OBSERVATIONS OF THE EFFECTS OF 17 α -METHYLTESTOSTERONE (MT) ON THE GONADS, TESTES OF *Clarias gariepinus* BROODSTOCKS AFTER HORMONE-TREATED FEEDING TRIAL.

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Abstract: Seven hundred and sixty-five *Clarias gariepinus* fry were stocked in nine concrete tanks. Stocking density was eighty-five fry per 2 X 2 X 1 metre square tank. Experiment duration for hormone usage was three hundred and sixty-six (366) days. Feeding with the hormone stopped at 366 days and normal feeding without the use of the hormone picked up for another 365 days before findings were carried out. Fry were fed 0.2mm coppers feed mixed with 17 α -Methyltestosterone (MT) at 10% of the fish' body weight from start of the experiment till month one. Fish were fed 0.8mm coppers feed in month two and month three at 10% of the fish' body weight. Fish were fed 2mm coppers in month four at 10% of the fish' body weight. 6mm coppers feed was administered from month five till twelve months at 5% of the fish' body weight. Feeding without the hormone continued for another 365days at 5% of the fish' body weight. Mean initial weight ranged from 0.049g to 0.06g. Mean final weights ranged from 6.4g to 8.5g. Weights of the gonads ranged from 31.5g to 117.8g for the C₅₀ treatment. Weights of the testes however, ranged from 0.8g to 11.5g also for the C₅₀ treatment. Weights of the gonads for the C₁₀₀ treatment ranged from 87.2g to 244.4g, while weights of the testes for the C₁₀₀ treatment were from 3.9g to 18.6g. There were three treatments: C₅₀, C₁₀₀, C₀. Treatments were in triplicates.

Keywords: *Clarias gariepinus*, fry, 17 α -Methyltestosterone (MT), sex-reversal, gonads, testes.

INTRODUCTION

Aquaculture production is synonymous with ensuring food security for the teeming Nigerian populace and indeed, Africa at large (Ataguba *et al.*, 2010). The use of hormones in aquaculture production is reported to be essential in the production of monosex populations, enhancing sex- reversal and increasing weight gain and growth in cultured fishes (Hoga *et al.*, 2018). Treatments for sex-reversal are conducted at the beginning of the fish' development before gonadal sexual differentiation takes place (Hoga *et al.*, 2018; Almeida, 2013). The hormone, 17 α -Methyltestosterone (MT) has reportedly yielded great results and ensured the production of all-male populations or, near all- male populations (Robert *et al.*, 2019c). This study was undertaken to document research findings on the effects of 17 α -Methyltestosterone (MT) on the gonads and testes of *Clarias gariepinus* broodstocks reared from fry stage to broodstock stage. Previous reports often detail microscopic observations only making it difficult for the local fish farmer to understand scientific terms; while leaving out the effects of the hormone on fish organs after usage through visual observations.

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MATERIALS AND METHODS

Study area, source of experimental fish and water source

This research was conducted using the indoor concrete tanks and outdoor concrete tanks of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger state. New Bussa is located on latitude 9° 53' N and longitude 4° 31' N (Robert *et al.*, 2019a). Fry of *Clarias gariepinus* were obtained through induced breeding of *Clarias gariepinus* male and female broodstocks in NIFFR indoor hatchery. The Kigera Dam of NIFFR supplied water for this research duration.

Experimental design

The experimental design was a 1 x 3 x 3 factorial design.

Experimental procedure

Male and female *Clarias gariepinus* were selected by examining their external features which showed swollen, distended abdomens, reddish vents, easy release of eggs when pressed gently for the females; and reddish pointed genital papillae for the males. These characteristics indicated maturity in male and female *Clarias gariepinus*. Females were injected intramuscularly with 0.1ml to 0.2ml of ovaprim per kg according to the fish' body weight for treatments C₅₀; and 0.25ml to 0.4ml of ovaprim per kg of the fish' body weight for treatment C₁₀₀. Fishes were then weighed and returned to the holding vats for a twelve-hour latency period. After the latency period, the males were sacrificed and the milt obtained by dissecting the testes of the male fish using a surgical blade. 0.9% saline solution was added to the mixture of milt and eggs. The mixture of the eggs, milt and saline solution were stirred to aid fertilization. The eggs were then deposited on spawning mats in nine concrete 2 X 2 X 1m² indoor tanks. After the fry had absorbed their yolk sac four days after fertilization, artemia feed was introduced. This starter feed (Artemia) was mixed with 50mg/kg inclusion level of 17 α -Methyltestosterone (MT) for the first three indoor hatching concrete tanks labelled as C₅₀. Artemia mixed with 100mg/kg dosage of 17 α -Methyltestosterone (MT) were fed the fry in the next three hatching concrete tanks labelled as C₁₀₀. The three hatching tanks for the control treatments were fed artemia with no mixture of 17 α -Methyltestosterone (MT) in their feed. For the purposes of this research, only the results from the treatments fed with 17 α -Methyltestosterone (MT) will be reported, as comparison studies will only reflect studies from the C₅₀ and C₁₀₀ tanks. On the seventh day, fry were transferred to the outdoor tanks. Fry were fed with 0.2mm coppers feed mixed with 17 α -Methyltestosterone (MT). From month two to month three, 0.8mm coppers feed was administered to fishes. Feeding continued using 2mm coppers feed in the fourth month. While, 6mm coppers feed were fed fishes from the fifth month to the twelfth month. After feeding the fishes for 366 days with feed mixed with 17 α -Methyltestosterone (MT), feeding using 9mm coppers continued for another 365 days without mixing with 17 α -Methyltestosterone (MT) and at 5% of fish' body weight. Monthly manual

exchange of water was carried out throughout the sampling period. Fish' genital papillae were checked during sampling every month with the aid of a biological microscope (XS Series 500) to ascertain sex-reversal.

RESULTS AND DISCUSSION

Table 1 Sex reversal of *Clarias gariepinus* into all-male using 17 α -Methyltestosterone (MT) for the duration under study

Treatment	Sex reversal %
C ₅₀	94%
C ₁₀₀	93%

Table 2 Observations of the Gonads and Testes of the male and female *Clarias gariepinus* using a biological microscope

Treatments	Sex (<i>Clarias gariepinus</i>)	Gonad weight (g)	Testes weight (g)	Biological observation via a microscope (XS Series 500)
C ₅₀	♂, ♀	31.5	11.5	-Motile eggs observed to be very active (eggs moving) under the microscope, brownish eggs, circular, reddish gonad (abnormal looking). -Watery milt
C ₅₀	♂, ♀	86.7	5.5	-Reddish gonad, non- motile eggs, greenish brown eggs. -Non-motile milt.

C ₅₀	♂, ♀	58.8	0.8	-Reddish gonad, motile eggs, brownish eggs. -Motile milt but slow-moving.
C ₅₀	♂, ♀	117.8	4.0	-Reddish gonad, motile eggs, slow moving. -Non motile milt.
C ₅₀	♂, ♀	70.0	4.0	-Reddish gonad, motile eggs. -Non motile milt.
C ₁₀₀	♀, ♂	165.4	18.6	-Reddish gonad, no motile eggs, greenish eggs, oval gonads. -Watery milt.
C ₁₀₀	♀, ♂	166.5	8.9	-Reddish gonad, motile eggs, slow moving eggs, brownish eggs. -Motile milt, ripe milt.

C ₁₀₀	♀, ♂	188.6	3.9	-Reddish gonad, motile eggs, light- green eggs, but slow moving. -Ripe, motile milt.
C ₁₀₀	♀, ♂	244.4	3.9	-Reddish gonad, non- motile, greenish eggs. -Abnormal testes. -One testes visible
C ₁₀₀	♀, ♂	143.3	8.0	-Reddish gonad, motile eggs yet slow- moving, brownish eggs. -Motile milt

♂ = male ♀ = female

Plate 1 Dissecting a male *Clarias gariepinus* to remove the testes

Plate 2 Pictures of some testes observed under the study

Plate 3 Picture of a watery milt observed under the study

Plate 4 Picture of an abnormal looking testes

Plate 5 Dissecting a *Clarias gariepinus* female fish

Plate 6 Abnormal looking gonads observed in the study

Plate 7 Pictures of some of the eggs released from the female *Clarias gariepinus* under study

DISCUSSION

Morphological study of the gonads revealed abnormal looking testes and gonads (one particular gonad had its eggs actively moving); an indication of a damaged organ structure due to the hormone, 17α -Methyltestosterone (MT) for which said fish was subjected to, during feeding. This does not agree with Turan and Cek (2007) in whose study, testes and gonads showed no damaged organ structure when *Tribulus terrestris* popularly called Gokshura was used on *Clarias gariepinus*. *Tribulus terrestris* is used in Europe, the Far East, China, India as a medicinal herb, helpful in treating sexual deficiencies, increasing testosterone and LH (Leutinizing hormone) levels in *Clarias gariepinus*. *Tribulus terrestris* is a steroidal sapronin responsible for sex-reversal in fishes. This study cannot conclude that the abnormalities were due to increased potency of the hormone in the treatments under study, as the abnormal gonads were seen in C₅₀ treatment and the abnormal testes seen in C₁₀₀ treatment. Previous reports have indicated higher levels of hormones are responsible for abnormalities in fishes (Robert, 2021c). There were, reddish gonads seen in the study. Microscopic examination also revealed motile eggs and milt for most of the *Clarias gariepinus* fishes. This was indicative of the non-effectiveness of 17α -Methyltestosterone (MT) in the gonads, testes of the fishes after feeding with the hormone ceased. This agrees with Guererro, (2008) who opined that hormone levels dropped in tilapia five days after hormone feeding stopped. Johnstone *et al.*, (1983), Megbowon and Mojekwu, (2014) further revealed that 17α -Methyltestosterone (MT) was not detected in the whole body of a fish 100 hours after hormone treated diet. Amounts of 17α -Methyltestosterone (MT) ingested by tilapia in a previous study was equal to 0.0 – 0.2mg/kg MT/fish. This amount represents less than 0.01% of the normal daily dose of MT prescribed in human medicine which is 20-40mg. This amount they suggested, reduces to less than 0.00001% of everyday human dosage a week after stopping hormone treatment. They, further reported that the testes of an adult human(man) gives out 15mg of endogenous testosterone per day; yet only 10mg of androgen is excreted daily.

CONCLUSION

This report therefore recommends that, the fishes can be eaten or are safe for eating after hormone usage in their feed had stopped. This is because of the quick metabolism and subsequent excretion of hormones (especially 17 α -Methyltestosterone (MT) and 17- β Estradiol (E₂), making it safe to consume hormone-treated product thereby facilitating the production of marketable sized fishes.

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