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Growth Performance Comparison of Sex Reversed *Oreochromis Macrochir* (Boulenger, 1912), *Oreochromis Andersonii* (Castelnau, 1861) and *Oreochromis Niloticus* (Linnaeus, 1758) Juveniles Under Pond Culture

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ABSTRACT: A study was carried out at the National Aquaculture Research and Development Centre, Fish Farm, to evaluate the effect of 17α-Methyltestosterone on the growth performance of fry of *Oreochromis andersonii*, *Oreochromis macrochir* and *Oreochromis niloticus* raised for a period of ninety day. The first 21 days, the fry (0.04g±0.001) across all treatments were fed on commercial feed incorporated with 17α-Methyltestosterone for the purpose of achieving sex reversal to all males and were held in 1m *x* 1m *x* 1.3 m hapas installed in a 400 m^2 semi concrete pond, at a stocking density of 250 fry per hapa and replicated three times in a completely randomized design. There were no significant differences (P>0.05) in the final weight gain among the species of *O. andersonii* (1.8240±0.067g), *O. macrochir* (2.6830±0.3220g) and *O. niloticus* (2.3960±0.2230g). The Body weight gain (g), Specific growth rates (SGR) and Apparent feed conversion ratios didn't significantly differ (P>0.05) among the treatments. There were also no significant differences (P>0.05) in the Final standard lengths (mm) at termination which ranged from 32.485±0.8600 to 37.5330±2.9320 whose stocking mean length ranged from 9.58±0.41 mm to 10.17±0.31 mm. Survival rates (%) ranged from 97.00±0.5774 to 99.00±1.000 and did not significantly differ (P>0.05) among treatments. These results indicate that the effect of 17α-Methyltestosterone on the growth of fry of the three species produce similar result. The present study demonstrates that each species can substitute the other and still produce similar results, hence a farmer having a wider choice.

KEYWORDS: Aquaculture; 17α Methyltestosterone; *Oreochromis species*; Commercial feed; Feed conversion ratio

INTRODUCTION

In aquaculture, tilapia species mature quite early and hence breeds at small sizes making it to be a major problem as it affects their body growth (Loya and Fishelson, 1969). Early maturity poses a threat to the carrying capacity of a cultural facility, such that, there is a serious competition for food and oxygen, which eventually impacts negatively to the growth and development of aquaculture industry (Toguyeni *et al*., 1996). FAO (2009) reported that, all male tilapia (mono-sex) grow twice faster than females and further alluded to the fact that, the weight of recruits may constitute 70% of the total harvest weight at the expense of growth for the targeted species. However, if one has to make a meaningful gain from fish farming, there is need to venture into mono-sex fish production to avoid over recruitment, competition for food and oxygen which will lead to stunting of the original stock, through use of hormones.

Hormones in aquaculture are used for induced breeding for fish that normally fails to reproduce naturally in enclosures and also they are used in achieving single sex reversal (Jensi *et al*., 2016; Olufemi *et al*., 2015). Artificial reproduction sustains the production chain with constant production of fry seeds, while, with sex reversal technique is used when the growth rate are different between the male and female offspring so as to make them even. Hormonal use in aquaculture for sex reversal has significantly proved to increase the growth rate of tilapias (Robles-Basto *et al*., 2011). The use of hormones in fish farming for sex reversal aims at the production of monosex population to increase growth rate, i.e., weight gain, especially when one sex of a species has the capacity to grow bigger and faster than the other sex. The technique to increase fish production based on sexual dimorphism mostly uses estrogens and androgens. Commercially, it is advantageous to rear individuals of the most profitable gender, thereby achieving more uniform lots and controlling undesirable breeding (Singh, 2013; Taranger *et al*., 2010). Several techniques have been employed to produce monosex fish, and these includes; manual sexing (Guerrero, 1982), hybridization (Ambali and Malekano, 2004;Hickling, 1960), chromosome set manipulation (Ambali and Malekano, 2004), and hormonal sex reversal (Pillay, 1990). However, two methods for producing sex reversal monosex fish populations are: (i) direct, by which the fish are treated with hormones for the purpose of developing the desired sex, and (ii) indirect, by which parents or breeders are treated with hormones to obtain neomale (XX♂), neofemale (XY♀, ZZ♀), or supermale (YY♂) populations, and with them getting the entire batch of larvae of the same sex (Piferrer, 2001).

The administration of hormones for sex reversal treatment can be done using systemic (direct injection and silastic implantation), immersion, or dietary supplementation (hormone incorporated in fish feed) (Pandian & Sheela, 1995). Commercially, the most successful treatments use immersion and diet, as both methods reach a large number of fish, while the systemic transfer method is expensive and requires technical ability to be applied on the fish. In the immersion technique, the dose administered not only affects the efficiency of hormonal treatments but also other parameters, such as the type of hormone, water temperature and exposure time. The addition of hormones in the feed is more efficient because it is easily controlled, and allows optimum steroid dose to induce the complete sex reversal of all individuals (Pandian & Sheela, 1995; Piferrer, 2001). Therefore, the use of hormones in sex reversal and artificial reproduction generally improves the profitability of the fish industry. However, different fish species reacts different in growth rate as a result of hormone induction, hence the need to carry out the research on growth performance and survival on sex reversed among the three species, for recommendation to fish farmers.

MATERIALS AND METHODS

Study area

The study was carried out at National Aquaculture Research and Development Centre (NARDC) (12°49'0" South and 28°12'0" East), in Kitwe district of Zambia. The study centred on three species of fish, *Oreochromis andersonii* and *Oreochromis macrochir* brooders where collected from within NARDC breeding ponds, whilst *Oreochromis niloticus* brooders where sourced from a farmer within Kitwe district. The brooders of each species were conditioned by sex separation for three weeks, then later brought together to breed at sex ratio of 1:1. The brooders were used to brood the fish for sex reversal. Fry at egg York stage where collected from the mothers' mouth and placed in incubation trays for them to finish up the egg York before administering the sex reversal hormone incorporated in feeds for a period of 21 days.

Sex-reversed Feed formulation

The hormone (60mg 17α-methyl testosterone/kg) was used to mix in the feed (Jensi *et al*., 2016; Kef *et al*., 2012). A stock solution was prepared by dissolving 6g of 17 α Methyl testosterone (MT) in 1 litre of >95% ethanol (Kefi *et al*. 2012). Then 10mls of the stock solution was further diluted with 60mls of >95% ethanol which was then used to mix with 1kg of a commercial feed with crude protein levels of 45%. The feed was then placed under a shade for a day to dry up and later stored at room temperature.

Pond and hapa preparations

The study was undertaken in semi-concrete pond measuring 400m^2, which was drained completely and allowed to dry for three weeks, in order to eliminate some microorganisms, removal of excess silt from the bottom and installation of hapas. The hapas, were made of a nylon material and sown into 2m *x* 1m *x* 1.5m, and set at 1m between the rows and 2m apart. Sticks were cut and drilled into the pond bottom for anchoring the hapa

Feeding of brood stock fish and the fry

During the conditioning period, the brood fish were fed a commercial fish feed pellets at 5% of their total body weight containing 32% crude protein with a feeding regime of 2 times per day by broadcasting in the cage (De Silva and Anderson, 1995). While, the fry were fed a commercial feed mash containing 38% crude protein for both 17 α Methyltestosterone (MT) incorporated feed and non-incorporated feed, and were fed four times per day at 10% body weight, through broadcasting on the water surface in the hapas (De Silva and Anderson, 1995).

Fry stocking

Fry of average weight (0.04±0.0913) in all the three treatments were subjected to a commercial feed incorporated with17 α Methyltestosterone. After 21 days of hormonal administration, ninety fry from each species (Treatment) weighting approximately 0.2±0.1621grams were placed in the hapas (Treatments) and replicated three times and feeding continued with non-incorporated 17 α Methyltestosterone. Sampling consisted of 10% from each treatment.

Experimental layout and data collection

The experiment was laid in a Completely Randomized Design (CRB) consisting of three treatments and replicated three times. Treatment 1 was *Oreochromis andersonii*, Treatment 2 was *Oreochromis macrochir* and Treatment 3 was *Oreochromis niloticus*. The treatments were randomly assigned to the hapas in the pond. Nine hapas measuring 2m x 1m x 1.5m depth were used during the experimental study. The fry of each species where then evaluated for growth indices, AFCR and survival for the period of ninety (90) days, sampled every two weeks. The initial body weight (g) of the fry where measured using a volumetric method for the first sampling of the juvenile fish. Then later in subsequent sampling a digital scale was used to measure individual weight (g). The standard length (mm) measurements were taken using a vernier calliper and a total of 6 samplings were done.

DATA ANALYSIS

Analysis of Variance (ANOVA) was performed to test the hypothesis for any significant differences at alpha level (0.05). Data computation and analysis was performed using Excel, R version 3.5.1. (R Core Team, 2018) and Scientific Package for Social Scientist (SPSS), using a Completely Randomized Design using the following statistical model:

$Y_{ij}=\mu+\tau_i+\varepsilon_{ij}$

Where:

Y_{ij} = j^{th} observation on i^{th} treatment

μ = overall mean

τ_i = effect of i^{th} treatment

ε_{ij} = error associated with the j^{th} on i^{th} treatment

In this study, the growth rate was determined on Final body weight (FBW) (g), Body weight gain (BWG) (g), Specific growth rate (SGR % day^{-1}), Survival rate (%) and AFCR were determined using the following formulas;

Final bodyweight:

$$\mathrm{FBW(g)} = \mathrm{Final\ \ wt(g)} - \mathrm{Initial\ \ wt(g)}$$

Body weight gain:

$$\mathrm{BWG(\%)} = [\frac{\{\mathrm{F.wt(g)}-\mathrm{Int.wt(g)}\}}{\mathrm{Int.wt(g)}}]\mathrm{X}100$$

Specific growth rate:

$$\mathrm{SGR(\%)} = [\frac{\mathrm{Ln(F.wt)} - \mathrm{Ln\ (int.wt)}}{\mathrm{time(days)}}]\ \mathrm{X}100$$

Survival rate:

$$\frac{\text{Number of fish at the end of experiment}}{\text{Number of fish at start}}\mathrm{X}\ 100$$

Apparent Feed Conversion Ratio (AFCR):

$$\mathrm{AFCR} = \frac{\text{Total feed administered (g)}}{\text{weight gained (g)}}$$

Water parameters determination

Water quality parameters were recorded both before stocking the fry into the hapa and during the experimental period, specifically observing temperature, dissolved oxygen and pH, at 7am and 2pm the same times of the day using standard water sampling protocols (Geypens *et al*. 2012). This was done to ensure that water quality is maintained at levels that could not compromise growth of the fish (Cline, 2019).

RESULTS

There were no significant differences (*P>0.05*) between the growths (weight gain) of sex reversed fingerlings treated with 17 α Methyltestosterone for 21 days and later fed on a commercial feed for a period of 60 days.

Results of growth of sex reversed juveniles of *Oreochromis andersonii*, *Oreochromis macrochir and Oreochromis niloticus* are presented in

Table 1

Table 1. Growth performance parameters of *Oreochromis andersonii*, *Oreochromis macrochir* and *Oreochromis niloticus* sex reversed fry (Mean ± SE)

Parameter	*Oreochromis andersonii*	*Oreochromis macrochir*	*Oreochromis niloticus*
IW (g)	0.04±0.1612	0.04±0.152	0.04±0.091
FBW (g)	1.8240 ± 0.067	2.6830±0.322	2.3960±0.223
BWG (g)	1.784±0.0670	2.643±0.322	2.356±0.223
FSL (mm)	32.485±0.8600	37.5330±2.932	36.137±0.645
SGR (%/day^{-1})	1.8641 ±0.0669	2.7233±0.322	2.4356±0.223
AFCR	1.7421±0.8238	1.1228±0.185	1.2574±0.132
Survival (%)	97.00±0.57735	97.67±0.667	99.00±1.000

Means values across the rows are not significantly different (P>0.05)
Note: IW – Initial weight; FBW – Final body weight; BWG – Body weight gain; FSL – Final standard length; SGR – Specific growth rate; AFCR – Apparent feed conversion ratio; SR – Survival.
The final body weight (g) highest was recorded in *Oreochromis macrochir* (2.6830±0.3220) and seconded by *Oreochromis andersonii* (2.3960±0.2230) and lastly in *Oreochromis andersonii*, though there was no significant difference. Other growth parameters, AFCR and survival were also not significantly different among the three fish species (Table 1).

Water quality parameters

Water quality parameters measured during the experimental periods were within the range for growth of the Oreochromis species in ponds (Table 2).

Table 2. Water quality parameters measured in the treatment pond stocked with *Oreochromis andersonii*, *Oreochromis macrochir* and *Oreochromis niloticus*

Parameter	Morning	Afternoon
Temp (ºC)	24.85 ± 0.11	26.70 ± 0.15
Oxygen (mg/L)	2.84 ± 0.02	3.87 ± 0.02
pH	7.12 ± 0.10	8.60 ± 0.08
Turbidity NTU	106.10 ± 2.89	112.2 ± 1.46
Ammonia (NH3, mg/L)	0.010 ± 0.06	0.010 ± 0.08
Alkalinity (mg/L)	98.20 ± 0.91	97.4 ± 0.80

During the experimental period, the average water temperature ranged from 24.85 to 26.70ºC in the experimental pond. Mean dissolved oxygen (DO) ranged from 2.82 to 3.87mg/L. The pH ranged from 7.12 to 8.60. All water parameter recordings were within the desired range for culture of Oreochromis species (Table 2).

DISCUSSION

The growth parameters (FBW, BWG, SGR and FSL) did not differ significantly after 40days of been weaned from 17 α Methyltestosterone, and this results are similar with Kefi *et al*. (2012) on *Oreochromis andersonii* after 30days of been weaned from the treatment of 60mg/kg of 17 α Methyltestosterone. Nagaraju & Devi (2020) observed that 17 α Methyltestosterone improves the growth performance of *Oreochromis mossambicus,* similar to what this study found. SGR in this study was also in the same range with those reported by Kikamba and Kang'ombe (2022) for *Coptodon rendalli* (1.87% day^{-1}) raised in ponds fertilized with chicken manure and fed on maize bran, *Amaranthus hybridus* and a combination of maize brand and *Amaranthus hybridus*. Survival of the fish was generally high in all the treatments and did not differ significantly (*P>0.05*) from each other. The good survival rates recorded can be attributed to acceptability of the hormonal treated feed by the fish. This is in tandem with studies conducted by Jensi et *al*. (2016), where the authors determined effect of 17 α Methyltestosterone on sex reversal and growth of Nile tilapia. This means that Oreochromis species under this study can thrive well on 17 α Methyltestosterone treated feed as well as on a commercial feed. The survival rates in this study are similar with what Nsonge (2014) reported for *Oreochromis macrochir* cultured in cages. The feed conversion ratio (FCR) recorded was similar in range with what was recorded by Nsonga (2014) for *Oreochromis macrochir* (1.56 day^{-1}). In this study, mean temperatures (24.85 ºC to 26.70 ºC) were within the recommended range for Tilapias good growth and better yield (Lucas and Southgate, 2003). Josiah *et al*. (2014) also observed and concluded from their study that, the optimum range for growth and good food conversion for tilapia fish was between 21- 28ºC. However, the levels of DO were below the recommended 5.0 mg L^{-1}, however, this did not affect the growth of the fish as tilapias are known to strive in low oxygen levels (Siddiqui, *et al*., 1989) hence, it was within the desirable limits for tilapia species (Ridha and Cruz, 2000). The pH ranged from 7.12 – 8.60 in the holding pond, and this pH range in this study was within the recommended range of between 6.7 and 9.5 according to Santhosh and Singh (2007) and, Kikamba and Kang'ombe (2022). Bryan *et al*. (2011) also observed that, most fish species do better in pH of between 6.0 - 7.0 and levels outside this would result in stunting and poor fish production.

In this study the ammonia levels were low due to been utilized by algae and other aquatic plants as a nitrogenous source for their protein synthesis. Most studies have observed that ammonia in earthen ponds, is not much of a problem since it is quickly used up by phytoplankton and aquatic macrophysics (Knud-Hansen, 1998). Levels of un-ionized ammonia (NH3) around 1.0 mg L^{-1} are considered toxic to fish and ionized ammonia (NH4+) values ranging from 0.2 to 2 mg L^{-1} are considered favorable for fresh water fish (Barker *et al*., 2003). In this study, NH3 was ideal for propagation of plankton and growth of fish, and was within the range as reported by (Brummett, 2000). However, total alkalinity in this study was within the recommended range of 50 to 300 mg CaCO3 L^{-1} (Boyd *et al.*, 2016). Normally alkalinity gets used up by snails as they incorporate it in the shell formation as carbonates (Knud-Hansen, 1998).

CONCLUSION

As indicated by this research, there were no significant differences in the overall performance of Oreochromis species fingerlings treated with 17MT and later fed on a commercial feed. This has demonstrated that sex reversed fingerlings of the three species performs the same in growth when treated with 17 α Methyltestosterone. The use of 17 α Methyltestosterone has the same growth effect on *Oreochromis andersonii*, *Oreochromis macrochir* and *Oreochromis niloticus* meaning that, these three species can substitute each other and still give the same growth performance and therefore, farmers have a wider choice to choose from on what is available to them.

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