PRELIMINARY INVESTIGATION OF *Tribulus terrestris* (Linn., 1753) EXTRACTS AS NATURAL SEX REVERSAL AGENT IN *Oreochromis niloticus* (Linn., 1758) LARVAE

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ABSTRACT

Five pairs of broodstocks of *Oreochromis niloticus* (average weight of 350±25.8g) in a ratio of 1:1 male to female were used for the fry production to examine the prospect of utilization of safer and natural extract (*Tribulus terrestris*) as sex reversal agent in tilapia population control.. Four hundred and fifty day-old fry were randomly distributed into 18 experimental tanks (30 litres capacity) representing five test diets (50% crude protein) containing varying inclusion levels of *Tribulus terrestris* extract at 0.0, 1.0, 1.5, 2.0, and 2.5g per 1kg of basal feed while diet six contain 50mg per 1kg of basal feed of 17-α-methyltestosterone (MT) served as the control diet. The growth performance parameter indices measured were Mean Weight Gain (MWG), Food Conversion Ratio (FCR) and Specific Growth Rate (SGR). At end of the study, the survival rates and sex ratio were also measured and recorded. Data collected were subjected to 2-way analysis of variance (ANOVA) where significant means were tested using the Least Significant Difference (LSD) test at $p = 0.05$.
Key words: Sex reversal, *Tribulus terrestris* and *Oreochromis niloticus*

INTRODUCTION

Tilapia constitute one of the most productive and internationally traded food fish in the world (Lim and Webster, 2006) and one of the major sources of protein to most developing countries. Despite having many good characteristics, one of the major drawbacks in commercial tilapia production is its precocious maturity. This has led to uncontrolled reproduction, resulting in increasing competition for feed followed by stunted growth and low commercial value (Jegede and Fagbenro, 2007). In populations of tilapia, males grow faster and are more uniform in size than females. This has led to the production of all male populations through sex reversal. Sex reversal is the process of administering male steroids to recently hatched fry so that the undifferentiated gonadal tissue of genetic females develops into testicular tissue, producing individuals that grow and function reproductively as males (Phelps and Popma, 2000). Monosex production systems show several advantages over mixed sex production systems like the choice for the faster growing sex of the species to be produced among other advantages (Beardmore *et. al.* 2001). There is however public health issues in relation to the use of synthetic sex reversal hormone. This study therefore examined the prospect of utilization of safer and natural extract (*Tribulus terrestris*) as sex reversal agent in tilapia population control.

MATERIALS AND METHODS

Five pairs of broodstocks of *Oreochromis niloticus* (350±25.8g) were collected from the Department of Aquaculture and Fisheries Management Fish Farm, University of Ibadan and stocked at a ratio of 1:1 (male to female) in 1m X 1m hapas and monitored regularly for fertilized eggs. After 21 days, fertilized eggs were noticed in the mouth of the fish and were collected and incubated in the hatchery for 18-32hrs. The hatchlings were transferred to the laboratory 24hrs after hatching.

Tribulus terrestris extract (Trib 60) was procured from Tonvara Premium Natural Supplements, United Kingdom. The extract contained 60% protodioscin, while the 17-α-methyltestosterone which was used as control was purchased from Kingdom Aquarium Nigeria Ltd, Lagos, Nigeria.

A 50% crude protein diet was prepared using soy bean meal, maize, fish meal, vitamins premix, di-calcium phosphate, and salt purchased and milled at Bodija Market, Ibadan;. The 50% C.P was based on the protein requirement of *O. niloticus* fry (30-56%) as recommended by Jauncey, (2000). The milled feedstuffs were passed through a 200 µm sieve mesh.

The experiment was a completely randomized design with six treatments and three replicates respectively representing different inclusion rate of 0.0, 1.0, 1.5, 2.0, and 2.5g *T. terrestris* extract to 1kg basal diet (T1-5) treatment 6 containing 50mg/kg of 17-α-methyltestosterone to basal diet as control. 450 day-old fry of *O. niloticus* were randomly distributed into 30L capacity plastic aquaria and were acclimatized for 3 days before the commencement of feeding with experimental diets in the laboratory.Each aquarium was adequately aerated. The fry were fed with the diet *ad libitum* from day four thrice daily for 42. Settled fish wastes were siphoned with half of aquarium's water, fresh aerated water was used to replenish daily while the water in the aquaria were changed completely twice a week. At the end of reversal stage, the fish were transferred into the Departmental fish farm into

hapas rigged in an earthen pond and were fed for 70 days with commercial diet. The fish were weighed biweekly and water quality parameters monitored. The fish were sexed on the 70th day using a hand binocular. The following growth performance parameters were determined; Mean Weight Gain, Length of fish, Food Conversion Ratio and Specific Growth Rate. Growth performance was determined and feed utilization was calculated according to Ogunji *et. al.* 2008 and Ahmad *et. al.* 2002Water quality parameters (temperature, dissolved oxygen, pH, ammonia and nitrite) were determined weekly. Dissolved oxygen, Ammonia and Nitrite were measured using Hach's Model FF-1A Fish Farmer's Water Quality Test Kit. Temperature was measured using a mercury-in-glass thermometer. The pH was monitored using Hanna model H1-98107.

RESULTS AND DISCUSSION

Fish in all the treatments during the laboratory phase and pond phase of the experiments exhibited high survival rates, though this survival was not uniform, it was observed that fish showed increase in survival rate with increase in *T. terrestris* level in the feed used in the experiment. This is similar to the observations of Kavitha and Subramanian (2011), and Cek, *et al.* (2007). This study further showed that *T. terrestris* has no negative effect on the survival of *O. niloticus*. The steady weight gain observed during the phase one of this experiment, with increase in *T. terrestris* inclusion, is similar to the observations of Keith and Subramanian (2011), CAC, *et al.* (2007a) and Cek *et. al.* (2007b) on *Peocilia latipinna, P. reticulata* and *Cichlasoma nigrofasciatum* respectively. They all reported that fish had better growth rate with increase in the concentration of *T. terrestris* in culture water. *Tribulus terrestris* has been reported by (Akram *et. al.* 2011) to be a testosterone enhancer, *T. terrestris* treated fish showed better growth rates with increase in the levels of *T. terrestris*. This agrees with the report of Mateen, (2007) that since the androgens have both sex reversal and anabolic effects, the sex reversed tilapia shows a better growth performance as compared to untreated tilapia. Water quality parameters of experimental set up are in conformity with the requirements for adequate growth of fish as reported by Omitoyin, (2007).

Table 1: Growth parameters of *O. niloticus* under different levels of *Tribulus terrestris* extract in the laboratory

	T_1	T_2	T_3	T_4	T_5	T_6
Initial weight (g)	0.03 ± 0.0012^a	0.03 ± 0.0011^a	0.03 ± 0.0011^a	0.03 ± 0.0010^a	0.03 ± 0.0013^a	0.03 ± 0.0011^a
Initial length (cm)	0.6 ± 0.0013^a	0.6 ± 0.0012^a	0.6 ± 0.0011^a	0.6 ± 0.0013^a	0.6 ± 0.0012^a	0.6 ± 0.0012^a
Final weight (g)	0.76 ± 0.028^a	0.82 ± 0.030^{ah}	0.85 ± 0.032^{ab}	0.95 ± 0.037^h	0.98 ± 0.036^b	0.77 ± 0.029^a
Final length (cm)	4.5 ± 0.36^a	4.9 ± 0.39^a	5.5 ± 0.43^{ab}	5.8 ± 0.53^{ab}	6.0 ± 0.62^c	4.8 ± 0.39^a
Weight gain (g)	0.73 ± 0.08^a	0.78 ± 0.09^{ab}	0.82 ± 0.09^{ab}	0.92 ± 0.1^b	0.95 ± 0.11^b	0.74 ± 0.08^a
FCR	2.12 ± 0.14^b	2.03 ± 0.17^b	1.89 ± 0.21^{ab}	1.7 ± 0.36^a	1.53 ± 0.17^a	2.056 ± 0.10^b
SGR	14.48 ± 0.15^a	14.59 ± 0.19^a	14.75 ± 0.24^{ab}	15.08 ± 0.50^{bc}	15.26 ± 0.25^c	14.55 ± 0.12^a
Survival	81.3 ± 2.31^a	80 ± 4.00^a	80 ± 4.00^a	85.3 ± 2.31^{ab}	89.3 ± 4.62^b	82.7 ± 2.31^a

Means with the same superscript along the row are not significantly different (p□0.05)

Table 2: Growth parameters of *O. niloticus* under different levels of *Tribulus terrestris* extract in the pond

	T_1	T_2	T_3	T_4	T_5	T_6
Initial weight (g)	0.76 ± 0.028^a	0.82 ± 0.030^{ab}	0.85 ± 0.032^{ab}	0.95 ± 0.037^b	0.98 ± 0.036^b	0.77 ± 0.029^a
Initial length (cm)	4.5 ± 0.36^a	4.9 ± 0.39^a	5.5 ± 0.43^{ab}	5.8 ± 0.53^{ab}	6.0 ± 0.62^c	4.8 ± 0.39^a
Final weight (g)	12.37 ± 1.37^a	14.62 ± 1.32^{ab}	15.96 ± 1.47^b	17.06 ± 1.42^c	18.04 ± 1.8^c	14.83 ± 1.38^{ab}
Final length (cm)	11.3 ± 1.26^a	13.8 ± 1.53^a	15.1 ± 1.68^{ab}	16.1 ± 1.79^b	17 ± 1.89^b	14.2 ± 1.58^{ab}
Weight gain (g)	11.35 ± 1.26^a	12.18 ± 1.33^{ab}	12.94 ± 1.43^{ab}	14.14 ± 1.52^{bc}	14.29 ± 1.59^c	12.06 ± 1.34^{ab}
FCR	1.4296 ± 1.34^a	1.303 ± 1.16^a	1.1356 ± 0.93^a	0.9768 ± 0.83^a	0.8554 ± 0.57^a	1.368 ± 0.84^a
SGR	5.3150 ± 2.64^a	5.3242 ± 2.83^a	5.3618 ± 2.48^a	5.3032 ± 2.35^a	5.2968 ± 2.78^a	5.3582 ± 2.83^a
Survival	91.8 ± 3.40^a	91.7 ± 3.40^a	95 ± 3.52^b	95.3 ± 3.53^b	95.3 ± 3.54^b	90.3 ± 3.34^a

Sex ratio						
Males	33.33±0.58[a]	55.67±3.06[b]	67.67±2.08[c]	79.33±2.52[d]	83.67±4.04[e]	73.00±1.73[d]
Females	66.67±0.57[c]	44.33±3.05[d]	32.33±2.08[c]	20.67±2.52[a]	16.33±4.04[a]	27.00±1.73[b]

Means with the same superscript along the row are not significantly different ($p \geq 0.05$)

Table 3: Water quality parameters monitored during laboratory phase and pond phase

Treatment	Temperature (^{0}C)	pH	D. O (mg/l)	Nitrite (mg/l)	Ammonia (mg/l)
Lab Phase					
T_1	28.9±2.13	7.8+0.061	8.13±0.065	0.015±0.0001	0.070+0.0005
T_2	28.6±2.11	8.0±0.063	7.65+0.061	0.014±0.0001	0.076±0.0006
T_3	28.8±2.13	8.0±0.063	7.48±0.06	0.014±0.0001	0.077±0.0006
T_4	29.2+2.16	8.0±0.063	7.46±0.059	0.016±0.0001	0.077±0.0006
T_5	28.5±2.10	7.9+0.062	7.46±0.059	0.016±0.0001	0.078±0.0001
T_6	29±2.14	7.9+0.061	7.71±0.061	0.015±0.0001	0.061±0.0004
Pond Phase					
	27.2+1.82	7.8±0.14	9.01±2.54	0.082+0.068	8.71±2.98

It is believed that *T. terrestris* affects androgen metabolism, significantly increasing testosterone or testosterone precursor levels (Neychev and Mitev, 2005) and oral administration of *T. terrestris* increased sexual behavior in male rats (Gauthaman *et. al.* 2003).

In this study, the inclusion of MT and *T. terrestris* extract to the fish feed contrary to the addition of *T. terrestris* in the culture water by Kavitha and Subramanian (2011) and Cek *et. al.* (2007a) showed similar results. As against the usual 1:1 ratio of males to females of *O. niloticus*, the treatment with 0.0g/kg of *T. terrestris* extract gave a ratio of 1:2 (males to female); the percentage of males was however, significantly higher than females in all *T. terrestris* and MT treated fish. The percentage of males recorded in the MT treated fish in this experiment was 73% which is similar to the results obtained by Marjani *et. al.* (2009) at the same concentration for Mozambique tilapia. Kavitha and Subramanian (2011), Cek *et. al.* (2007ab) and Kavitha *et. al.* (2012) also reported that percentage of males increased with increase in the concentration of *T. terrestris* in *Peocilia latipinna, P. reticulata* and *Cichlasoma nigrofasciatum*. *Tribulus terrestris* treated 0-day-old larvae showed successful sex reversal and spermatogenesis than untreated progenies.

In the present study, there was a higher recorded percentage of males, growth rates and better feed conversion ratio in the fish treated with *T. terrestris* at 2.0g/kg concentration which compares very well with those recorded for the fish treated with MT. It can therefore be concluded that 2.0g/kg and above concentration of *T. terrestris* can be used successfully to induce sex reversal in *O. niloticus* in place of MT which is more expensive, unavailable and has public health issues related to its use.

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