CULTURE OF CATFISH (*Clarias gariepinus*) FED WITH DIET CONTAINING 17-ALPHA METHYL TESTOSTERONE

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ABSTRACT
The study examines the sex-reversed population of Clarias gariepinus after its fry were fed with 17 alpha-methyl testosterone. Broodstock of Clarias gariepinus which consists of three females and one male were collected and used for fry production. The volumetric method was used in the stocking of 200fry into four experimental units; (T1, T2, T3, and Control) in triplicates. The different experimental units T1, T2, and T3 were fed with feed containing 0.03, 0.035, and 0.04g of 17 alpha-methyl testosterone, and the control was devoid of the hormone. The fry was fed with the experimental diet for 30 days. The gonadal analysis was carried out after 51 days of culture. Results showed that the mean survival rate in Clarias gariepinus varied in the different units. T1 (65%), T2 (70%) T3 (75%) and control (85%). Experimental unit T3 (0.04g) had the highest number of sex-reversed males at 43(86%) while the control has the lowest with 23(46%). The lowest quantity of the hormone that produces sex-reversed males was in T1 (0.03g). Sex reversed fishes with a good survival rate were produced in the experimental unit treated with hormone while the controls produce a relatively small number of male fish.

Keywords: Totipotent, Testosterone volumetric, Sex reversed

INTRODUCTION

Fish is an important and cheapest source of animal protein (Goni *et al*., 2020). Fish provides approximately 16% of the animal protein consumed by the world population (Kumar *et al*., 2015: Béné *et al*., 2015). It is particularly a major source of animal protein and an essential food item in the diet of many people in Nigeria (Adeyeye and Oyewole 2016). Fish is additionally an important source of Thiamine, Riboflavin, Vitamin A and D, Phosphorus, Calcium, and Iron. Zárate *et al*., (2016) believe that fish has a proportion of very high polyunsaturated fatty acids contents which are important in lowering blood cholesterol levels. Hence, we can infer that Fish is suitable for complementing high carbohydrate diets typical of the low-income group in Nigeria (Hammed *et al*., 2019).

The rapid growth of Nigeria's population has led to insufficiency in the supply of animal protein sources of food. Consequently, it is paramount to increase efforts that are made towards increasing animal production (Lugert *et al*., 2014). Farming of Fish is now a general practice across the globe in recent years. The state of captured fisheries has been on the decline in recent times indicating the need to increase aquaculture. Overfishing has damaged most fishing grounds because of apathy and pollution (World Bank, 2005). Aquaculture, therefore, remains the only viable alternative for increasing fish production to meet the protein demand of people (Nwachi and Yuzine 2016). Aquaculture unlike capture fisheries requires human intervention in increasing productivity and yields that exceed those from the natural environment (Mustafa *et al*. 2017).

Aquaculture genetics shows immense potential for enhancing production in a way that meets aquaculture developmental goals for the new millennium. The application of these genetic tools is known to have increase fish production (Nwachi and Dasuki 2017; Akinwande et *al*., 2009). The use of 17α-Methyl Testosterone (MT) in fish farming dates from the late 1960s when various hormones and treatment methods were experimented with the mandate to produce single-sex stocks (Jensi *et al.*, 2016; Ritesh *et al.*, 2019). Hormone treatment does not alter the genotype of the fish but it affects the phenotype that is expressed. Researchers like Rima *et al*. (2015) Olufeagba *et al*., (2017) consider the production of an all-male population through the administration of 17-α methyltestosterone. They infer that it is the most effective and economically feasible method for obtaining all-male fish populations. Feeding small amounts of 17-α methyl testosterone-treated feed to fry before sexual differentiation, results in virtually all the treated fish developing into males morphologically. The potential of the stock to breed is thereby eliminated. This form of sex control has the added benefit that male fishes generally grow faster than females, with a result that all male fishes are larger and more uniform in size than mixed-sex fish (Hossain *et al*., 2005). The larger size and greater uniformity of MT treated fish make them highly suited for consumers, especially to supply the fast-growing demand for fish. Energy expenditure on male-male and male-female behavioral interactions and gamete production are also minimized, thereby maximizing growth potential. Production of monosex fish ensures uniformity of size at harvest because they do not waste energy in gonadal development (Kim *et al.*, 2020). Hence this study examined the optimal quantity of the hormone that is required to transform female stock to an all-male population, determine the effect of the hormone on survival and growth.

MATERIALS AND METHODS

Experimental site

This research was carried out at the Department of Fisheries, Delta State University, Abraka located in Oshimili South Local Government Area of Delta State. This study was carried out for a period of three months, from November 2018 to January 2019.

Collection of broodstock

A total of four broodstock consisting of one male and three females were collected from Adaeze fish farm, Asaba, and transported to the Wet Laboratory of the Department of Fisheries and Aquaculture, Delta State University, Asaba. At the farm mature female broodstock was ascertain by a mild press on the abdomen with thumb towards the vent, thinly separated greenish and brownish eggs were seen oozing out freely from the vent of the reproductive organ and the male papilla was reddish indicating that they were gravid (Onyia *et al*. 2019). They were disinfected with concentrated common salt solution (30g/L) and brought into the hatchery.

Hormonal administration

The number of hormones used was commensurate with the weight of the female fish injected. The hormone was administered at 0.5ml per kg of female fish. The hormone was injected into the dorsal muscle of the female broodstocks at an angle of 45^o. A total of 8 hours latency period was observed.

Artificial fertilization

The milt was collected by sacrificing the male. The two lobes of the male's testes were removed, cleaned with tissue paper, and kept in a cleaned petri dish. The abdomen of the female was well cleaned with tissue paper to avoid contact between the eggs and water. Then the females were stripped of their eggs by a mild application of pressure on the abdomen to release the eggs. The eggs were collected in a dry, well-cleaned plastic bowl. Milt solution was prepared from the gonad of the male after which the milt was used in fertilizing the already stripped eggs. Fertilized eggs were incubated for up to 24 to 48 hours, after which the fish fry was produced. The fertilized egg masses were incubated in the spawning tanks for a period of 24 to 40 h. After this period, most of the larvae surfaced. The percentage hatching rate was estimated at 40 h after fertilization.

Hormonal Feed Preparation

The 17-alpha methyltestosterone was prepared by diluting in absolute alcohol and the mixture was added into a shell-free artemia at 0.03, 0.035, and 0.04g. The mixture was dried in shade and was used to feed the fish at the totipotent level for 30 days.

Experimental setup

A total of 12 bowls was used for raising the fry, the volumetric method was used to count 200 fry. The fry was stocked in triplicates in one experimental unit representing T1 (0.03g), T2 (0.035g), T3 (0.04g), and control that was not fed with the hormone-treated feed.

Gonad Examination

Gonadal histology was done to determine the percentage of sex ratio.

Survival rate

$$= \frac{No. of hatchlings\ alive\ up\ to\ larvae\ stage \times 100}{Total\ no. of\ hatchlings}$$

(Ferosekhan *et al.*, 2018)

Data analysis

The data were analyzed with the aid of SPSS version 22. Data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means at 5 % level of significance.

RESULTS

Water quality parameter

A total of five water parameters were examined in the experimental unit. They were temperature (ºC), Ammonia (mgl^{-l}), Dissolved oxygen (mgl^{-1}), pH, and total dissolved solids (mgl^{-1}). The experimental unit was divided into four and was labeled as T1, T2, T3, and control as shown in Table 1. The temperature in T1 was 27.77 ^{0}C, T2 had 26.78^{0}C, T3 27.28^{0}C, and control 26.87^{0}C It was observed that T1 the highest temperature at (27.77^{0}C) while the lowest temperature was observed at T2 (26.78^{0}C). The highest number of ammonia was at the control unit, while T1 at (0.2987mgl^{-1}) had the lowest number of ammonia. A total of 0.2997 mgl^{-1}of ammonia was recorded for T1 while T3 had 0.2988 mgl^{-1} ammonia. In the experiment, it was observed that T3 had the highest dissolved oxygen (7.65mgl^{-1}) while the lowest dissolved oxygen found in T1 was (7.21mgl^{-1}). The dissolved oxygen found in T2 was at (7.43mgl^{-1}) and (7.63mgl^{-1}) was found in the control. There was little variation in the pH value of the different tanks. The tank observed to have the highest pH value is the control at (6.71 mgl^{-1}) and 6.54 mgl^{-1} T1 was observed to be the lowest pH value, the pH value present in T2 was observed to be 6.55 mgl^{-1} while T3 had 6.61 mgl^{-1}total number of dissolved solids present in T1 was 0.24 mgl^{-1}, while 0.241 mgl^{-1} was observed in T2 T3 had 0.236 mgl^{-1}and 0.239 mgl^{-1} was found in the control. From the experiment, it was observed that the highest number of total dissolved solid was found in T1 (0.235 mgl^{-1}) while T3 had the lowest number of dissolved solids (0.236 mgl^{-1})

Table 1: Mean Water Quality Parameters in the Experimental units of *Clarias gariepinus*

S/N	Parameters	T1	T2	T3	Control	Standard Error
1	Temperature (^{0}C)	27.77	26.78	27.28	26.87	± 0.043
2	Ammonia (mgL^{-1})	0.29	0.29	0.29	0.30	± 0.069
3	Dissolved Oxygen (mgL^{-1})	7.21	7.43	7.65	7.63	± 0.008
4	pH	6.54	6.55	6.61	6.71	± 0.081
5	Total dissolved solids (mgL^{-1})	0.24	0.241	0.23	0.24	± 0.012

The number of fry collected from the female broodstock of *Clarias gariepinus*.

A total of 4 broodstock consisting of three females and one male was used to produce the fry as shown in Table 2. The parent stock weighing 700 g and length 36.8 cm produced the highest number of fry. The female stock of 600g and length 30.5 cm produced a total of 2100 fries while the lowest value of 1579 was produced by the female that weighs 500 g with length 28.2 cm.

Tables 2: Number of fry collected from the female broodstock of *Clarias gariepinus*.

Length (cm)	Weight (gm)	No. of Embryo collected
18.5	600	2100
17.2	500	1579
19.8	700	3000

Mean survival in *Clarias gariepinus* with different doses of 17-alpha methyl testosterone

The experimental unit was divided into different segments consisting of treatment T1, T2, T3, and control as shown in table 3. Each unit was fed with different doses of 17 x methyl testosterone T1 (0.03g), T2 (0.035g) T3 (0.04g) with a control (shell-free artemia) for 30 days. It was observed that the survival rate varied between 65% and 85%. Control had the highest number of fry at (85%), and the lowest was T1 (65%). A total of 70% was observed at T2 while T3 had 75%.

Table 3: Mean survival in *Clarias gariepinus* with different doses of 17-alpha methyl testosterone for 30 days

Treatment	Survival
T – 1	65.00
T – 2	70.00
T – 3	75.00
Control	85.00

Sex proportion of *Clarias gariepinus*

A total of 50 fries were used from each experimental unit for gonadal histology as shown in table 4. The number of males was observed in T3 (86%) and female (4%) with a sex ratio of (1.0.17). The control experimental unit had the lowest number of males (70%) and females (30%) in the ratio of 1:0.5. T 2 had (80%) of male and (20%) of female in the ratio of (1:0.25) whileT1 had (76%) of males and females (24%) with a sex ratio of 1:0.3

Table 4: Sex proportion of *Clarias gariepinus* after 30 days of 17 alpha methyl testosterone treatment

Treatment	No. of fish sexed	Males	Female	Sex ratio
T -1	50	38(76)	12(24)	1:0.3
T -2	50	40(80)	10(20)	1:0.25
T -3	50	43(86)	7(14)	1:0.17
Control	50	23(46)	27(54)	1:0.02

DISCUSSION

Anabolic steroids, both androgens, and estrogens improve growth and feed conversion efficiency when administrated at an optimal level in the diet of fish (Olufeagba *et al*., 2017; Shamsuddin *et al*., 2012). The effects of the most frequently synthetic steroid use are 17-alpha methyl testosterone which has been proven to be dose-dependent and was reported by several authors (Celik *et al.*, 2011; Donaldson *et al*., 1979). In the present study, there was variation in the weight and length of fishes due to their different hormone doses. The fry fed with the diet mixed hormone at 0.04g, showed significantly higher weight and length gain than those that received 0.03g, 0.025g per diet.

Pandian and Varadaraj (1990) found that there was high mortality of *Oreochromis mossambicus* as a result of a rise in methyl testosterone. Yamazaki (1976) believes that using 17 alpha methyl testosterone at a concentration of 1ppm gives the best growth rate of goldfish, although the growth rate slows down when there was a decrease in the concentration of hormone 30 ppm. The effect of the hormone cannot be considered as the only factor for the high survival rate of the fries because even the control unit that was fed without the hormone-treated feed had the highest percent survival rate (85%). Celik *et al*. (2011) reported a higher survival rate in the control group indicating that the hormone could interfere with the survival rate.

In this study, the synthetic androgen methyl testosterone was observed to play a role in altering the sex of *C. gariepinus* into males. Among the three doses administered, the dose of 0.04g mixed in the diet of *C. gariepinus* was observed to be more effective in 86% of male fish. Komen *et al*. (1989) reported that the administration of 50 ppm of 17 alpha methyl testosterone for 5 weeks (from 6 to 11 weeks), resulted in 92.7% of males in *Cyprinuscarpio.* However, the administration of (0.04 g) to T3 of 17 for 30 days resulted in 86% male in the present study.

The results from this study are contradictory to the report of (Guerrero and Guerrero, 1975; Woiwode and Adelman, 1991) which infer that the higher dose of the hormone might not necessarily produce a higher result in sex-reversal. This could be explained by the fact that the quantity applied could only be regarded as high when compared to the quantity used in the experimental set-up. From the present study, it appears that the best dose of methyl testosterone in *C. gariepinus* lies within the range of 0.035 – 0.04 g/kg. From this study, the highest frequency of males obtained was 86% which shows that it is very possible to reverse the sex of fry in their early stage after hatching by dietary administration of methyl testosterone in *C. gariepinus.*

CONCLUSION AND RECOMMENDATION

Based on the results of this study, it can be concluded that T3 had the highest number of males with a dose of 0.04g. Variation of survival rate as observed during the cause of the study. There were differences in the weight and length of fry in the different experiments.

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