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Sex Reversal of Nile Tilapia (Oreochromis Niloticus) Using 17α-Methyl-Testosterone, Wet Testes from Selected Animals and Plant Extract

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Abstract: **The study was conducted for 42days to evaluate the potency of wet testes of bull *(Bos. indicus*), boar *(Sus. domesticus)*, bull/mud catfish testes (*B. indicus* / *C. gariepinus)* and pawpaw seed powder (*Carica papaya*) on sex reversal of Nile tilapia (*O. niloticus)* fry in indoor experimental ponds. The estimates of their efficacy in producing phenotypic males were determined after 4months. A total of 825 fry were randomly allocated to 15 experimental ponds. Phyisco-chemical parameters like temperature, dissolve oxygen, PH were checked weekly while ammonia was checked bi- weekly. The hormonal diets were formulated by adding 17α- methyl-testosterone (MT) T1 , wet testes from bull (T2), boar (T3), bull/catfish testes (T4) and pawpaw seed powder, PSP(T5) at an inclusion of 0.06g, 17.47g, 25.59g, 20.48g (80:20% ratio), and 20g per kg of feed respectively . The fry were fed at 20% body weight during a 42day feeding trial and a gradual reduction of feed from 10% to 5% during the 4months rearing period. . The results of water parameters showed that there were significant differences among all treatments. The results were within the recommended range for fish rearing. Results from the gonad examination revealed that Nile tilapia fry fed with MT-treated (T1) diet gave the highest percent phenotypic males of 100% while T2, T3, T4 and T5 gave male of 80, 86.7, 83.3% and 73% respectively. The use of wet animal testes and pawpaw seed powder can be a natural alternative which is cheap and readily available and also be of great relevance to organic tilapia production. Production of all male will help to reducing prolific breeding in Nile tilapia, consequently leading to an increase in the sizes of the fish which is excellent for market value.**

Keywords: **Sex reversal, *Oreochromis niloticus*, wet testes, 17α- methyl-testosterone.**

I. INTRODUCTION

Nile Tilapia, *Oreochromis niloticus* is an African freshwater fish that belong to the family Cichlidae. It is a commonly cultured fish species. Its productions play an important role in global food security especially amongst rural communities of developing countries. It is likely the most important cultured fish in the 21st century [1]. Fish farming makes important role in the world [2]. Decrease in fish production as a result of pollution and over fishing by fish farmers gave rise to aquaculture [3]. With the purpose of achieving more productivity in growing tilapia *Oreochromis niloticus*, it is important to produce mono-sex culture that constitutes totally of males [4]. Different methods are used to direct sex ratios and increase the number of males produced. The first method was by hand sexing i.e. separation of males from females [5]. The most efficient method of producing only males is by the use of steroid hormones fed to newly hatched fry with unknown sex [6] This is done by fish exposure to hormones (testosterone or estrogen) in their diets orally for several weeks .

Tilapia fish is generally accepted and eaten by many, but most aquaculturists especially in Nigeria, are discourage to culture tilapia because of their high fecundity rate, overpopulate ponds [7], they mature sexually at 20 - 30g which results in over spawning and reduced growth rate [8]. For this reason all male population of tilapia are desirable to female because of their fast growth. The use of 17α-methyltestosterone is by far the most common practice for many aquaculturist in view of the fact that it is efficient and relatively a cheap means of sex reversed fry of at least 95% for various tilapia specie [9; 10]. However, Synthetic hormones are more expensive and not readily available in Nigeria. Also, experienced level of expertise is required for handling the hormone when compared to that of local animal testes and plant extracts. The alternatives that can be considered to reduce the use of synthetic steroid hormone for sex reversal of tilapia is the use of testes from farmed animals like bull, boar, cat fish and plant extract (pawpaw seed powder) which are available from local market and abattoirs in the country. Little literature is available in the use of hormone from animals, there is therefore need to add to the existing knowledge in this area of interest which has been given less attention in this nation. Plant sources with potential reversing mechanism and growth-promoting effects could be worth studying since they have not been subjected to licensing for use in food animals. The main objective of this study is to determine the effect of 17α-methyl-testosterone, wet testes from catfish (*Clarias gariepinus)*, boar *(Sus domesticus)*, bull *(Bos indicus)* and pawpaw seed powder in masculinisation of Nile Tilapia.

II. MATERIALS AND METHODS

The study was carried out in 15 different experimental concrete ponds of approximately $1m^3x1m^3x1m^3$ at the African Regional Aquaculture Centre Aluu, (ARAC) Community, Port Harcourt, Rivers State. A total of eight hundred and twenty five (825) Nile tilapia (a day old) fry obtained from ARAC were weighed using an electronic weighing balance (Model M P 2001). Fifty five (55) fry where subjected to five treatments with three replicates in a completely randomized designed.

Test Materials:

The testes of boar *(Sus. domesticus)*, and bull (*Bos. indicus*), were procured from the Abuloma abattoir in Port Harcourt, Rivers State (Nigeria). The matured African mud catfish (*Clarias gariepinus*) male testes were obtained from the Nursery Unit of African Regional Aquaculture Centre, Aluu. The pawpaw fruit (*Carica papaya*) was collected from the experimental farm of the Faculty of Agriculture, University of Port Harcourt (Nigeria).The synthetic steroid hormone (17α methyl-testosterone) was obtained from Prof. Fashina Bombata at the University of Lagos, Nigeria.

Preparation of test materials:

The fresh testes of the catfish, bull and boar were skinned, weighed, sliced, completely minced without dilution using an electric blender and stored at a temperature 4^OC. The seeds from ripe pawpaw fruit were removed, sun dried, milled into powder using an electric blender and stored at room temperature. 60 mg of 17 α-Methyl testosterone, 10g of bull, 10g of boar, and 4g of mud catfish testes were respectively dissolved in a beaker containing 500ml of ethanol (95%). Each of the solutions was analysed for its testosterone content using Enzyme Immunoassay Testosterone test kit (BioCheck, Inc.) 5.7g of pawpaw seed powder was also dissolve in water for hormonal analysis (testosterone).The testosterone level is shown in the table 1 below.

The feed composition:

The feed was formulated based on the working composition obtained from the feed mill of ARAC. 40% crude protein was used. The feed was composed of soya bean, fish meal, wheat bran, processed cassava flour (garri), palm oil, and vitamin premix in various percentages.

Treatment 1(the control) 17α- methyl-testosterone based diet – 0.06g/1kg of compounded feed

Treatment 2 Bull wet testes based diet – 17.47g/1kg of compounded feed

Treatment 3 Boar wet testes based diet – 25.59g/1kg of compounded feed

Treatment 4 bull/African mud catfish based diet – 20.48g/1kg of compounded feed (80:20)

Treatment 5 pawpaw seed powder based diet – 20g/1kg of compounded feed

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Exposure of test organisms:

The sex reversal of Nile tilapia fry was done through oral administration of the experimental diets for 42days, three times daily. The experimental feed were administration at the rate of 20% of fish body weight during the 42days and gradually reduced to 10% and 5% of fish body weight during an addition 4months rearing period.

Sex determination:

At the end of the four month trial, sex of the tilapia fish was determined anatomically by examining their internal reproductive organ [12]. A total of ten fish in each treatment were randomly collected in all the replicates and sex was confirmed by dissection through examination of their gonads (Fig I and II) as described by [12].

Figure.I: Nile tilapia Ovary

Figure.II: Nile Tilapia testes

Data Analysis:

Results were analyzed using SPSS (Statistical Package for Social Sciences) version 21. Data gathered were subjected to Analysis of Variance (ANOVA) to determine significant differences among treatments. The relationship and differences between treatment mean (±SE) were determined using Duncan multiple range test (DMRT) at 5% level of probability.

III. RESULTS AND DISCUSSION

Table.1: Total testosterone from 17α- methyl-testosterone, testes of bull, boar, catfish and pawpaw seed powder (testosterone/estrogen)

Treatments	Sample(gram)	Concentration(ng/ml)
Treatment 1(MT)	0.06g	16.25
Treatment 2(bull testes)	10g	9.3
Treatment 3(boar testes)	10g	6.35
Treatment 4(catfish testes)	4g	1.8
Treatment 5(PSP)	5.7g	0.3/263

Physico-Chemical parameters of Experimental Pond:

Temperature (^oC): The temperature of all treatment ponds ranged from 28.72±0.22 to 29.36±0.31^oC. **Dissolved Oxygen (Mg/L)**: the dissolved oxygen (DO) in all treatment ponds ranged from 3.95 -4.78mg/l. **pH**: The pH treatment ponds ranged from 6.95±0.02 – 7.15±0.08. **Ammonia**: The values range between 0.10±0.00 to 0.18±0.04: **Conductivity (µs/cm)** Conductivity of all treatment ponds ranged from227.92±3.4 (µs/cm) to 155.64±0.23 (µs/cm) **Total Dissolved Solid (pp/m)**: Total dissolved solid (TDS) of all treatment ponds ranged from 227.92±3.4pp/m to 155.64±0.23pp/m.

All treatment were significantly different ($p<0.05$) from each other with respect to PH, Conductivity, TDS, DO. However, there were no significant differences with respect to Temperature. The range of values observed for Temperature, PH, DO, TDS, Conductivity and Ammonia within the various ponds may lie within or around the optimum suitable for sex reversal of tilapia fry. This is because [9] had earlier reported that the optimum temperature for sex reversal of tilapia fry

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is between 26-28^{o}C. Furthermore, [13] had earlier reported that the average temperature suitable for sex reversal of tilapia fry should be around 27^{0}C. Popma *et al*. [14] reported that tilapia should be able to survive at a pH range of 6.0-9.0, which is in accordance with [13] permissible limit of 6-9, [15] permissible limit of 6.5 - 8.5. Furthermore, [16] reported an average pH of 7.0 for sex reversal of tilapia fry. The result in this present study is in line with [9] who recommended that the dissolve oxygen concentration should remain above 4mg/l to ensure a strong feeding response. The result of Conductivity from this study which ranged from 155.41 -213.21 µs/cm was in accordance with [15] who reported an acceptable limit of 250 µs/cm, The TDS result from our study was in line with [13] acceptable limit of 500 mg/l.

Table.2: Mean water quality parameters monitoring during the 42days treatment period

PARAMETERS	TREATMENTS				
	T1	T2	T3	T4	T5
Temperature(^{o}C)	28.72±0.22^{a}	28.92±0.23^{a}	29.05±0.13^{a}	29.36±0.31^{a}	28.82±0.12^{a}
Dissolve oxygen(Mg/L)	3.96±0.03^{b}	3.95±0.03^{b}	4.70±0.06^{a}	4.78±0.07^{a}	3.96±0.03^{b}
PH	7.11±0.05^{a}	7.01±0.02^{ab}	7.07±0.03^{ab}	6.95±0.02^{b}	7.15±0.08^{a}
Ammonia	0.18±0.04^{a}	0.10±0.00^{b}	0.11±0.01^{b}	0.10±0.00^{b}	0.10±0.00^{b}
Total dissolve Solid (pp/m)	143.55±0.5^{b}	209.08±3.4^{a}	113.92±4.4^{c}	117.93±0.26^{c}	105.95±0.35^{d}
Conductivity(pp/m)	212.88±0.33^{b}	227.92±3.4^{a}	167.25±1.09^{d}	180.78±1.22^{c}	155.64±0.23^{e}

Mean values (mean ± standard error) in the same row with different superscript are significantly different ($p<0.05$)

Sex reversal:

Gonad examination of treatments on sex ratio:

The result of gonad examination in the various treatments is presented in Table 3 and it showed that each of the treatment groups gave a high male: female ratio. The highest percentage (100) sex reversal of tilapia fry was observed in the ponds fed with MT–treated diet (T1). This was followed by the ponds fed with boar (T3) testes treated diet with a percentage sex reversal of 86.70 while, the ponds fed with bull testes treated diet had a percentage sex reversal of 80. The ponds fed with bull/ catfish testes treated diet (T4) had a percentage sex reversal of 83.3 and the least was observed in ponds fed with pawpaw seed treated diet with a percentage sex reversal of (T5) 73. There were significant differences (at $p<0.05$) within the means of Treatments. The highest result observed in the pond treated with MT- treated diet is in line with the findings of [17] and [18] who reported 100% reversal but disagrees with the findings of [19],[20] and [21]. However other treatment groups were able to skew sex to male, deviating from the theoretical 50:50 sex ratio [22]. The ponds fed with boar testes treated diet (T3) had a male to female ratio of 86.67:13.33. However, [22] reported a percentage of 79.33 male when tilapia fry were fed with lyophilized boar testes treated diet. Meyer *et al*. [23] obtained males of 87 and 83% of tilapia fry fed *ad libitum* with fresh bull testes and fresh hog testes respectively but [24] obtained 93 percentage sex reversals from *ad libitum* feeding of tilapia fry with frozen bull testes after a 30-day treatment period. The high performance of boar testes treated diet in this study might be as a result of the wet nature of the testes used while compounding the diet which makes it resistance to temperature variation. Furthermore, it might also be as a result of the semi flow-though system maintained, duration of experimental trial (42days) and the hormone concentration from boar testes included in the diet. Gomelsky *et al*. [25] and [26] reported that hormones administered for sex reversal are metabolized and eliminated from the body of fish. Odin et al. [27] observed sex reversal of untreated fish reared within a system previously used with hormone treatment. They also reported that sex reversal treatments are more successful in closed water systems where metabolites and leachates can build up. However, these results are higher compared to the reported 74% male population of sex-reversed tilapia fry fed with carp testes for 24 days by [28].

Table.3: Gonad examination of Treatments on sex ratio of *O. niloticus*

Parameters	Treatments				
	T1(MT)	T2(BT)	T3(BO)	T4(BT/CA)	T5(PSP)
Total no	30	30	30	30	30
Male	10.00±0.00^{a}	8.00±0.00^{bc}	8.67±0.58^{b}	8.33±0.58^{b}	7.33±0.33^{c}
Female	0.00±0.000^{c}	2.00±0.00^{ab}	1.33±0.33^{b}	1.67±0.33^{b}	2.67±0.58^{a}
%Male	100±0.00^{a}	80±0.00^{bc}	86.67±3.33^{b}	83.33±3.33^{bc}	73±2.60^{c}
%Female	0.00±0.00^{c}	20±0.00^{ab}	13.33±3.33^{b}	16.67±3.33^{ab}	26.67±2.60^{a}

Mean values in the same row with different superscript are significantly different ($p<0.05$)

The failure to obtained 100% sex reversal using animal testes might be a result of the method of preparation of the testes that were added to diet. This is because the tough and fibrous septa inside the testes, which are very hard to digest, were not removed while compounding the feed. As a result, it may have been possible that some of the testosterone in the testes was not available for assimilation. Furthermore, the lower percentage (73%) of sex reversal observed in the tilapia fry in ponds fed with pawpaw seed powder (PSP) might be as a result of the high estrogen found in PSP as compared with the concentration of testosterone. Although our result of 73% sex reversal was higher than the 65% reversal reported by [29]. However, we had an inclusion level of PSP at 20g/kg as against the 15g/kg inclusion used by [29]. Omeje *et al*. [30] had earlier reported that pawpaw seed extract had masculinizing effect on fish but inclusion level varied their efficacy.

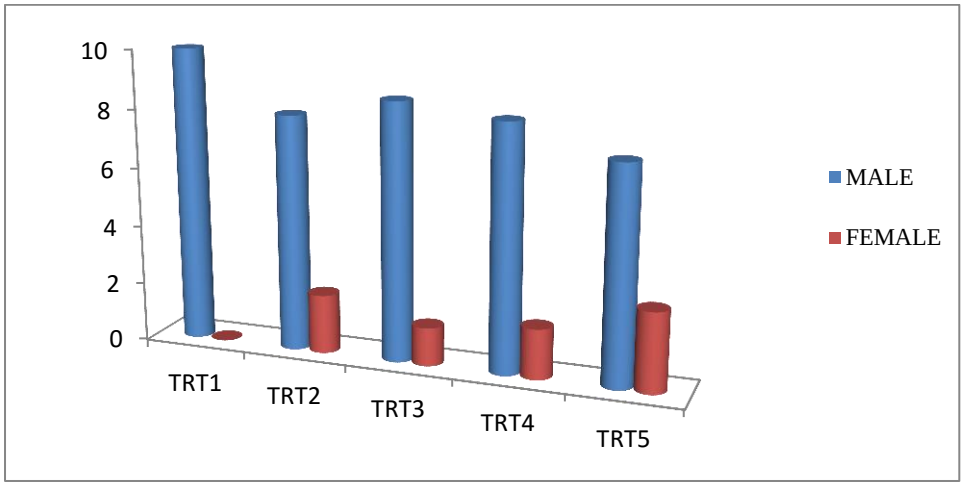

Figure.III: male to female ratio of Nile tilapia (*O. niloticus*) fry in each treatment observed by gonad examination (anatomically)

IV. CONCLUSION

The findings of this study, it can be concluded that the various hormonal treatment (such as wet testes of bull, boar, bull/catfish 80:20% and pawpaw seed powder) were able to reverse the sex of *O. niloticus* , deviating from the theoretical 50:50 sex ratio after a 42day experimental trial. Production of all male will help to reducing prolific breeding in Nile tilapia, consequently leading to an increase in the sizes of the fish which is excellent for market value.

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