

Aspects of a Monosex Population of *Oreochromis Niloticus* Fingerlings Produced Using 17- α Methyl Testosterone Hormone

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Abstract

The study investigated the efficacy of 17- α methyl testosterone hormone in producing a mono male sex population in *Oreochromis niloticus* fry under tropical conditions in ponds in Zimbabwe. Fry were randomly stocked in ten fry tanks at 15000 fry per tank. Five randomly selected tanks received androgenised feed with 17- α methyl testosterone (M T hormone). The other five tanks received feed which had not been treated with 17- α methyl testosterone. The sex ratios, feed conversion ratio, growth rate and survival were established after three months. Fry fed to a diet treated with MT had a significantly higher growth rate as compared to those fed to a non hormone treated diet. Individuals whose diet was androgenised had a higher survival rate as compared to those individuals whose diet was not treated. We concluded that MT has a high efficacy in producing a male population in *O. niloticus* under tropical conditions and that treatment duration during cold periods be increased to 30 days as compared to the usual 21 days currently being used at Lake Harvest Aquaculture Zimbabwe so as to produce a male population above 95% for optimality.

Keywords: Sex reversal; *Oreochromis niloticus*; 17- α methyl testosterone; Androgenisation; Feed conversion ratio

Introduction

Tilapias (*Oreochromis niloticus*) are a paradox in reproduction. The relative fecundity of *O. niloticus* species is low; 6,000–13,000 eggs/kg/spawn. However, this is compensated by high survival rate and its iteroparity nature. Ideally, a fish species used in aquaculture is not allowed to reproduce in the culture environment before reaching market size. This phenomenon presents a significant challenge to the fish culturist. Most tilapia species often reach maturity within 6–8 months of hatching at a size often less than 100 g. Under favourable conditions tilapia will start to reproduce, leading to intraspecific competition hence stunted growth and become unmarketable.

Tilapia has numerous advantages as an aquaculture species, some of which include; sturdiness, an almost catholic diet and general adaptability [1]. However, its ability to reproduce in the production setting has resulted in various techniques being developed to control unwanted reproduction. Techniques such as stock manipulation [2], polyculture of tilapia with predatory fish [3] and monosex culture [4] have been used to control tilapia overpopulation.

An all male culture of tilapia is preferred because of their fast growth. Several techniques have been used to produce monosex tilapia, and among these include; manual sexing [5]; hybridization [6]; genetic manipulation [7]; and sex reversal through sex oestrogenic hormone administration [5,8,9]. However, monosex culture achieved through manual selection often results in half of the potential fish seed being rejected. Popma and Phelps [10] developed an efficient system to produce hand-sexed fingerlings, but 30% of a farm's acreage had to be devoted to brood fish and fingerling while the remaining 70% is set aside for food fish production.

The use of hormones to alter the sex ratios of fish was first demonstrated in species other than tilapia. Sex hormones were observed to affect the gonads in addition to other secondary characteristics such as masculinisation [8]. In an experiment, Yamamoto [8] produced 100% females in medaka (*Oryzai latipus*) with an oestrogen hormone. The hormone technique successfully altered the sex ratio of rainbow trout (*Salmo gairdneri*), goldfish (*Carassius auratus*), Zebra fish (*Danio rerio*)

[11], grass carps (*Ctenopharengodon idella*) [12] and tilapia [13]. Following the techniques outlined by Popma and Green [14], less than 15% of a farm's acreage is devoted to brood stock and fingerling production, leaving the remaining 85% for food. Such efficiency and simplicity in production techniques has resulted in hormone sex reversal becoming the commercial procedure of choice to produce male tilapia fingerlings, and has been a significant factor in the dominance of tilapia as an aquaculture product.

Hormone treatment does not alter the genotype of the fish but directs the expression of the phenotype. Production of all male population through administration of androgen (17- α methyl testosterone) is considered to be the most effective and economically feasible method for obtaining all male tilapia populations [15]. Recently hatched tilapia fry do not have developed gonads such that it is possible to intervene at this early point in the life history and direct gonadal development to produce monosex populations. Exogenous steroids given during the gonadal development period can control the phenotype overriding the expression of the genotypically determined sex. Lake Harvest has been practicing sex reversal of fry for years, but the efficiency of the sex reversal hormone 17- α methyl testosterone (MT) has never been checked. Instead the company just adopted the technique basing on results from researches done in countries like Ecuador, Philippines and Israel and at present the company is failing to reach their expected target of 95% males as published by researchers elsewhere. Therefore this study focused on assessing the efficacy of androgenisation with 17- α methyl testosterone, with survival rate, growth rate, feed conversion being monitored between sex reversed and non-sex reversed fish.

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Materials and Methods

Study area

The study was carried out at Lake Harvest Aquaculture in the resort town of Kariba, located in the North West Province of Zimbabwe; 16° 30' - 17° 00'S and 20° 00' - 29° 40'E and at an altitude of 490 m above sea level. The climate is generally tropical with three distinguishable seasons, a hot rainy season from late November to March, a cool dry season from May to August and a hot dry season from September to November. Annual rainfall ranges from 400 to 700 mm whilst temperature ranges between 13 and 40°C.

Fry tanks preparation and stocking

Fry tank preparation was done through scrubbing off algae from the wall linings, as it might interfere with the fry feeding regimes. Harvested fry were graded using a standard grading box. The grading process entailed blowing pure oxygen from one end of the box and a mesh size allowing fry of less than one gram was used to screen the fry down an oxygen gradient. The graded fry with average body weight of 0.02 ± 0.001 g were randomly stocked in ten fry tanks at 15000 fry per tank. Five of the ten tanks were randomly selected and received androgenised (17- α methyl testosterone) fry meal eight times a day for 21 days. The other five stocked fry tanks received un-androgenised feed using the standardized Lake Harvest Aquaculture (LHA) Growth Chart feeding regimes, as with the first treatment. MT was added to a powdered commercial feed with a pellet size of 1 mm, containing > 40 percent protein, by dissolving it in 95-100 percent ethanol, and was mixed with the feed to create a concentration of 60 mg MT/kg feed after the alcohol had evaporated. The alcohol carrier was added at 200 ml/kg feed and mixed thoroughly until all the feed became moist. The moist feed was dried out of direct sunlight and then stored under dark, dry conditions; this is because androgens break down when exposed to sunlight or high temperatures. An initial feeding rate of 20-30 percent body weight per day which was gradually decreased to 10-20 percent by the end of 4 weeks was adopted in each hapa. Rations were adjusted daily, and feed administered automatically eight times per day and this was done consistently so that the feed would float. Otherwise a considerable amount of feed would be lost as it settles through the bottom of the hapa.

Harvesting fry for stocking into hapas

As a stress control measure, fry were initially starved for 24 hours prior to harvesting. After harvesting, the wet weight method was then used to obtain the Average Body Weight (ABW) of the fry as given by the formula reference below:

$$ABW = \frac{\text{total wt. of fish randomly sampled}}{\text{No. of fish sampled}}$$

Harvested fry from each treatment were transferred to five production hapas, each stocked at 4608 ± 31.88 fry per hapa. Feeding of fry in the hapas followed the standardized LHA Feed Chart. For the first week in hapas, fry were fed to fry meal while in the second week, fry were fed a mixture of fry meal (40% Crude Protein) and crumbles (30 % Crude Protein) at a ratio of 1:2 dry mass respectively. From the third week up to the termination of the experiment, 30% crude protein crumbles were fed to the fry. The daily feeding rate after the LHA feed chart was calculated as follows:

$$DFR = ABW \times \text{stocking density} \times \text{feeding rate}$$

Feed Conversion Ratio (FCR) determination

The amount of feed fed to fry in each hapa was recorded on a daily

basis throughout the experimental period. The volumetric method was used to determine the initial and final stocking biomass for each hapa. The FCR of each hapa was calculated by adapting the procedure by Dey [16] as below:

$$FCR = \frac{\text{Amount of feeds consumed (kgs.)}}{\text{Wet weight gain of fish (kgs.)}}$$

Survival rate

Survival rate of the fry was determined using the formula below:

$$\text{Survival Percentage} = (H / S) \times 100$$

Where, H = number of fish

S = number of fish stocked

Temperature and dissolved oxygen were recorded on daily basis throughout the course of the experiment (21 days). Water temperature and dissolved oxygen were measured using a HACH dissolved oxygen meter (HACH, LDO, Germany). Water was exchanged daily at a rate (up to 120 percent of the hapa volume daily) and continuous aeration (up to 10 HP/ha).

Sex examination

Standard laboratory methods for sex examination in fish were employed to ten random samples from each hapa at the end of the experimental. Each sample comprised 40 ± 5.6 fry.

Data analysis

All data were tested for normality using Kolmogorov-Smirnov test at a 0.05 level significance. Data were found to be satisfying the normality assumptions and the paired t-test was used to check for any significant differences in the sex ratios, survival rates, feed conversion ratio and the weight gain by fry under t experimental conditions. A Pearson correlation test was carried out to investigate any relation between the weight gain of fry whose diet was not treated with hormone and those with hormone treated feed. All data were analyzed using SPSS version 16.00.

Results

Water parameters

The water dissolved oxygen and temperature for the two treatments during the entire 21 days treatment period are shown in Table 1. The dissolved oxygen in the water for all the treatments were not significantly different ($F = 1.319$, d. f = 4, $p = 0.268$) for the entire treatment period. There were no significant differences ($F = 1.116$, d.f = 4, $p = 0.353$) in the water temperature for the two treatments.

Sex ratios and survival rates

The results obtained showed a relatively high efficacy of MT in producing a mono sex fry population in *O. niloticus* culture systems. Fry fed to a diet treated with MT had a significantly high (t test: $t = -20.862$, $df = 9$; $p < 0.05$) male population of 90.060 ± 1.103 % as compared to those fed to a non hormone treated diet $50.630 \pm 1.09\%$. We observed a significant high survival rate (t test: $t = -35.109$, d. f = 4; $p < 0.05$) in

Treatment	D.O (mg/l)	Temperature (°C)
Non Androgenized	6.16 ± 0.09	22.54 ± 0.19
Androgenized	6.25 ± 0.07	22.26 ± 0.25

Table1: Mean (Mean \pm SE) dissolved oxygen (D.O) and temperature for the water during the treatment period.

individuals fed to a diet treated with MT (77.00 ± 0.707) as compared to those individuals whose diet was not treated (59.80 ± 0.663) over the three months study period.

Weight gain, growth rate and FCR

The weight gain by the fry for both treatments was observed over a three month period. The Pearson correlation tests showed a significant relation ($p < 0.05$, $r^2 = 0.901$) between the weight gain of fry whose diet was not treated with hormone, and similarly ($p < 0.05$, $r^2 = 0.996$) with those that were fed with hormone treated feed. There was a consistent weight gain by individuals whose diet was hormone treated (Figure 1).

The observed grand mean weight for fry fed with an untreated diet and those with MT treated diet were 1.513 ± 0.063 g and 2.241 ± 0.136 g respectively. The results revealed that the difference in the weight gain by individuals was not due to chance variation and as such can be attributed to the absence or presence of MT in the diets. When the growth rate of the fry from the two treatments were computed, we observed that fry whose diet was treated with MT had a relatively high growth rate (0.0428 g/month) as compared to those whose diet was not treated (0.0304 g/month). We observed a significant difference (t test: $t = 21.729$, d. f = 4, $p = 0.00$) in feed conversion ratio (1.686 ± 0.008) in individuals whose diet was androgenised as compared to (1.918 ± 0.007) of those without hormone in their diet.

Discussion

Producing a monosex population of *O. niloticus* for aquaculture is high priority since males have a higher growth rate as compared to females. In this study, we observed a relatively high male percentage (90:18%) compared to the females in those groups fed with androgenised feed compared to those not subjected to the hormone. These results in terms of male to female percentage are similar to other findings [17-19] implying that 17- α methyl testosterone hormone is an effective androgen in sex reversal even in open environments. However, our findings male; female sex ratios (90 ± 1.10 : 18 ± 1.54 %) were rather low compared to the other researchers [17,20]. Even though the feeding rate was similar, fed at 20% body weight four times a day, a low male percentage observed in our study seem to be highly correlated to the low temperature (19-24°C) compared to 26°C used by Phelps et al. [17].

The major environmental factors influencing sex reversal are temperature and dissolved oxygen [21-23]. The dissolved oxygen in the water under the treatment period was above the recommended 4 mg/L for optimality [22]. We therefore regarded this environmental parameter as constant in this current study. The observed mean water temperature

for the two treatments was not significantly different and as such, the variation in the sex ratio, feed conversion ratios and weight gain by the fry is not related to temperature. Therefore for tilapia aquaculture in the tropics we recommend that the water temperature be not colder than 25°C as the growth rate will decrease if the temperature goes any lower. Tilapia will normally start dying at roughly 10°C (50° F) [24]. It is also important to keep in mind that a temperature below the recommended range will have a negative impact on the immune system of the fish. The fish might survive at first, but it will be highly susceptible to possibly fatal attacks from bacteria, viruses and parasites.

The temperatures observed in this study (19 - 24°C) were relatively lower than general optimum range (26 - 28°C) which produced more desirable results [24]. The overall temperature during the entire treatment period was less than 24°C and we assume that this reduced the feeding rate of fry. A reduced feeding rate tends to affect the hormone dose assimilation rate hence gonadal differentiation would be compromised [19]. Popma and Green [14] produced a 99.5% all male population when they subjected fry to treatment at 34°C in doors over a period of 30 days. Nevertheless, at 21- 23°C over a period of 30 days; Bocek et al. [20] obtained a 98% male. Though the average temperature was lower than the optimum, by extending the treatment period to 30 days, the effect of low temperature was compensated by an extended dosage period.

The treatment period for the current study was only 21 days, seven days less than those used by Bocek et al. [20]. This corresponds to the findings of Mbarerehe [25] who found a 95% male population at temperatures between 18 and 23°C over a period of 40 days. However, when the same trial was done over a period of 20 days, only 69% were males. It is therefore apparent that under low temperature conditions, especially towards winter, there is need to increase the treatment time that would increase the dose rate in relation to the feeding rate [26]. Accordingly, we suggest that treatment duration is important in attaining the proper dose rate and consequently a high male percentage. The dose rate used in this study was rather lower than 0.52 g MT/g fish used by Okoko [19] to produce a male population greater than 95 %. We suggest that towards winter, when the temperatures are low, the dose rate and the treatment duration need to be increased for optimality. Since quantity of feed is positively correlated with the operational costs, manipulating the dose rate during cooler months is highly recommended.

We observed a relative high growth rate in individuals treated with MT hormone compared to the one without androgenised diet. The former had a significantly more male proportion signifying that males have a higher growth rate compared to females. This corresponds to other findings that males are the most desirable crop for cage aquaculture as they grow faster than females [9,17,27]. Males are thought to grow twice faster than females in most situations [14]. In a population where males constitute a greater percentage, the feed conversions are more favourable. Most of the feed protein consumed by females is not spent towards growth. According to Lone and Ridha [28], female fish use most of their feed protein for production of eggs and little is converted to flesh that is why males are relatively big as compared to females. Moreover females do not eat properly when incubating eggs or caring for the fry and there is also a compensation effect of utilising lipids for egg production which ultimately makes the female tilapia leaner and thinner than the male fish [29].

Variations in the growth rates in this study may also be attributed to the differences in the feed conversion ratios of these two groups. High FCR values for individuals whose diet was androgenised may have contributed to better conversion of feed and the consequent weight gain as

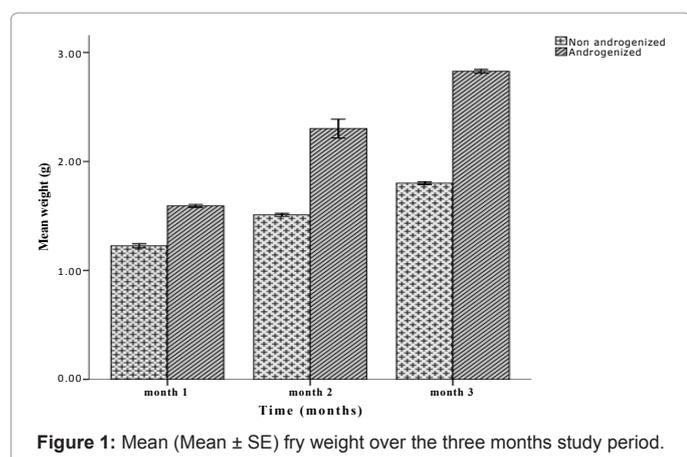


Figure 1: Mean (Mean \pm SE) fry weight over the three months study period.

compared to the other group. MT is an accelerator of mineral uptake into the body as well as their retention and hence males are likely to have a better conversion of food [30]. Although we observed a relatively high feed conversion ratio in individuals, whose diet was androgenised than the ones without, the FCR established in this study are rather low as compared to the findings of Watanabe et al. [31]. This could have been due to the fact that the hormone can degrade during storage and on its passage through the digestive tract. Furthermore, the lack of uniformity of the hormone in the feed and hierarchies among fish can cause significant variability in dose among treated individuals making estimates of amounts ingested very difficult. Excessive doses of some hormones can lead to sterility or paradoxical feminization following aromatization of androgens to estrogen.

The survival rates of the fry in the two treatments were significantly different with the highest survival in the one which receive MT. Such findings have been noted by Shelton et al. [9], who established that males have high survival rates of up to 80% in fry tanks and about 95-100% in grow out ponds. Fry at LHA are stocked in grower ponds where they are put in hapas which are covered by predator nets thereby reducing predation. Common fish predators are African fish Eagle (*Haliastur vocifer*), the crocodile (*Crocodylus niloticus*) and the monitor lizard (*Varanus niloticus*) [32]. In future studies tracing the survival and growth rate of these two groups of fish (males and females) produced using steroids would be beneficial to establish any variations in the survival rates even among same sexes [33-36].

Conclusion and Recommendations

Males have relatively high feed conversion ratio and consequently growth rate as compared to females. Higher survival rates in individuals with androgenised feed indicate that males tend to be more resilient to environmental shifts than females. We therefore recommend that in tilapia aquaculture there may be continued use of a consistent dose rate during summer (warmer conditions) and increase the treatment period to compensate the reduction in feed intake during (cooler periods) winter in order to achieve a male percentage of above 95% for optimum production. And for tilapia aquaculture in the tropics (an area which has consistently high temperatures 21-35°C for at least three quarter of the year) we recommend that the water temperature be not colder than 25°C as the growth rate will decrease if the temperature goes any lower. Also in our study the sex-reversed fry reached an average of 0.2 g after 3 weeks and 0.4 g after 4 weeks thus we found 3-4 weeks to be the most sensitivity phase of sex reversed tilapia fry growth. The average efficacy of sex-reversal ranged up to 90% in each hapa. And hence we recommend continuous hormone application until 8 weeks at a temperature range of 22-28°C. We conclude that 17- α methyl testosterone is effective in producing a mono sex population of *O. niloticus* under both the tropical conditions (an area which has consistently high temperatures 21-35°C for at least three quarter of the year) and unenclosed environments (open hapa) with varying environmental conditions.

Hormonal treatments are very efficient, but they could pose environmental problems in the future, due to uncontrolled discharge of the resulting waste water (i.e. steroids and / or metabolites are not removed from the water before discharge). The potential for localized pollution downstream of the discharge has not yet been investigated in Zimbabwe and moreover the major risk is a lack of specific legislation on steroid amounts to be used for androgenisation, such that steroids may be used without regulation, handled incorrectly and applied without the necessary care if operators have not been properly trained.

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