EFFECTS OF 17α-METHYLTESTOSTERONE (MT) ON THE LIVER OF MALE AND FEMALE *Clarias gariepinus* BROODSTOCKS AFTER WEANING OFF THE HORMONE-TREATED FEED.

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Abstract: *Clarias gariepinus* fry numbering four hundred and fifty, were stocked in nine concrete tanks. Stocking density was fifty fry per 2 X 2 X 1 metre square tank. The duration for the experiment was three hundred and sixty-five (365) days. Feeding with the hormone ended at 365 days from which normal feeding without the use of the hormone commenced for another 365 days. *Clarias gariepinus*. fry were fed 0.2mm coppens feed mixed with 17α-Methyltestosterone (MT) at 10% of the fish' body weight from start of the experiment till they were one month old. Subsequently, fish were fed 0.8mm coppens feed from two months old till three months old. Feeding was administered at 10% of the fish' body weight. *Clarias gariepinus* fish were fed 2mm coppens at four months old. Feeding was also done at 10% of the fish' body weight. 6mm coppens feed was administered to fishes from five months old till twelve months at 5% of the fish' body weight. Thereafter, fish were fed 5% of the fish' body weight for another 365 days. Mean initial weights ranged from 0.053g to 0.065g. Mean final weights ranged from 6.7g to 8.8g. Liver weights for the CLA_{50} treatments ranged from 3.7g to 8.0g for the male *Clarias gariepinus;* while the female *Clarias gariepinus* also in the CLA_{50} treatment showed liver weights of 4.0g to 12.6g. The CLA_{100} treatments showed the following weights for the male *Clarias gariepinus* and the female *Clarias gariepinus* under the study - 6.6g to 14.2g and 5.9g to 18.2g respectively. There were three treatments under study: $CLA_{50,}$ $CLA_{100,}$ CLA_{0}. Treatments were triplicated.

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

INTRODUCTION

In Nigeria, aquaculture production is responsible for 70% of fish production (Williams *et al.,* 2008). *Clarias gariepinus* is reported to be the most popular fish cultured in Nigeria noted for its adaptable characteristics under culture systems. The popularity of the culture has therefore endeared many, leading to increased demand for high quality fish seed which has led to the springing up of many hatcheries in Nigeria (Oyebola and Awodiran, 2015). One way to manipulate the sex of a fish is to use steroid hormones. For increased efficiency, steroid hormones are to be used before sexual differentiation occurs. This is because at that stage, gonadal tissue becomes sensitive to the action of steroids (De Carvalho *et al.,* 2014). This research was undertaken to document research findings on the visual observations of the effects of 17α-Methyltestosterone (MT) on the liver of broodstock male and female *Clarias gariepinus;* cultured from fry to fingerling to juvenile and, to broodstock stage. Previous reports focused on sex- reversal leaving out the effects on fish organs after

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hormone application in feed and for a duration detailing 365 days after. This report therefore, seeks to bridge this gap as effective documentation of fishery management is essential (FAO, 2006) for the growth of the fishery industry.

MATERIALS AND METHODS

Study area, source of experimental fish and water source

This research was carried out using both indoor concrete tanks and outdoor concrete tanks of the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger state. New Bussa is reportedly located on latitude 9° 53' N and longitude 4° 31' N (Robert *et al., 2*019a). Fry of *Clarias gariepinus* were procured by induced breeding of *Clarias gariepinus* male and female broodstocks in NIFFR hatchery. NIFFR's Kigera Dam supplied water for this research duration.

Experimental design

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

Experimental procedure

On the selection of broodstocks for induced breeding, this study looked at the fish' external features which were as follows: swollen, abdomens which were distended, reddish-looking vents, easy release of eggs when pressed gently for the females. For the males, reddish pointed genital papillae for the males were the distinguishing trait for sex selection. The traits mentioned, show the fishes selected were matured. Female *Clarias gariepinus* were injected intramuscularly with 0.2ml to 0.3ml of ovulin per kg according to the fish' body weight for treatments CLA_{50}; and 0.25ml to 0.65ml of ovulin per kg of the fish' body weight for treatment CLA_{100}. 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 gotten, by dissecting the testes of the male fish using a surgical blade or a razor blade. The solution of 9 grams of salt and 1liter of water equaled 0.9% saline solution also known as normal saline. This saline solution was added to the mixture of milt and eggs. The mixture of the eggs, milt and saline solution were stirred. This process was carried out to enhance fertilization. The eggs were finally deposited on spawning mats in nine concrete 2 X 2 X $1m^2$ 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 17α-Methyltestosterone (MT) for three indoor hatching concrete tanks labelled CLA_{50}. Artemia mixed with 100mg/kg 17α-Methyltestosterone (MT) were fed the fry in hatching concrete tanks labelled CLA_{100}. The control hatching tanks were also fed artemia with no mixture of 17α-Methyltestosterone (MT) in their feed. For the purposes of this study, only the results from the treatments fed with 17α-Methyltestosterone (MT) would be compared. On the seventh day, fry were transferred to the outdoor concrete tanks. 0.2mm coppens

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feed mixed with 17α-Methyltestosterone (MT) was fed fish at 10% of the fish' body weight from start of the experiment till they were one month old. Thereon, fish were fed 0.8mm coppens feed from two months old till three months old. Feeding was administered at 10% of the fish' body weight. *Clarias gariepinus* fish were then fed 2mm coppens at four months old. Feeding was carried out at 10% of the fish' body weight. 6mm coppens feed was given to the fishes from when fishes were five months old till twelve months at 5% of the fish' body weight. After feeding the fishes for 365 days with coppens feed mixed with 17α-Methyltestosterone (MT) for the CLA_{50} and CLA_{100} treatments, feeding resumed yet again. 9mm coppens was given fish at 5% of the fish' body weight for another 365 days without mixing with 17α-Methyltestosterone (MT) as fishes were taken off feeding with hormones (weaned). Monthly manual exchange of water was carried out throughout the research duration. The fish' genital papillae were checked for sex-reversal during sampling monthly.

RESULTS

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

Treatment	Sex reversal %
CLA_{50}	96%
CLA_{100}	91%

Table 2 Visual observations of the liver of the male and female *Clarias gariepinus*

Treatments	Sex (*Clarias gariepinus*)	Liver weight (g)	Visual observation of the liver from random five selections
CLA_{50}	♂, ♀	8.0, 12.6	Dark red liver for both sexes
CLA_{50}	♂, ♀	6.5, 4.0	Blackish liver, Dark red liver.
CLA_{50}	♂, ♀	3.7, 4.3	Bright red, dark red liver with white blotches.

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CLA_{50}	♂, ♀	6.1, 6.1	Dark red liver for both sexes
CLA_{50}	♂, ♀	6.0, 4.5	Dark red liver
CLA_{100}	♂, ♀	8.6, 19.2	Bright red liver for both sexes
CLA_{100}	♂, ♀	14.8, 17.1	Dark red liver for both sexes
CLA_{100}	♂, ♀	10.3, 5.9	Dark red liver, Bright red liver
CLA_{100}	♂, ♀	6.6, 9.6	Dark red liver for both sexes
CLA_{100}	♂, ♀	6.5, 9.2	Dark red liver for both sexes

♂ =male, ♀= female

Plate 1 Dissecting *Clarias gariepinus* fishes to extract the liver

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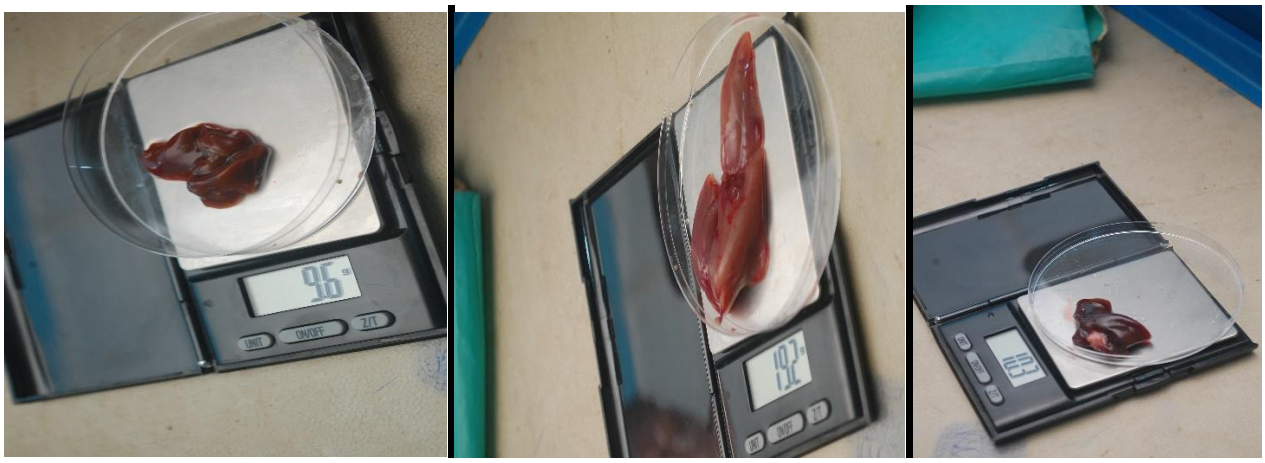

Plate 2 Pictures of some livers extracted under the study

DISCUSSION

Kefi *et al.*,(2013) reported red liver coloration (and not brownish liver coloration) for *Oreochromis andersonii* treated with 0mg/kg , 40mg/kg and 60mg/kg in their short study on the effects of 17α-Methyltestosterone (MT) on haematology and histology of the liver. The results in this study support their findings. Onuoha (2010) however reported results showing yellowish looking liver as a result of infection by river metacacercariae after pathology examination. The white blotches on one of the livers observed in the study in the treated group CLA_{50} did not indicate degeneration, as the liver was bright red with a normal appearance. It is however suggested that the white blotches could be a sign of a mild inflammation brought on by the hormone-treated diet ingested by *Clarias gariepinus* in the study. The findings of Olurin e*t al.,* (2016) support this line of thought as their study of the effects of sub-lethal concentration of phostoxin on *Clarias gariepinus* juveniles suggested. A contrary opinion by Onuoha (2010) suggested that the white blotches may be a sign of necrosis-an infection in the liver.

CONCLUSION

It is recommended however, that further studies on visual observatory effects of 17α-Methyltestosterone (MT) on fish liver be carried out; while fish is still being fed with 17α-Methyltestosterone (MT) to compare observations and results.

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