the onset of maternal mouthbrooding behavior which is characterized by a dilation of the mouth. On the first day of incubation, all other individuals were removed to leave the mouthbrooding female on her own in the breeding aquaria. Five days after hatching, i.e., nine days post-fertilization (dPF), the fry were removed from the mouth. Each brood, identified by the fertilization date and the marks on the respective parents, was divided into two to five batches of at least 100 fry reared separately in 200-l aquaria.

Hormone Treatment

Steroids were administered through the feed. A first feeding salmonid feed (Aqualim) was impregnated with an alcohol solution containing steroids. Concentrations of 1 to 45 ug of steroids per gram of feed were tested. For the control batches, the same preparation was applied except that the feed did not contain any steroid.

Fry were fed ad libitum six times daily using an automatic feeder during the 12-hour photoperiod, seven days a week.

Fry at 10-15 dPF were treated thus for 45 and 21 days. To avoid a potential masculinizing effect from the temperature (Baroiller et al., in press), the water, which was filtered and aerated, was maintained at 28±1.5°C.

Nursing and Sexing the Fry

At the end of the treatment, fry older than 31 dPF were transferred to 1.5-m^3 outdoor tanks where they were fed the same diet until sexing was possible. At 60-90 dPF, when the histological differentiation of the male and female gonads had already taken place (Baroiller 1988a and b), all fry were sexed by microscopic examination of the gonad squash (x125). The presence of previtellogenic (auxocytosis) or vitellogenic oocytes, and the lobular configuration showed the female and male sexes, respectively.

A χ^2 test was used to compare the sex ratios of the batches that had received treatment and the control batches (α=0.05).

Results

After treatment and at the moment of sexing, there was no significant difference in the survival rate of the fry between the controls and the batches that underwent hormonal sex reversal, regardless of the treatment duration and the concentrations used (Table 1).

The microscopic examination of the gonad squashes of fry treated with 11ß-OHΔ4 (1,631 sexed animals) or 17α-MT (416 fry) did not show any hermaphrodite characteristics, sterility, or structural abnormality. The gonads of the individuals treated with 11ß-OHΔ4 were functional, irrespective of their genotype: functional sex reversed male XX and normal males were obtained and identified by progeny testing at the end of these treatments (Baroiller, this vol.).

Of the 17 batches treated with 11ß-OHΔ4, only one batch showed no deviation of sex ratio compared to the controls (Table 2). The progeny of the XY4 male underwent hormonal sex reversal only after treatment 15 dPF whereas the 13 other families received treatment after 10-14 dPF. Since sex reversal was produced in the four other batches also treated with 5 $\mu g \cdot g^{-1}$, this concentration did not seem to be the cause of absence of a deviation in sex ratio. This result could instead indicate a critical period of hormonal sensitivity.

All treatments using 11ß-OHΔ4 on fry of less than 15 dPF significantly affected the sex ratio towards the male sex compared to the controls, regardless of the concentration used (Table 2).

In contrast, 17α-MT had masculinizing effects only in a range of concentrations between 5 and 45 $\mu g \cdot g^{-1}$.

The 21-day treatment yielded all-male populations using hormonal concentrations between 10 and 35 $\mu g \cdot g^{-1}$ (11ß-OHΔ4) and at 5 $\mu g \cdot g^{-1}$ (17α-MT). With concentrations lower or equal to 5 µg of 11ß-OHΔ4 per gram of feed administered over a period of 21 days, the percentage of males was proportional to the concentration used (Fig. 1).

There was no significant difference in masculinizing potency between the two hormones for concentrations of 10-45 $\mu g \cdot g^{-1}$. With concentrations of 5 $\mu g \cdot g^{-1}$ and higher, significant differences were observed in the results of both treatments: using 5 $\mu g \cdot g^{-1}$, only 17α-MT yielded 100% male populations against a maximum of 88% with 11ß-OHΔ4. Inversely, 17α-MT was not effective if used at 1 $\mu g \cdot g^{-1}$, whereas 11ß-OHΔ4 used at the same concentration significantly altered the percentage of males compared to the controls.

Replicates (2-4) were conducted for four different concentrations of 11ß-OHΔ4 (5, 20, 30 and 35 $\mu g \cdot g^{-1}$) (Table 2). There was no significant difference in sex ratios between replicates of the same treatment.

Discussion

The hormones used for masculinization of fish are generally artificial molecules derived from testosterone: 17α-MT, 17α-ethynyltestosterone, dihydrotestosterone acetate and testosterone propionate. These synthetic androgens are considered more potent than natural androgens for the hormonal sex reversal of gonochoristic teleost fish species (Hunter and Donaldson 1983).

Many authors have also produced 100% male tilapia populations using these steroids, despite a great heterogeneity in the experimental conditions: especially concentrations varying from 10 to 240 $\mu g \cdot g^{-1}$ of feed (Baroiller and Jalabert 1989). The optimum concentrations usually suggested are 30 $\mu g \cdot g^{-1}$

Table 1. Survival of *Oreochromis niloticus* fry according to hormone treatment applied for sex reversal.

Treatment	Survival after treatment (%)	Number of batches tested	Survival at sexing (%)	Number of batches tested
11ß-OHΔ4	83.8	15	55.2	17
17α-MT	85.6	4	56.7	4
Control	79.6	12	50.2	14

Table 2. Sex reversal treatments administered to *Oreochromis niloticus* fry, the progeny of normal male (XY_n) or sex reversed male (XX_n) broodfish pairs.

	Treatment characteristics				Sexing characteristics			
Broodfish male type and no.	Steroid	Level in feed ($\mu g \cdot g^{-1}$)	Initial age of progeny (dPF)	Final Age (dPF)	Duration days	Number of males	Number of females	Males (%)
XY1	11ß-OHΔ4	35	12	57	45	81	0	100
	Control	0	12	57	45	45	55	45
XY2	11ß-OHΔ4	35	12	57	45	92	0	100
	Control	0	12	57	45	45	41	52
XX1	11ß-OHΔ4	35	12	57	45	128	0	100
	Control	0	12	57	45	64	56	53
XX2	11ß-OHΔ4	30	13	55	42	64	0	100
	11ß-OHΔ4	20	13	55	42	51	1	98
	Control	0	13	55	42	0	74	0
XX3	11ß-OHΔ4	30	14	42	28	129	0	100
	11ß-OHΔ4	30	14	35	21	168	0	100
	Control	0	14	42	28	47	69	41
XX4	11ß-OHΔ4	30	12	54	42	112	2	98
	11ß-OHΔ4	20	12	54	42	103	1	99
	Control	0	12	54	42	0	86	0
XX5	11ß-OHΔ4	20	11	32	21	74	0	100
	17α-MT	20	11	32	21	110	0	100
	Control	0	11	32	21	23	80	22
XX6	11ß-OHΔ4	10	10	31	21	100	0	100
	17α-MT	10	10	31	21	172	1	99
	Control	0	10	31	21	34	118	22
XX7	11ß-OHΔ4	5	10	31	21	24	3	89
	17α-MT	5	10	31	21	47	0	100
	Control	0	10	31	21	6	21	22
XX8	11ß-OHΔ4	5	11	32	21	79	30	73
	Control	0	11	32	21	28	96	23
XX9	11ß-OHΔ4	5	11	32	21	71	30	70
	Control	0	11	32	21	16	69	19
XY3	11ß-OHΔ4	5	11	32	21	46	20	70
	Control	0	11	32	21	15	70	18
XY4	11ß-OHΔ4	5	15	36	21	79	30	73
	Control	0	15	36	21	71	70	70
XX10	11ß-OHΔ4	1	13	34	21	41	72	36
	17α-MT	1	13	34	21	12	74	14
	Control	0	13	34	21	11	59	15

dPF=days post-fertilization.

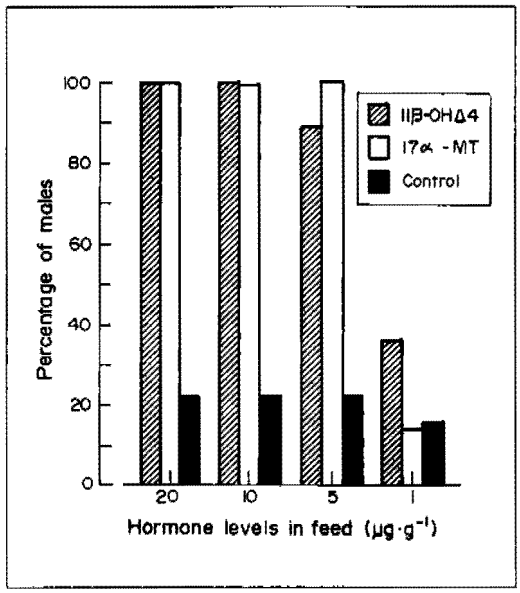

Fig. 1. Efficiency of hormonal sex reversal using different steroids and different concentrations in tilapia feed.

for 17α-MT and 60 μg·g^{-1} for ethynyltestosterone (Pandian and Varadaraj 1987; McGeachin et al. 1987; Rothbard et al. 1987; Baroiller and Jalabert 1989). For *O. mossambicus*, the minimum concentration for the production of all-male batches using 17α-MT is 5 μg·g^{-1} (Pandian and Varadaraj 1987). Other artificial androgens such as 17α-methyl-5-androsten-3ß-17ß-diol (Varadaraj and Pandian 1987) and mibolerone (Guerrero and Guerrero, this vol.) have also been used.

The present study indicates that in *O. niloticus*, the minimum concentration of 17α-MT yielding a 100% male population is also 5 μg·g^{-1} for a 21-day treatment period; there was no deviation in sex ratio with concentration of 1 μg·g^{-1}.

As the steroidogenesis in the period of gonadal differentiation has not been extensively studied in teleost fish, particularly in tilapia (Baroiller 1988a and b; Baroiller et al. 1988), only a limited number of natural androgens have been tested as masculinizing agents. Among these, two 11-oxygenated derivatives, administered through the rearing water and through the feed, respectively, can modify the differentiation process in tilapia: adrenosterone causes a destruction of the ovarian structures in *O. niloticus* at a concentration of 5 mg·l^{-1} (Katz et al. 1976) and 11 ketotestosterone, at 200 μg·g^{-1}, inhibits the formation of the ovarian cavity in *O. mossambicus* but does not prevent the formation of young oocytes that will subsequently degenerate (Nakamura 1981). However, considering the high levels used, the hypothesis of toxic or paradoxical effects cannot be discounted (Hunter and Donaldson 1983). In *O. niloticus*, the masculinizing effects of adrenosterone have also been demonstrated after treatments through the feed at 45 μg·g^{-1} (Baroiller 1988b).

Until then, 11ß-OHΔ4 had not been used in hormonal sex reversal treatment in tilapia. This natural androgen shows a masculinizing potency comparable to that of 17α-MT at concentrations of 10-35 μg·g^{-1}. In addition, deviations of sex ratio are observed at concentrations lower than 5 μg·g^{-1}, which is not the case for 17α-MT.

The microscopic examination of the gonads of fry treated for 60-90 days did not show any abnormality in the course of gonadal ontogenesis. Moreover, the functional reversal of the gonads was demonstrated by the identification of the sex reversed males after progeny testing of the individuals treated in the present study (Baroiller, this vol.).

The steroid 11ß-OHΔ4 was identified *in vitro* in three species of teleost fish in the early stages of differentiation: this 11-oxygenated steroid can be specifically synthesized by the testes of the rainbow trout *Oncorhynchus mykiss* (van den Hurk et al. 1982), the catfish *Clarias*

gariepinus (van den Hurk et al. 1989) and *O. niloticus* (Baroiller 1988b) during the early gonadal ontogenesis.

Administered through the feed (60 and 6 $\mu g \cdot g^{-1}$) and through the rearing water (300 $\mu g \cdot l^{-1}$), 11ß-OHΔ4 produces populations with high percentages of males, yielding 76-78% in the trout (van den Hurk and Lambert 1982; van den Hurk and van Oordt 1985) and 77% in the catfish (van den Hurk et al. 1989), respectively, against 48 and 50% males in the controls, respectively. In *C. gariepinus* (van den Hurk et al. 1989), 17α-MT significantly biases the sex ratio towards males (65%) at concentrations of 30 $\mu g \cdot l^{-1}$ and towards the female sex at 100 $\mu g \cdot l^{-1}$. In *Oncorhynchus mykiss*, testosterone derivatives may not be essential for testicular differentiation: testosterone and its 11-oxygenated derivative can be synthesized by the testis only stages beyond those at which 11ß-OHΔ4 has been identified (van den Hurk et al. 1982). Furthermore, a treatment using cyproterone acetate did not affect the sex ratio of the batches of trout (van den Hurk and van Oordt 1985) and tilapia fry (Hopkins et al. 1979). The androstenedione 11-oxygenated derivatives may be involved in some stages of testicular differentiation in these three species.

In *O. niloticus*, response to the hormone treatment is observed during a determined critical period. To be efficient, treatment must begin between nine and 13 dPF. Beyond this period, differentiation seems definitively according to the genotype; from then on, it could only be affected by exogenous steroid factors.

The 21-day treatment is therefore applied between 9-30 dPF. From a histological perspective, at 27°C, oogonia proliferation occurs in females between 20-28 dPF and is followed by their first meiotic prophase at 28-35 dPF (Baroiller 1988a and b). In males, a highly progressive multiplication of somatic cells and spermatogonia is observed during the same period (Baroiller 1988 a and b). Exogenous hormones are therefore administered to the fry before the establishment of these histological processes.

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Edited by

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