Vol.66: e23210792, 2023
https://doi.org/10.1590/1678-4324-2023210792
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

Effect of Fluoxymesterone on Sex Proportion and Growth Performance of Nile Tilapia (*Oreochromis niloticus* L.).

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Marcelo Ricardo Vicari

Received: 08-Dec-2021; Accepted: 26-Jul-2022

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HIGHLIGHTS

- Fluoxymesterone is ineffective at a low concentration six days after hatching.
- Growth is not promoted by feeding the fry with fluoxymesterone.
- Survival is not negatively affected by fluoxymesterone.

Abstract: One of the main problems to be solved in the commercial production of Nile tilapia is the use of large volumes of steroids during the sex reversion process. The precise evaluation of the time after hatching to start the steroid treatment could help reduce its use in the short-term. The main objective of this work was to evaluate the sex proportion and the growth obtained in a mixed population of Nile tilapia fed fluoxymesterone at different days (3, 6, 9, 12, and 15) after hatching. A one-factor experiment was designed, with the day, after hatching, that the fluoxymesterone (5 mg) began to be supplied as factor (3, 6, 9, 12 and 15). Initial stocking density was 0.4 larvae L^{-1}. All groups were evaluated by triplicate. Steroid treatment finished at day 25 after larvae stocking. Squash technique was used to determine sex proportion at the end of the experiment (45 days). Growth evaluation was performed based on wet weight and total length obtained from each biometry. The results showed the highest percentage of males (92%) in the fish fed fluoxymesterone from day three after hatching. No anabolic effect on growth was observed in any steroid-treated fish. A negative effect on growth was observed after withdrawal of the steroid in the fish that were fed fluoxymesterone for the longest time. These results show that it is necessary to supply the fluoxymesterone prior to the onset of sex differentiation (5-6 days after hatching) to achieve a permanent reversion of the sex differentiation process.

Keywords: fluoxymesterone; sex reversal; hatching; time; growth; survival rate.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is responsible for more than 99% of the world tilapia production [1], thanks to its fast growth and high tolerance to a wide range of water temperatures and salinities [2]. However, in mixed-sex cultures reproduction during grow-out is a major problem, leading to the presence at harvest of a wide range of fish sizes instead of the larger and more uniform fish expected from the original stocking [3-5].

All-male Nile tilapia cultures show better growth than mixed cultures and eliminates uncontrolled reproduction allowing for the production of marketable-sized fish [3,6,7]. Therefore, for many years the production of monosex, all-male populations, has been recognized as the most effective technique to increase Nile tilapia production under commercial culture conditions [3,7,8,9]. Several approaches have been developed to achieve all-male populations, the most commonly applied in the industry today being direct hormonal sex-reversal by feeding fry with different steroids [3,10,11,12].

Since the start of its commercial farming, a great deal of research effort has been invested in the development of the Nile tilapia´s all-male populations to exploit their benefits for aquaculture [13,14]. However, in recent years the use of large volumes of sex steroids to obtain all-male populations has generated growing concern on the part of environmental groups since its accumulation in water bodies near farms (rivers, lakes, coastal areas) can alter the sex ratio of wild animals that inhabit these areas [15,16].

This has made sex reversal an increasingly controversial technique, prompting the search for techniques such as the production of YY males [3,17]. However, the production of YY males has not proven to be a short-term solution as originally planned. A wide-ranging and immediately applicable solution would be the optimization of the protocols currently used to reverse sex. These were designed without knowing the exact period where the sexual differentiation of the Nile tilapia occurs [18,19], using a reduced number of potential steroids for reversing sex and probably need to be revisited, especially in highly domesticated Nile tilapia strains. Under this scenario, steroids such as 19-norethyltestosterone, ethyltestosterone, testosterone propionate, mesterolone, androstenedione, trenbolone acetate, testosterone, 17α-ethynyltestosterone, dihydrotestosterone, 17α-methyldihydrotestosterone and fluoxymesterone [20-23] are documented alternatives to 17α-methyltestosterone. In the case of fluoxymesterone, its advantages include that is a product commonly used in human medicine, readily available, cheaper than 17α-methyltestosterone and without importation restrictions [20]. Additionally, according to the information reported in several works, is highly effective in reverting sex at low concentrations (2.5, 5 and 7.5 mg Kg^{-1}) in Nile tilapia and its hybrids [24-28]. Therefore, using the knowledge of the time after hatching where sexual differentiation occurs [29], is possible that using fluoxymesterone at a low concentration could optimize the percentage of males obtained. Taking this into account, the present study aimed to evaluate the sex proportion and growth obtained in the Nile tilapia fed fluoxymesterone at different days after hatching.

MATERIAL AND METHODS

Larvae

Nile tilapia larvae at late yolk-sac stage (three days of age) were acquired from the Unidad de Producción del Tesechoacan (La Cuatezona, Veracruz, México), a fry production farm that developed its own strain of Nile tilapia (Isaluma strain), adapted to the climatic conditions of the Papaloapan region. To ensure that the larvae were of the same age, they were collected at the egg stage from the female's mouth and incubated in a McDonald-type hatching jar (6 L, Pentair Aquatic Eco-systems, Florida, USA). According to the sex-reversal protocols of the aforementioned fry producing farm, the larvae were delivered at the onset of the exogenous feeding (before they completely finished their yolk sac). In total, 612 larvae were transferred to the Laboratorio de Acuicultura of the Universidad del Papaloapan. Upon arrival at the laboratory, a salt treatment (> 35 psu) was applied for approximately 10 minutes in order to avoid the introduction of external parasites to the experimental system.

Experimental design

A completely randomized one-factor experiment was designed, with the day that the feed added with the exogenous steroid began to be supplied as factor, with five groups (3, 6, 9, 12 and 15 days after hatching) and as a control a group fed commercial diet without added steroid. All groups (treatments) were evaluated by triplicate. Fluoxymesterone (Pharma, Hamshire, UK) treatment finished, regardless of the day that it began, at day 25 after larvae stocking (28 days after hatching). In total, the experiment lasted 45 days.

For the experiment, the sexually undifferentiated larvae were transported to a closed recirculating system and randomly divided into 18 acrylic aquaria of 85 L at an initial stocking density of 0.4 larvae L^{-1} (34 larvae

per aquaria). The water in the recirculating system was filtered using a mechanical filter (Hayward, Model S310T2, Hayward Pool Products Inc., Elizabeth, New Jersey, USA) and a biofilter containing only plastic bio-balls (Aquatic Eco- System, Model CBB1, Pentair Ltd. Apopka, Florida, USA). During the experiment, a photoperiod of 12L: 12D was used and water temperature was thermostatically (Intensity 12000 BTUS, Carrier, Florida, USA) maintained at 26 ± 1°C.

Preparation of the steroid added feed

The synthetic hormone fluoxymesterone (FM) was added to one kilogram of commercial fish meal (<0.35 mm, 53% protein, 15% lipids, Purina, Agribrands) using the method described by Guerrero [18]. In brief, selected concentration of FM (5 mg) was dissolved in 500 mL of 95% ethanol, sprayed over the feed, which was distributed on a thin layer over a laboratory table and mixed several times until the food was completely moistened. Sprayed food was maintained at room temperature for six hours to allow the alcohol to evaporate. The food for the control group was treated in exactly the same manner with the exclusion of the added steroid. Once dried, the food was stored in a plastic container (DFR-9010, Daewoo, Seoul, KOR) at 4°C.

Development of the experiment

Larvae and then fry were fed at 2-h intervals at a feed ratio of 10% of their total body weight. Water and air flow were closed in all aquaria 10 minutes before feeding and for 15 minutes after offering the feed, in order to encourage feeding. The aquaria were siphoned daily to remove feces and dead fry. Once the FM treatment was completed, the fry were fed with untreated commercial diet (53% protein, 15% lipids, Purina, Agribrands) for five more days until the fry period was finished (day 30 after larvae stocking). Juveniles were fed four times a day with untreated commercial diet (<1.0 mm, 50% protein and 1.5 mm, 44% protein, Purina, Agribrands) at a feed ratio of 10% of their total body weight for another 15 days until the end of the experiment (day 45).

Random samples of 35% of fry per replicate were collected every 15 days. Mean wet weight (WW) was obtained using a digital scale (±0.01 g) (Ohaus Cor., Scout Pro Model Sp 202, Parsippany, NJ, USA), and total length (TL) was recorded from a digitized image using an imaging software (ImageJ version 1.36).

Growth performance

The following fish performance indices were calculated from the wet weight and total length obtained from each biometry:

Gained biomass (GB):

GB = Final biomass (g) – Initial biomass (g)

Average daily weight gain (ADW):

ADW = [Final wet weight (g) – Initial wet weight (g)] / Culture days

Average daily length gain (ADL):

ADL = [Final length (g) – Initial length (g)] / Culture days

Specific growth rate (SGR):

SGR (% per day) = [Ln FWW (g) - Ln IWW (g) / Culture days] × 100

Where: FBW = Final wet weight, IBW = Initial wet weight.

Fulton´s condition factor (CF)

CF = [Fish wet weight (g)] / [Fish length^3 (cm)] × 100

Survival

Final survival (FS%) was obtained using the following formula:

FS = NFE / NFS × 100

Where: NFE = number of fish at the end of the experiment, NFS = number of fish stocked.

Evaluation of sex proportion

The sex of 25% fish per replicate-treatment was determined by the squash technique proposed by Guerrero and Shelton [30]. Fish were sacrificed and the collected gonads were mounted individually on a glass slide, added a few drops of acetocarmine stain and lightly squashed with a cover slip. The criteria to identify the sex of the organisms under the microscope (40x) was the following: If uniform tissue with fine like granular structure (spermatogonia) was observed, it was classified as male, while if large and circular structures (oogonia) were observed in the tissue, it was classified as a female.

Statistical analysis

Differences in total length, wet weight and growth indices were analyzed using a one-way analysis of variance, with a Tukey test performed on treatment means *a posteriori*. Final survival was analyzed using a chi-square test. The proportion of males identified in each treatment was tested against the 1:1 (male: female) expectation using a chi-square test at a probability of 0.1% (P<0.001).

RESULTS

Wet weight and total length

WW and TL for all groups are given in Table 1. Significant differences (P<0.001) were observed in WW at days 30 and 45. At day 30, WW was significantly higher (P<0.001) in the 9G and 15G groups in comparison to the rest of the treated and the control group. At 45 d of age, the 15G group showed the significantly higher (P<0.001) value of WW, compared to the 6G and 9G groups. No significant differences were observed between any of the FM-treated groups or the control group.

TL registered significant differences (P<0.001) only at 30 d of age. The 12G group showed the significantly higher (P<0.001) value of TL compared to the 3G and 9G groups, and the control group. No significant differences were observed between the 0G, 6G and 12G groups.

Table 1. Wet weight (WW, g) and total length (TL, cm) (± S.E.) (n = 3) of Nile tilapia (*Oreochromis niloticus*) fed fluoxymesterone at days after hatching. Values in each row superscript with different letters indicate significant differences between groups (P<0.001).

	Treatment						P value
	CG	3G	6G	9G	12G	15G	
0							
WW	0.16±0.01	0.13±0.01	0.11±0.02	0.10±0.02	0.12±0.02	0.11±0.01	0.095
TL	1.56±0.05	1.54±0.06	1.54±0.03	1.51±0.07	1.54±0.01	1.50±0.01	0.212
15							
WW	0.88±0.09	0.76±0.05	0.74±0.09	0.84±0.08	0.77±0.10	0.79±0.07	0.091
TL	3.14±0.09	2.81±0.06	2.85±0.08	2.88±0.08	2.85±0.07	3.12±0.04	0.075
30							
WW	1.65±0.20^{a}	2.37±0.23^{a}	2.21±0.10^{a}	2.64±0.19^{b}	2.09±0.11^{a}	3.13±0.17^{b}	<0.001
TL	4.20±0.07^{a}	5.01±0.01^{bc}	4.69±0.09^{ab}	5.03±0.18^{bc}	4.50±0.01^{ab}	5.38±0.16^{c}	<0.001
45							
WW	8.61±0.49^{ab}	7.59±0.43^{ab}	7.36±0.44^{b}	7.52±0.55^{b}	8.18±0.42^{ab}	9.49±0.48^{a}	<0.001
TL	7.42±0.15^{a}	7.17±0.12^{a}	7.14±0.21^{a}	7.04±0.22^{a}	7.33±0.08^{a}	7.66±0.02^{a}	<0.001

CG = group that received not fluoxymesterone treatment, 3G = group fed fluoxymesterone from day three after hatching, 6G = group fed fluoxymesterone from day six after hatching, 9G = group fed fluoxymesterone from day nine after hatching, 12G = group fed fluoxymesterone from day 12 after hatching, 15G = group fed fluoxymesterone from day 15 after hatching. Number of fish analyzed per treatment= 36.

Growth performance

Gained biomass (GB), average daily weight gain (ADW), average daily length gain (ADL) specific growth rate (SGR) and condition factor (CF) for all groups is shown in Table 2. No significant differences were observed at day 15 between any of the FM-treated groups or the control group. At day 30, BG and ADW values were significantly higher (P<0.001) in the 15G group in comparison to the control group. No significant differences were registered between the rest of the FM-treated groups and the 15G or the control group. A significantly lower (P<0.001) value for ADL was observed in the control group in comparison with the 3G, 9G and 15G groups. No significant differences were registered between any of the FM-treated groups. At day

45, on the contrary, the control group showed a significantly higher (P<0.001) value of ADL than those observed in the FM-treated groups, except for the 12G group.

Table 2. Biomass gained (BG, g), average daily weight gain (ADW, g), average daily length gain (ADL, cm) specific growth rate (SGR) and condition factor (CF) (± S.E.) (n = 3) of Nile tilapia (*Oreochromis niloticus*) fed fluoxymesterone at different days after hatching. Values in each row superscript with different letters indicate significant differences between groups (P<0.001).

	Treatment						P value
	CG	3G	6G	9G	12G	15G	
D15							
BG	22.36±1.67	20.01±1.87	19.46±2.40	23.53±1.61	20.51±2.48	21.60±2.24	0.094
ADW	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.401
ADL	0.10±0.01	0.08±0.00	0.08±0.01	0.09±0.00	0.08±0.00	0.10±0.00	0.215
SGR	0.11±0.00	0.11±0.01	0.12±0.00	0.14±0.01	0.12±0.01	0.13±0.01	0.102
CF	2.87±0.38	3.49±0.46	3.17±0.12	3.57±0.50	3.36±0.47	2.63±0.40	0.082
D30							
BG	21.30±4.11^{a}	40.63±6.52^{ab}	36.97±5.82^{ab}	42.63±4.14^{ab}	34.27±7.75^{ab}	55.15±6.33^{b}	<0.001
ADW	0.04±0.00^{a}	0.08±0.01^{ab}	0.08±0.01^{ab}	0.09±0.01^{ab}	0.07±0.01^{ab}	0.12±0.01^{b}	<0.001
ADL	0.07±0.01^{a}	0.14±0.00^{b}	0.12±0.00^{ab}	0.14±0.01^{b}	0.11±0.01^{ab}	0.15±0.01^{b}	<0.001
SGR	0.04±0.00	0.06±0.00	0.06±0.01	0.06±0.00	0.06±0.01	0.08±0.01	0.175
CF	2.13±0.10	1.62±0.11	1.95±0.23	1.83±0.33	2.06±0.19	1.74±0.27	0.060
D45							
BG	181.85±9.07	152.24±2.98	146.87±19.62	135.54±28.27	173.00±11.00	179.54±9.84	0.051
ADW	0.39±0.02	0.34±0.00	0.33±0.04	0.30±0.05	0.37±0.02	0.40±0.02	0.183
ADL	0.21±0.00^{a}	0.14±0.00^{c}	0.16±0.00^{bc}	0.13±0.01^{c}	0.18±0.00^{ab}	0.15±0.01^{bc}	<0.001
SGR	0.10±0.00	0.08±0.00	0.08±0.00	0.07±0.00	0.09±0.00	0.08±0.00	0.291
CF	1.82±0.09	1.93±0.03	1.91±0.03	1.94±0.05	1.91±0.01	1.95±0.04	0.478

CG = group that received not fluoxymesterone treatment, 3G = group fed fluoxymesterone from day three after hatching, 6G = group fed fluoxymesterone from day six after hatching, 9G = group fed fluoxymesterone from day nine after hatching, 12G = group fed fluoxymesterone from day 12 after hatching, 15G = group fed fluoxymesterone from day 15 after hatching. Number of fish analyzed per treatment= 36.

Sex proportion and final survival

Sex proportion obtained showed that sex-reversed males were produced in almost all FM-treated groups, however, only the 3G and 9G groups produced a progeny significantly skewed toward male with a sex ratio that deviated significantly (P<0.001) from the 1:1 sex ratio expected in the Nile tilapia crosses between normal males (XY) and normal females (XX) (Table 3). A 100% masculinization rate was not achieved in any treated group.

No significant differences were observed in final survival between any of the FM-treated groups or the control group (Table 3).

Table 3. Sex proportion and final survival (FS) of Nile tilapia (*Oreochromis niloticus*) fed fluoxymesterone at different days after hatching. *Significantly different from the expected 1:1 distribution (P<0.001).

Treatment	Sex proportion		FS
	Males	Females	
CG	50	50	92
3G	92*	8	91
6G	63	37	90
9G	67*	33	91
12G	56	44	91
15G	50	50	90

CG = group that received not fluoxymesterone treatment, 3G = group fed fluoxymesterone from day three after hatching, 6G = group fed fluoxymesterone from day six after hatching, 9G = group fed fluoxymesterone from day nine after hatching, 12G = group fed fluoxymesterone from day 12 after hatching, 15G = group fed fluoxymesterone from day 15 after hatching. Number of fish analyzed per treatment= 26.

DISCUSSION

The steroid 17α-methyltestosterone has been recognized for years as the most efficient androgen for sex-reversal in Nile tilapia, however, lately it has become more difficult to acquire in some countries, including Mexico. This has prompted the search for alternative steroids that produce high reversion rates without negatively affecting growth or survival. One of the steroids that have been identified as potential substitutes is the FM, which is easily obtained and according to several authors [24-28,31-36] show high percentages of sex reversal at low concentrations.

In our work, the steroid used showed a significant effect on sex proportion in only two of the six treated groups (3G and 9G). However, only the 3G group produced a percentage of males higher than 90%. This confirms that for a successful sexual reversion, the implementation of the steroid should start before the beginning of the labile period, which in the case of Nile tilapia is around five (in females) to six (in males) days after hatching [37,38]. The high levels of FM maintained since day three after hatching allowed a 92% males to be obtained, indicating that starting the hormonal treatment at this age is sufficient to suppress the action of the *cyp19a* gene (responsible for producing the aromatase enzyme, which allows ovarian differentiation), by stimulating the *dmrt1* gene that is involved in testicular differentiation [37,39]. Delaying the application of the steroid, in this case, at the onset of the labile period for males (six days after hatching) is enough to cause a significant decrease (29%) in the proportion of males, indicating that the hormonal treatment must be carried out prior to the onset of the labile period in order to efficiently induce a permanent change in the genetic expression of the genes involved in the sexual differentiation of the gonads. This is probably related to the concentration of the steroid in the bloodstream, which requires time (days) to reach a high concentration enough to alter permanently the action of the genes involved in the sexual differentiation process. However, Budd and coauthors [40] reports that treatments applied outside the labile period require higher doses of steroid and longer treatment times than those that are administered during the labile period in order to successfully reverse gonadal sex. It is probably that to obtain high male percentages six days after hatching, it is necessary to increase the steroid concentration or use a more powerful steroid or aromatase inhibitor. In Nile tilapia, Bertolla and coauthors [41] obtained 100% males using high concentrations of fadrozole (75 and 100 mg Kg^{-1}) nine days after hatching, and Bhandari and coauthors [39] obtained 100% males eight days after hatching, using 50 mg Kg^{-1} of 17α-methyltestosterone. In our experiment, we used 5 mg Kg^{-1} of FM, which can be considered a low concentration, so it is likely that to obtain a high percentage of males six days after hatching, it may be necessary to increase the concentration of FM to 10 or 20 mg Kg^{-1}.

Although the duration of hormonal treatment has been pointed out as an important factor to successfully induce high rates of sex reversal, in recent years, both feminization and masculinization experiments with Nile tilapia, have shown that it is not necessary to administer the hormone for more than 20 days after the onset of the labile period to induce high rates of sex reversal [12,42,43,44]. Nakamura and coauthors [45] reports that under the light microscope, the first signs of gonadal sex differentiation appear in Nile tilapia fry at 23-26 days after hatching with the formation of the ovarian cavity in the XX gonad or the efferent duct in the XY gonad. So if a hormone is going to successfully reverse sex, it has to act several days or weeks before the gonadal differentiation begins. Tao and coauthors [38] reports that typically, when hormonal treatment is administered during the period covering 3-6 days after hatching, phenotypic sex can be successfully reversed in both sexes. In this regard, Trejo-Quezada and coauthors [44] reports starting the steroid treatment five days after hatching in Nile tilapia (Var GIFT) and obtaining a 100% males supplying 60 mg Kg^{-1} of 17α-

methyltestosterone during only 10 and 15 days. Using FM, Manosroi and coauthors [32] in a Nile tilapia hybrid obtained 96% males supplementing 40 mg Kg^{-1} to 4-days old fry while Calzada-Ruíz and coauthors [46] using commercial fry of similar age, report 100% males using a concentration of 20 mg Kg^{-1}. In our work a percentage of 92% males was obtained in 3-days old fry using only 5 mg Kg^{-1}. These results illustrate not only the importance of the age of the fry in a successful sex reversal, but the variability of male percentages obtained after using different concentrations of FM.

The idea behind this work and the project of which it is a part, is to reduce the use of steroids in the process of sex reversal in Nile tilapia. That is the reason, behind the duration used for the hormonal treatment, which was reduced by delaying the onset of steroid application, because according to the information available for Nile tilapia, after 28 days of age the gonadal differentiation has already begun, making very difficult a successful sex reversal regardless of the steroid or concentration used. The work of Trejo-Quezada and coauthors [44] was a successful part of this project, which was able to provide data that contribute to reducing the use of steroids in the process of sex reversal in Nile tilapia. However, the present work, failed to successfully move the onset of the hormonal period using a low concentration of FM. For future experiments it will be necessary to use another steroid and/or a higher concentration to contrast the results obtained.

Although the age after hatching is widely used as an indicator of the sex determination/differentiation process in Nile tilapia, there is a few works where the size of the larvae or fry is addressed as a key factor. Vera-Cruz and coauthors [8], recommend that the fingerlings be less than 10 mm in length before starting the hormonal reversal process to ensure percentages close to 100% while Hiott and Phelps [47] and Jiménez and coauthors [48], evaluated aspects related to initial length and success in sexual reversion, reaching the conclusion in both studies that the optimum length to ensure a percentage close to 100% of masculinization is less than 11 mm. In our work, the larvae used showed a length greater than the recommended one (15 mm), which could have contributed to reducing steroid efficiency when administered several days after hatching. However, it is important to mention that the average size of the Nile tilapia´s offspring has been increasing as the domestication of this species has advanced worldwide [49], which probably requires that the optimal length to start the sex-reversal process be evaluated again, since the fish used in the present experiment still had remnants of the yolk sac at the beginning of the experiment, which, in theory, guarantees an optimal start of the sex-reversal process. However, recent works using FM and other steroids [20,25-28,31,33,35,46], generally do not report initial size, making it hard to keep track of the evolution of optimal initial fry size for future works.

At the end of the experiment, no higher growth was observed in the groups treated with FM compared to the control group, which indicates that there was no anabolic effect on growth, as reported when using other steroids [10]. The results obtained in the present work agree with those reported by the few authors that have used FM to reverse sex in Nile tilapia or his hybrids [20,24,26,31,33,36]. Only Manosroi and coauthors [32], in a Nile tilapia hybrid and Gutiérrez-Sigueros and coauthors [50], in a native cichlid have reported a positive effect on growth by FM at the end of the sex-reversal process. The anabolic effect resulting from the use of steroids in Nile tilapia sex-reversal process appears to be almost exclusive to 17α-methyltestosterone [51-56]. However, in some experiments it may be difficult to identify the anabolic effect. Most studies that have analyzed improvements in the growth rate of sexually reversed fish and compare it to the one obtained in untreated fish, do so by comparing the growth of populations composed exclusively of male fish against a mixed sex population, in which the female show a significantly lower growth rate in comparison to the male. Phumyu and coauthors [9], report that in the Nile tilapia the reversed fish with the steroid show a growth similar to that of males, however, females fed the food added with the steroid show higher growth and better nutritional efficiency, which is attributed to features of the gastrointestinal tract. Phelps and Popma [4], suggest that any improved growth of the steroid-treated Nile tilapia is more related to the superior growth of the males than to the anabolic response related to increased protein synthesis and increased muscle.

The BG, as well as the ADW and the SGR did not show significant differences with respect to the control group, only the ADL, like the WW, showed differences, with the treated groups showing the lowest values at the end of the experiment, 20 days after completion of the steroid treatment. This is important because while the FM did not show an anabolic effect on the growth of the treated fish, indicates that the steroid has a negative effect once it is withdrawn from the commercial food provided to the fish, especially when supplied for a longer period of time, as the 3G, 6G and 9G groups showed the lowest values at the end of the experiment. Similar results have been observed after withdrawal of 17α-methyltestosterone by Abdelghany [52] in the Nile tilapia and by Ostrowski and Garling [57] in the rainbow trout.

The CF is an important parameter that indicates the nutritional status of the fish based on the amount of energy available to perform processes related to growth, maturity and reproduction, therefore is an indicator,

in a general way, of the health status of the reared fish [58,59,60]. Values close to 2.0 have been reported for the Nile tilapia and its hybrids grown under optimal [12,54,59,60]. The CF can also be implemented to assess the physiological state of fish under potential stress [55], such as feeding Nile tilapia fry with a steroid enriched food. In our work, CF values observed in the FM-treated groups did not show significant variations in comparison to the control group, indicating that the treatments were developed under optimal conditions and that the supplied steroid did not alter the metabolic balance or cause a decrease in the energy available for the fish physiological functions.

Survival at the end of a sex-reversal experiment depends on factors such as species, type of steroid and concentration, time of onset and duration of the treatment [10]. One of the reasons why FM is pointed out as an alternative for the use of 17α-methyltestosterone, in addition to its high rates of sexual reversal, is that usually does not cause adverse effects on survival. In our work, final survival showed no differences between the control group and the groups exposed to the steroid, which indicates that the FM had no negative effects on survival rate. Jiménez and Arredondo [31], and Chakraborty and coauthors [61], report a final survival after a steroid treatment similar to those observed in our work (~ 90%). However, other studies have reported lower survival rates than those obtained in the present study, ranging from 24% to 83% for groups exposed to similar steroids [62-68], supporting the idea that the steroid and the concentration implemented in the present work did not negatively affect final survival.

CONCLUSION

The results obtained indicate that delaying three days the onset of the sex-reversal process has a significant effect on the percentage of males obtained. This is probably related to the role of the exogenous steroid in the sex differentiation process at a genetic level, which once initiated is more difficult to reverse, especially at a low steroid concentration, such as that used in the present work. It will be necessary to evaluate this process using elevated steroid concentrations, which are probably more capable to reverse an advanced process of sex differentiation in the Nile tilapia.

Funding: This research was funded by the Universidad del Papaloapan under the internal project FO-UNPA/0001/19.
Acknowledgments: The authors would like to express their appreciation to the technical staff of the Laboratorio de Acuicultura of the Universidad del Papaloapan.
Conflicts of Interest: The authors declare no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

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