



## THE EFFECT OF GRADED LEVELS OF RAM TESTES ON SEX, GROWTH AND SURVIVAL OF *Oreochromis niloticus* (LINNAEUS, 1856)

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### ABSTRACT

The study investigated the effects of homogenized ram testes using shrimp meal as carrier on the phenotypic sex, survival and growth of *Oreochromis niloticus*. To determine the phenotypic males, specific growth rate and survival rate, 4 different ratios of shrimp to ram testes 1:0 (T0), 1:1 (T1), 1:2 (T2) and 1:3 (T3) were fed to 7 days-old fry for the period of 28 days in plastic tanks (45 cm diameter x 30 cm deep) indoors. The results showed that the highest (82.0 %) numbers of males were recorded in fry treated with 1:3 shrimp to ram testes, followed by those treated with 1:2 shrimp to ram testes. Significant variation ( $p < 0.05$ ) was observed between the number of males in fry treated with 1:3 shrimp to testes and those fed 1:2 of shrimp to ram testes. Survival was higher (92.3 %) in the 1:0 (control) shrimp to ram testes. However, fry treated with higher dosage of the ram testes were heavier (66.0g). Feeding 7 days old fry with ram testes at the ratio of 1:2 to 1:3 testes and fish diet could reduce the number of females in the cultured population thereby decreasing the precocious reproduction in fish ponds. Ruminant testes are cheap and readily available, hence could be utilized for the control of precocious reproduction in tilapia.

**Keywords:** Ram testes, Sex ratio, growth and survival, *Oreochromis niloticus*

### INTRODUCTION

Tilapia farming is now the most widespread type of aquaculture in the world with global production reaching about 4 million tons in 2011 (FAO, 2014). The impetus for this growth in tilapia culture are based on the many attributes of the species including tolerance to wide ranges of environmental conditions such as high water temperature, low dissolved oxygen and high ammonia concentration (Avault, 1996). Tilapia culture also thrives on low input cost because the animals can subsist on a wide variety of natural foods as well as artificial feeds or even without fishmeal (Gee and Singh, 2005; Khan *et al.*, 2008; FAO 2014). They are hardy, adapting to wide ranges of salinity because of their marine ancestry and reproduce quickly in captivity, making them one of the most widely cultured fresh water fish in tropical and sub-tropical countries (Kirk, 1972; Gee and Singh, 2005).

Tilapia has been given the names “aquatic chicken” and “fish of the 21<sup>st</sup> century” (Ramnarine, 2005), fish of the 1990’s (Costa-Pierce and Rakocy, 2000) because of its worldwide acceptability, versatility and palatability and potentials to revolutionize aquaculture the same way chicken did to traditional agriculture (Maclean, 1984;

Perschbacher, 2014). These potentials have not been realized given that *O. niloticus* reproduces at 3 months of age, filling ponds with small unattractive “baby” fish (Perschbacher, 2014), especially in Africa. However, from the most cultured farmed species in Nigeria from the 1970’s until recently, tilapia is now relegated to second position. As catfish production increased in Nigeria, tilapia production decreased. Catfish represents 70-80% of all farmed fish in Nigeria (Ponzoni and Nguyen, 2008) and she is the leading producer in Africa. Tilapia is highly preferred by large segment of the Nigerian population. Nigeria will play a significant interregional role in food security in West Africa by stepping up production of tilapia. To reverse this trend and encourage tilapia culture, cheap technology for production of fast growing fish must be developed.

Sexual dimorphism in fish is of great importance in aquaculture where there are differences in growth rate, size, behavior patterns, and breeding time between males and females (Manosroi *et al.*, 2004). Since sexual dimorphism for growth occurs in most farmed fish, techniques for the production of the fast growing sex will increase production (Dunham, 1990). Male tilapia exhibit faster and more uniform

growth rates than the females (Guerrero and Guerrero, 2001; Soto-zarazua *et al.*, 2001; Hines and Watts, 1995). This phenomenon has over the past several decades been exploited to manipulate sex and produce all-male tilapia to increase production. Several authors have reported different techniques to produce all-male Tilapia. These include Jo *et al.* (1988), Pandian and Varadaraj (1988), Mubarik (2011), amongst others. According to Afonso *et al.* (2001) steroid hormones such as androgen and estrogen administered before gonads are developed can skew sex to males or females in non-mammalian vertebrates. The synthetic steroids 17 $\alpha$ -methyltestosterone (MT) is a very effective and popular male specific hormone commonly used to induce sex reversal in teleost (Hunter and Donaldson, 1983), and it is thought to promote both muscle growth and the development of male sexual characters.

The main challenges of its use in Nigeria include technical considerations and high costs because it has to be imported. Other implications of use of these synthetic substances on the environment and man are not well understood; however, there are concerns of negative environmental and health impacts. As a result, there are strong campaigns by environmental advocates against the use synthetic hormones in fish production and some developed countries require that farmed fish be certified and labeled as free. In the United States of America, for example, Penman and McAndrew (2000) reported that the use of methyltestosterone for sex reversal of food fish must be licensed by the U.S. Food and Drug Administration and in Europe it is prohibited.

The cost of production and perceived environmental effects can be reduced substantially by sourcing and utilizing locally available resources as replacement or substitute for synthetic hormones. Thus, steroids active materials derived from animals or plants substances are promoted as possible substitutes for 17 $\alpha$ -methyltestosterone. Ruminant testes are androgen-rich and can be easily and cheaply obtained by resourceful poor fish farmers. The objective of this study therefore was to investigate the efficiency of dietary ram testes on sex ratio, growth and survival of *Oreochromis niloticus*.

## MATERIALS AND METHODS

This study was conducted in the fish hatchery complex of the Department of Fisheries, University of Maiduguri. Maiduguri is located in the Sahel Savannah region of North eastern Nigeria located at latitude 11 ° 05' North and longitude 13° 05' East (BSBLS, 2004).

## Experimental fish

Matured brood stock of *Oreochromis niloticus* (150 to 200 g) were captured from Lake Alau using cast nets. They were acclimatized for three weeks before the commencement of the experiment in 2 x 2 x 1.2 m concrete tanks outdoors. During acclimatization, they were fed with 30 % crude protein diet. To obtain fry for the experiment, the broodstock were stocked at a ratio of one male to three females (1:3) in a net hapa of (1.5 m x 1.5 m x 1.2 m) fitted into polyethylene lined fish pond (7 m x 5 m x 1.2 m deep). The fish were fed with a 30% crude protein diet twice daily (9: 00 am and 4:00 pm) at 5% of their body weight. The broodstock were closely monitored for spawning until the eggs hatched.

## Collection and preparation of the ram testes

Dried shrimps were obtained from a local market in Maiduguri. The shrimps were re-dried, and ground into fine powder using manual grinding machine. The shrimp powder was used as the carrier of the ram testes. Fresh ram testes were procured from an abattoir in Maiduguri and transported to the hatchery complex in a dark polyethylene bag placed in an ice box. The fresh testes were skinned and freed from epididymis, sliced, weighed and completely homogenized using a Countertop Blender and kept in a refrigerator until required.

## Experimental design

The homogenized testes were mixed with the shrimp at the ratio of T0, T1, T2 and T3 representing 1:0, 1:1, 1:2 and 1:3 of shrimp to testes, respectively. T0 was the control, containing zero testes meal. These were thoroughly mixed and each spread on stainless plates and oven dried at 70 °C for 9 hours. The resultant crumbs were pulverized and sieved with 0.2 mm sieve separately and kept in opaque plastic containers stored at room temperature. Two hundred (200) 7 days old *O. niloticus* fry were stocked in twelve 50-litre capacity plastic basins (45 cm diameter x 35 cm deep) filled with 30 litres of water indoors, making a total of 4800 fry in a complete randomized design (CRD) manner. The *O. niloticus* fry were fed to satiation with various levels of the ram testes-shrimp for a period of 28 days. After the 28 days of feeding with ram testes, the fish were transferred to 2.0 m<sup>2</sup> outdoor concrete tanks partitioned into four and reared on 1.0 mm Coppen's feed for 4 weeks. Final weights and length were recorded.

### Assessment of the sex ratio

After 4 weeks of outdoor rearing, fingerlings from each treatment were sexed manually with the aid of Gentian Violet (GV). Cotton wool was dipped into the GV and applied on the vent region to expose the two openings for female and one opening for male. Fish of 15-20 gm were conveniently sexed. The numbers of male and female fish from each treatment were recorded.

### Histological examination of gonads

Fry identified through manual sexing from each treatment were collected at random, dissected and the gonads extracted. The gonads were fixed in 10 % normal saline solution, rinsed in distilled water and then transferred into different concentrations of alcohol first in ascending order and subsequently in descending order of 35, 50, 70 and 95 %. Blocks of tissues were cut using microtome and the sectioned microtome was then stained with hematoxylin-Eosin for 5-10 minutes. This was observed under the light microscope to confirm the sex and the characteristics of the gonads. The presence of ovarian or testicular structures was used to determine sex.

### Statistical analysis

The data obtained from the experiment were subject to one-way analysis of variance (ANOVA).

Differences between the means were determined using LSD at 95.5 confidence level ( $p \leq 0.05$ ).

### RESULTS

Table 1 shows the indoor water quality parameters during the study. The temperature ranged between 25.5 °C and 26.3 °C. The highest mean temperature ( $26.3 \pm 0.48$ ) was recorded in tanks containing fry fed with T0, while fry in T3 showed the lowest mean temperature ( $25.5 \pm 0.12$ ). The pH ranged between 7.7 and 8.1 with the highest pH observed in the control.

The range between 5.0 and 5.2 mg/l was observed for dissolved oxygen with the highest recorded in tank T2. However, no significant variation ( $p > 0.05$ ) was observed in the water quality parameters.

### Sex Expression

Table 2 shows the effect of ram testes on sex, growth and survival of *O. niloticus* fed graded levels of ram testes. The number of phenotypic males increased with elevated levels of ram testes. Male population were significantly ( $p < 0.05$ ) higher (82.0 %) in fry fed T3 compared to other levels. The lowest ( $45.0 \pm 1.5$  %) number of males was recorded in the control (T1), which differed significantly from other levels ( $p < 0.05$ ).

**Table 1: Water quality parameters (mean  $\pm$  SEM) during the indoor experiment**

Shrimp : Ram testes	Temperature °C	pH	Dissolved Oxygen (mg/l)
T0	$26.0 \pm 0.58^a$	$8.1 \pm 0.26^a$	$5.0 \pm 0.58^a$
T1	$26.3 \pm 0.48^a$	$7.8 \pm 0.42^a$	$5.1 \pm 0.08^a$
T2	$26.0 \pm 0.30^a$	$7.7 \pm 0.33^a$	$5.2 \pm 0.08^a$
T3	$25.5 \pm 0.12^a$	$7.9 \pm 0.12^a$	$5.0 \pm 0.03^a$

Means followed by the same super script in a row are not significantly different ( $p < 0.05$ ) at 5% level of significance

**Table 2: The effect of ram testes on reproduction control in *Oreochromis niloticus***

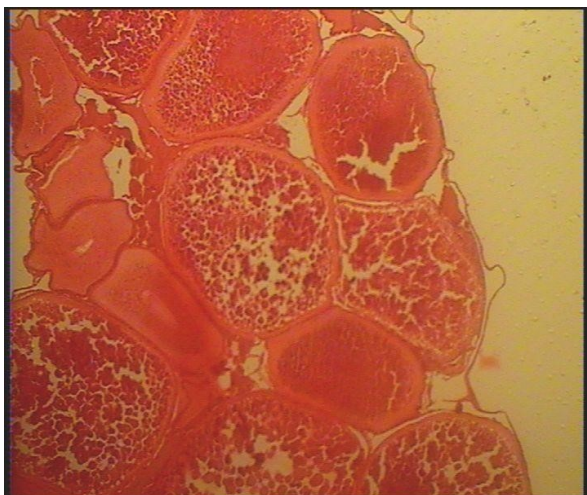
Parameters	Testes inclusion levels (ram testes to shrimp)			
	T0	T1	T2	T3
Initial Length (mm)	$2.3 \pm 0.12^b$	$2.40 \pm 0.38^b$	$2.6 \pm 0.03^b$	$3.2 \pm 0.5^a$
Initial Weight (mg)	$25.0 \pm 0.50^b$	$26.0 \pm 0.05^a$	$25.0 \pm 0.05^b$	$25.0 \pm 0.05^b$
Males (%)	$45.0 \pm 1.5^d$	$67.0 \pm 1.15^c$	$73.0 \pm 1.73^b$	$82.0 \pm 0.3^a$
Females (%)	$55.0 \pm 1.13^a$	$33.0 \pm 0.38^b$	$23.0 \pm 4.91^b$	$18.0 \pm 0.58^c$
Survival (%)	$88.6 \pm 0.33^b$	$92.3 \pm 1.20^a$	$82.3 \pm 0.33^c$	$80.0 \pm 0.38^c$
Final Length (mm)	$4.1 \pm 0.88^a$	$4.1 \pm 0.08^a$	$4.6 \pm 0.88^a$	$4.4 \pm 0.48^a$
Final Weight (mg)	$50.0 \pm 0.30^d$	$51.0 \pm 0.05^c$	$53.0 \pm 0.05^b$	$66.0 \pm 0.15^a$

Means followed by the same letter in a row are not significantly different ( $p > 0.05$ )

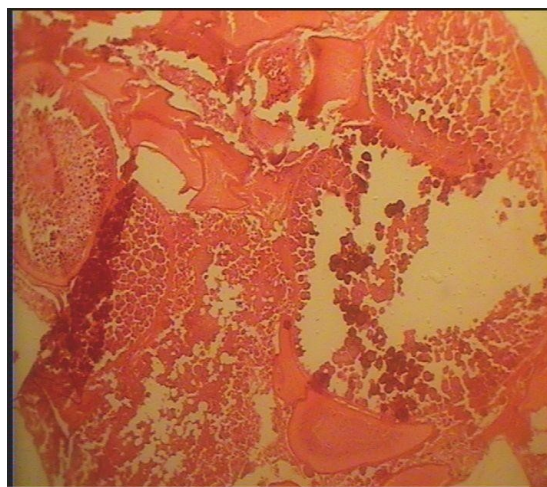
Plates 1-4 show the results of the histological analysis, which validated the manual sexing.

Plate 1: Histology of gonads of 52 day old *Oreochromis niloticus* fed only shrimp (control) showing normal developing ovary with polygonal shaped oocyte, while plate 2 shows mild degenerated oocyte in females treated with 1:1 shrimp to ram

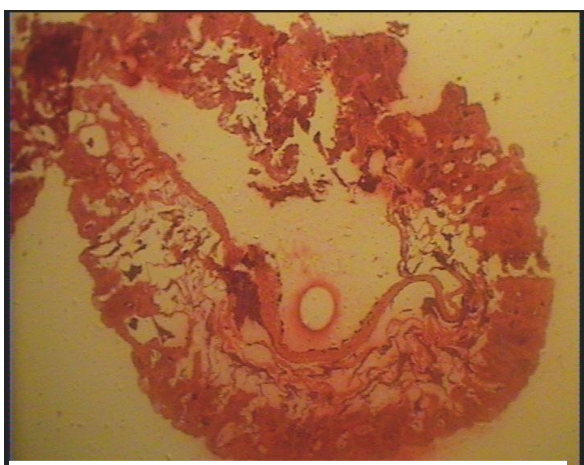
testes. The effect on ovary for other levels of inclusion of ram testis was similar to those of plate 2. Plates 3 and 4 show histology of testes of fry fed with T2 and T3, respectively had severe degenerated and disorganized tubular elements with increased intercellular space. The intercellular space indicates absence of developing organs.



**Plate 1: Histology of 52 days female fry fed the control diet (shrimp only)**



**Plate 2: Histology of *Oreochromis niloticus* fry fed shrimp- ram testes**



**Plate 3: Histology of *Oreochromis niloticus* fry 1:2 shrimp - ram testes**



**Plate 4: Histology of *Oreochromis niloticus* fry 1:3 shrimp - ram testes**

### Survival

Fry survival at the end of the experiment was higher (92%) in T1, followed by 88.6 % in those fed T0. There was significant variation ( $p < 0.05$ )

between the survival rates of T1 fry compared to the other treatments.

The weight of fingerlings after rearing on a normal diet outdoor increased with increase in the

level of testes administered to the fish. Fry exposed to higher levels (T3) had significantly heavier weight ( $< 0.05$ ) compared to other treatments.

The length of fish, however, was not significantly different from each other at the end of the experiment ( $P > 0.5$ ).

## DISCUSSION

The temperature, pH and Dissolved Oxygen recorded during this study fall within the acceptable ranges for fish culture recommended by Viveen *et al.* (1985) and for rearing tilapia (Popma and Masser, 1999). There was no deterioration in water quality during the experimental period. Phelps and Popma (2000) suggested that dissolved oxygen concentration must always be more than 4 mg/l to facilitate a strong feeding response.

## Sex Expression

The number of males recorded in T3 was higher than 80.67, 73.0 and 73.0 % males reported by Odin and Bolivar (2012) after treating *O. niloticus* with lyophilized testes of Carabao (*Babalos bubalis carabensis* L.), Bull (*Bos indicus* L.) and Boar (*Sus domesticus* L.), respectively. The differences could be due to the variation in species of animal from which the testes were obtained, testosterone level of the testes and the processing method. The lyophilized testes might have been affected by low temperature during freeze drying. Fashina-Bombata and Somotun (2008) reported some level of success in achieving phenotypic maleness of Tilapia (*Wesafu*) after feeding with the lyophilized ram testes as a starter feed, though they did not indicate the levels of the success. Phelp *et al.* (1996) reported lower number (65 %) of male population after oral treatment of *O. niloticus* with 57 % freeze dried bull testes. However, the number of males recorded in this study was lower than 85 % males reported by Haylor and Pascual (1991) after feeding *O. niloticus* with ram testes. It is possible that increasing the level of ram testes or feeding 100 % ram could totally skew the sex of populations towards all- maleness. This study, however, compares favourably with other results using synthetic and phyto-rich androgens. For example, Wafaa *et al.* (2011) revealed that *O. niloticus* treated with 100 and 200 mg of the chemical Fadrozole/kg of feed for 14 to 28 days recorded 95 and 98 % percentage male population, respectively. Ghosal and Chakraborty (2014) reported 70.3 % males after immersion of 3 days old mixed sex juveniles of Nile tilapia in 0.1 g/l aqueous leaf extract of *Basella alba*. Chukwu *et al.* (2012) also reported 92.86 % males after treating *O. niloticus* fry with 6

g/kg of *Carica papaya* seed meal per kilogram of feed.

The gonadal histology is similar to those of other studies and teleosts. Similar structure was also observed by Chukwu *et al.* (2012) after treating *O. niloticus* with 8 g of pawpaw seed meal/kg of feed 30 days.

## Survival

Fashina-Bombata and Somotun (2008) observed higher survival rate in the control when lyophilized goat testes meal was fed to “wesafu” tilapia. Popma (1998) reported reduced survival of tilapia fed with goat testes meal. The survival of fry during sex reversal are dependent on factors such as stocking density, feeding rate, temperature and other environmental conditions Bocek *et al.* (1992), type, form and sources of hormone or androgen (plant, animal or synthetic hormone) and the dosages.

Several studies have shown that both mammalian and non-mammalian testes promote growth when included in the diet of fish. Specifically, Suleiman *et al.* (2013) reported progressive improvement in growth rate of Nile tilapia (*O. niloticus*) fed diets containing graded levels of dried bull testicles. Lee *et al.* (2013) also found the testes of the fish, pink salmon as effective growth promoter in the Nile tilapia when used as partial replacement for fish meal. The reason for this was that androgens accentuates masculinization by increasing protein synthesis and improves growth by improving feed and protein efficiency ratio.

Therefore, mammalian testes may have triple positive action on tilapia: sex reversal, anabolism and growth. Farmed fish is sold in terms of weight and not length (Lawson and Ishola, 2010); therefore, fish growth and their consequent increase in biomass are basic breeding goals and therefore, of major interest to the fish farmers (Olele and Tighiri, 2013). Endogenous testosterone is produced by the testicles in rams. Testosterone is important in building muscles and weight. The differences in weight could be due to the high protein and testosterone in the trial containing 1:3 levels of ram testes.

## CONCLUSION

In conclusion, the potential adverse effects of synthetic steroids used in sex inversion, favours research on the utilization of environmental-friendly organic or natural substances to produce monosex populations in tilapia and other aquaculture species. Testosterone rich ram testes incorporated in shrimp meal have skewed sex differentiation of the ovary in Nile tilapia species towards testes.

It is likely that supplementation of fry diet with ram testes may stimulate growth and increase profit in tilapia culture.

There is, therefore, the need to launch more investigations into the effects and mechanisms of the action of livestock testes on growth of farmed fish. Standard procedures for processing the animal testes must be developed to encourage their use and expand and intensify the dwindling tilapia aquaculture in Africa.

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