

The Use of Sex Hormone in Sex Reversal of *Oreochromis niloticus*

Hassan Ali Osman Mohamed

A.R. Prof. Department of aquaculture and fish breeding, Fisheries and Aquatic Research Center
Animal Resources Research Corporation, Ministry of Animal Resources and Fisheries–Khartoum–
Sudan.

***Corresponding Author:** Hassan Ali Osman Mohamed A.R. Prof. Department of aquaculture and fish breeding, Fisheries and Aquatic Research Center Animal Resources Research Corporation, Ministry of Animal Resources and Fisheries–Khartoum–Sudan.

ABSTRACT

The Nile Bulti, *Oreochromis niloticus* topping the list in view of popularity among consumers and its availability throughout the year. But in spite of that it has some problems that require resolving. Of these are multi-spawning throughout the year and the risk of high degree of competition among generations connected with food consumption –which leads to stunted growth. The said problem has initiated this piece of research directed towards sex-reversal in order to re-direct activities towards fattening of the required sex. For the purpose two types of hormones at varying doses were used. These comprised test osterone and aromatase inhibitor. The well planned management has induced good and significant sex reversal in the range of 84-100%. Not only that but also the consumption safety was maintained and order of merit amongst the hormones was determined. In this way the results obtained can be left at the disposal of practitioner who needs to get involved in this exercise.

Keywords: *O. niloticus*, sexreversal, testosterone and aromatase inhibitor.

INTRODUCTION

The term sex reversal which is currently used worldwide was adopted in order to address problems attached to selected important target fish of economic importance. The intervention is dependent on the role, dose and concentration level of sex hormones whether used by man or resulting from the effect of surroundings where the fish lives. With the above in the background, the Nile Bulti, *Oreochromis niloticus*, which is a very popular fish among consumers, is the subject of this investigation. It is appropriate to note that the onset of breeding in this fish is started by multi factors including well feeding, elevation in temperature and salinity levels.

This situation leads to formation of multi generations that co-existed in a limited mass of water. Those generations compete on a limited source of food and the final result is limited growth rate (stunted growth). In this case only very limited individuals can grow to marketable size. \Thus, the use of the relevant hormone(s) will serve in resolving the problem through the one sex targeted. The advantage here will be in the direction of elevated growth rate i.e. fattening till required weight or size is obtained for marketing. Based on the above the objectives of the research problems are enlisted as below:

- Reconfirm the application of sex reversal in the solution of standing problems in the world of fisheries.
- The adjustment of dose, level and concentration that serve the purpose in the target fish selected.
- The identification of the problems attached to the selected fish and formulation of the plan for solution.
- Advices and recommendations that are passed to current and future practitioners and the note on hygienic consideration to eliminate and cut down hazards if any during the practice. This study is a continuation of what was started in those studies, and the expected results will be an addition for reconfirmation and solidification of previous results.

MATERIAL AND METHODS

The research programme was conducted at the Shagara Fisheries and Aquatic Research Center. The relevant components ranging from pond construction, aquaria, water quality, fish species selection, biological and physiological investigation, hormonal determinations...etc were considered as given in the following:

Ponds

- Earthen growth and fattening ponds each being 1.5×25×60 m. with a total area of 2250 m² were used.
- Twelve plastic aquaria, dimensions of each being 20×20×40cm
- Ten nylon hapa, dimension being 100 ×130×190 cm. with mesh size 2.5 mm. fixed in the earth expands with bamboo
- Ten glass aquaria with dimensions being 30×40×75 cm. (plate4) were also used.

Water Quality

The water level was continually observed through topping and recording the main characteristics as regards aeration (Hash), dissolved oxygen (Hanna), p H (Hanna) and temperature by using digital thermometer

Test Fish Species: The Term Bulti Applies to Three Species:

Oreochromis niloticus (Trewavas) Sarotho rodong alilaeus (Artemis) and Tilapia zilli (Gervais) which represent about 92%, 5% and 3% respectively of their presence (Abu Gideiretal., 2004) that belong to order Perciformes, family Cichlidae, taxonomically and later on they were divided on the basis of their reproductive behavior.

Brood Stock

150 Brooders stocked at a ratio of 1 male to 2 females fish in a hapa in earthen pond.

Eggs Collection

Five brooders from the above injected intramuscularly under the dorsal fin with human chorionic gonadotropin hormone 960 I.U. /kg/ body weight to induce spawning. After spawning, eggs were collected and incubated.

Eggs were also collected from the female's mouths after natural spawning and transferred to incubator, with continuous water supply (flow one L/minute), constant oxygen supply, which gives the eggs its required movements. After hatching, the hatched fry were transferred to test aquaria, with oxygen pump. Fry 6.5 mm in length were stocked at the rate of 25/l in the plastic aquarium. This stocking rate ensures that the fry feed only on the experimental diets. After the end of the treatment period the fry were moved to glass aquaria with stocking rate of 3fry/l. After 2 months the fry were

moved to the earthen pond in a hapa with stocking rate of 50fish/m.

Biological Investigations and Experiments

The O. niloticus, was sexed in order to differentiate between males and females. Not only that but also the percentages of ovulation, fertilizationetc were determined. Proper distinction between male and female sexes was carefully stuck to morphological and anatomical character.

Test Diets and their Preparation

The diets used were Cotton seed cake + rice (ground) +fish meal as a concentrate at a ratio of 1:1:2 respectively with a total amount of crude protein 33.6%) and Rizgalla and Abdel Rahim, 1999) mixed with Aromatase inhibitor. Brooders were fed three times a day at hour 08:00, 12:00 and 16:00.

Preparation of the Diets

a- The testosterone diet was prepared by dissolving 25mg, 50mg and 75mg of testosterone tablets each in a 250ml flask containing 250ml of a 94.5 methanol. The testosterone-diets mixture was prepared by mixing the contents of each flask with 1kg diets. The mixture was left for 24 hours at room temperature to allow the methanol to evaporate. (Hunter and Donaldson, 1983; Bock et al., 1992; Phelps et al., 1995 and Sanches and Hayashi, 1999)

Aromatase Inhibitor Diets

Aromatase inhibitor is water soluble. 50 mg; 100mg and 150mg were dissolved each in 500ml water then mixed with one kg diets, then left to dry a fonsoetal. 2001 and Known, 2003).

Determination of Food Yield: All Growth Parameters were Calculated

- Food conversion ratios: = $\frac{\text{Food consumed}}{\text{Fish body weight gain}}$
- Food conversion efficiency: = $\frac{\text{Total fish body weight gained} \times 100}{\text{Total weight of feed intake}}$
- Specific growth rates: = $\frac{\text{Gain weight} \times 100}{\text{Days}}$

Experimental Trials

Two thousand and four hundred O. niloticus fry four days post hatching about 6-6.5mm length at hatching, 7 mm at starting treatment and about

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0.006 mean weight, One hundred eighty fry were stocked in each aerated 16-liter aquaria in laboratory room. They were arranged into seven groups (table 1), and fed for four months, 30 days with diets treated with testosterone for (A groups) and diets treated with Aromatase inhibitor for (B groups), 15% of body weight for the first two weeks, then 10% for the rest two weeks, then at 4% for the rest period

Hormones

A-Testosterone is the most important representative of the male sex hormones collectively called androgens. Using cholesterol as a base, the male gonads, adrenal cortices and ovary produce it (Roger, et al., (1988.)) The commercial testosterone has many different names. The one used is Proviron from Schering AG/Germany/Allemagne, as tablets each one 25 mg. Testosterone itself is responsible for three major functions in animals including fish (Raymond, 2005) as given in the following:

- The development of secondary male sex characteristics, such as Sexual behavior, libido and the maturation of sperm.
- Promotion of the protein biosynthesis (anabolic characteristics) important function is accelerates muscle buildup, increases the formation of red blood cells, speeds up regeneration and recovery time after injuries or illness. It also stimulates the entire metabolism which results in the burning of body fat.
- Inhibition of the gonad regulating cycle, including the hypothalamo hyperphysical testicular axis which regulates the amount of testosterone produced in the organism.

Aromatase Inhibitors

Steroid hormones can influence sex differentiation in nonmammalian vertebrates. Male and female sex differentiation is driven by androgen and estrogen hormones, respectively. Estrogen biosynthesis is mediated by the steroidogenic enzyme cytochrome p450 aromatase, which converts androgen to estrogen (Afonso, et al. 2001), (breast cancer.Org. 2005). This enzyme is inhibited by aromatase inhibitors which keep androgen from being converted to estrogen in early embryonic stage. Commercial aromatase inhibitor is mainly used as anti-estrogen therapy for breast cancer to lower the amount of estrogen being produced by the

body in human. There are many commercial names; the one used was Fadrozole.

Sampling

25 fry from each treated and untreated fish were taken weekly for length and weight. The fry were put in a dish with water to keep them alive while working. The fry weighted by balance in a flask. At the 4th month 50 fish from each group were sexed firstly externally, then macroscopically and histologically (Guerrero and Shelton, 1974).

Blood samples were taken every month to determine the concentration of the testosterone hormone for blood sampling, once the fish is captured the caudal peduncle is cut and the free flowing blood is collected in syringe, allowed to clot at room temperature.

After that the sample should be centrifuged and the serum separated, and sent to the Lab. (Lionel and Mandalay, 1972).

- Hormonal determinations: The test used was Direct Human Serum Testosterone EIAF, EIA (2005).
- Histological preparations followed (Roy Mahoney, A.I.S.T., 1973; Bancroft and Stevns, 1975).

RESULT

The hormone injected intramuscularly under the dorsal fin in accordance with international unit per kg body weight or given with food per mg kg food play crucial roles throughout the life span of the fish over the consecutive phases including ovulation, spawning, fertilization, hatching, sex differentiation, survival, growth...etc. The results attached to those are outlined in the following:

A) HCG result

Spawning

The treated fish spawned and ovulated at different percentage. For individual fish respectively however, most fish spawned 1-2 days after injection none of the control spawned. The hormones: The hormones used were, commercial testosterone (proviron), and non-steroid aromatase inhibitor.

The goal of the experiment was to determine the masculinizing efficiency of these hormones on *O. niloticus* fry produced by females in controlled production system. The finding of the applications are summarized in table (1)

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Table1. To show general applications and results.

TRETMENTS	testosterone			Aromatase inhibitor			D/control
	A1	A2	A3	B1	B2	B3	
Amount of hormone	25 mg/kg	50mg /kg	75mg /kg	50mg/kg	100mg/kg	150mg/kg	-
No. stock	180	180	180	180	180	180	180
Av. L at starting	7	7	7	7	7	7	7
Survival %	87.7	88.8	88.	87.7	89.4	87.7	89.4
Initialfeed/kg/liveb.w.%	15	15	15	15	15	15	15
Final feed /kg b.w.%	4	4	4	4	4	4	4
Total food intake	26067	26685.12	25524.5	20755	21962.2	24560	30156.8
Total B.M.	13232	14044.8	13872	11280	12269.	12926.3	12160
F.C.R.	1.9	1.75	1.85	1.91	1.852	1.948	2.4
F.C.E	0.689	0.56	0.539	0.524	0.540	0.513	0.42
S.G.R	0.506	0.73	0.528	0.587	0.639	0.673	0.24
Male%	86	100	100	96	98	100	58

IB.M= Biomass, FCR=Feed Conversion Ratio, FCE=Feed Conversion Efficiency Ratio and SGR= Somatic Growth Rate.)

From The Table Crucial Results are

- The implementation of sex reversing trials gives a base for an application of large scale technology under farm conditions.
- Sex hormones play an important role in sex determination and differentiation as illustrated by anatomical and histological examination of the gonads and thereafter
- Hormones affect growth, length, FCR, FCE, SGR more than the survival rate.

The Metabolism and Tolerance

Analysis showed that testosterone was well metabolized and tolerant. After the end of the treatment the concentration of the hormone was 10.66ng /ml at the end of the 4th month it reached .288- 1.73 ng / ml. Testosterone: examination for 50 fish from the A1 group showed that about 40 fishes were assumed to be male .After the anatomical and histological examination of the gonads declared that there were 42 males, 7 were females and one was hermaphrodite. The Hermaphrodite seminiferous tubules with the ovarian follicle Externally 50 specimens from group A2 show that there were

about 43 fish male but after the anatomical and histological investigation of the gonads affirmed all of them were male. Sample from group A3 from there external fixture denoted that about 47 fish were male .After killing of the fishes all of them were males. The histological sampling and examination cleared that all fishes were males.

Aromatase Inhibitor

Aromatase inhibitor was used in three replicates in group B1, B2, and B3. Group D as control .The male % was as follows 96%, 98% and 100% for B1, B2 and B3 respectively. While it was 54% in the control group (Table 2). Macroscopically 45 out of 50 fish from group A assumed to be males but after more investigation by anatomical and histological differentiation 48 were male. External fixture of 50 specimens from group B denote that all fishes were male, but the anatomical examination of gonads revealed that there was one female. The histological treatments of samples confirm the result. All 50 samples from group B3 were male after external, decision and staining treatment confirms the result.

Table2. Morphologically, anatomically and histological male

Tretments	No. Examined	Morphologically Mal	Anatomically And Histological Male
D	50	32 (64%)	27(54%)
A1	50	40(80%)	42(84%)male ,7(14%)female 1(2%)hermaphrodite
A2	50	43(86%)	50(100%)
A3	50	47(94%)	50(100%)
B1	50	45(90%)	48(96%)
B2	50	50(100%)	49(98%)
B3	50	50(100%)	50(100%)

Follows from the above the Following Briefings

- The implementation of sex reversing trials using hormones give a base for an

application of large scale technology under farm conditions.

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- Sex hormones play an important role in sex differentiation as well as inducing sex reversal for commercial uses.
- Hormones affect growth, length, FCR, FCE, SGR but with no effect on survival rate.

DISCUSSION

This section has been designed as to compare the findings obtained in this investigation with what was carried out by other investigators elsewhere no matter the selected or target species. In this respect the focal position has been directed towards the confirmation of the process and the tools for application i.e. the sex hormones, their doses durability and ultimate results.

Here what has been achieved is not different from those obtained by others like Johnstone et al. (1979) Hopkins et al. (1979) Victor Bye. (1981). Obeid (1983) Hunter, and Donaldson, (1983), Alok et al. (1993), Argue and Phelps, (1996), Carvalho and Foresti (1996), Contreras Sanchez et al. (1997), Afonso et al. (1999), Ekwu and Sikoki (2001), Afonso et al., (2001), HELENA D'COTTA et al (2001). Contreras Sanchez, (2001), Ekwu et al., (2001), Morales et al., (2002), Bart et al., (2003), Karayuce et al., (2003), Kobayashi et al., (2003b), Yanget al., (2003), Wilfrido et al., (2005). Refat Nahla et al (2011)

It does worth mentioning that the findings related to the Nile Bulti are of special significance as it has multifactor for initiation and I believe that my findings, in this case are an addition to knowledge. The management of fish farms and even some water bodies fish can benefit from the implementation of the sex reversal exercise as long as above mentioned programme is stuck to and the precautions both hygienically and application are observed.

The outcome from the studies have yielded the growth rate detected in both treated and material from natural water i.e. this observation indicates that final product will not differ from the natural product at least in the White Nile at Shagara. This point is considered, to my judgments, as an advantage. It is appropriate to refer to the correlation between the hormone dose composition and the final percentage recorded.

And Phelps, (1996), Carvalho and Forest, (1996), Green, et al. (1997), Gale, et al., (1999), D'Cotta et al., (2001), Guiguen et al., (1999), Mainardes, et al., (2000), Afonso, (2001), Ekwu, et al., (2001), Bart, et al., (2003), Known, et al., (2003), Wassermann and Afonso, (2003),

From the findings it was noticed that the hazards of hormones which are likely to occur do not continue indefinitely but no trace is detected beyond the fourth month from the time taken from the hatchery.

CONCLUSIONS

From the study and the information collected in the field of applying steroids and non steroids for masculinizing *O. niloticus* the following conclusions are summarized:

- The activity as a whole confirms the practices undertaken elsewhere and on different species in regard of sex hormones and their dosage.
- Of the sex hormone testosterone and aromatase inhibitor used the percentage approaching 86-100%. This will get rid of the hazards of using other hormone,
- There is a correlation between dose composition and the percentage.
- The hazards described for any of the hormones enlisted disappear after a reasonable time before the attainment of the marketable size (4 months).

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