HISTOLOGICAL AND GONADAL CHANGES IN NILE TILAPIA (*OREOCHROMISNILOTICUS*) FED *TRAGIA BENTHANI* LEAF MEAL.

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Abstract

The study was carried out to investigate the effects of feeding Tragia benthani leaf meal (TBLM) diets (inclusion levels) on the reproduction (reproductive performance; Fecundity, relative fecundity, ovary weight, size of egg, milt count, motility and volume), histology and growth performance of Oreochromis niloticus for 42 days. Five treatments were used (0g; (control) treatment 1), 10g (treatment 2), 20g (Treatment 3), 30g (Treatment 4) and 40g (Treatment 5). Water volume of 25 litres was maintained throughout the period of the experiment and the fish were fed at 4% body weight per day: at 09:00 hr and 17:00 hr. A reduction in motility duration, milt count, milt volume and fecundity were observed as the treatment level increased ($P < 0.05$). The histology of the testis of O. niloticus fed 0g (TBLM)/ kg diet showed primary and secondary spermatocytes in the seminiferous tubules; connective tissue is also clearly seen. In the treatment 10g, 20g, 30g,40g of TBLM/kg diet. There were eroded connective tissue and necrosis. The histological examination of ovary in O.niloticus fed 0g of TBLM/kg diet showed normal histology. In 10g, 20g, 30g and 40g of TBLM/kg, O. niloticus showed increase in interstitial cell and abnormal gonadal development. The histology of testis at concentration 10g, 20g, 30g and 40g showed eroded connective tissue, increase in interstitial cell, disintegration in the seminiferous lobule and necrosis.The liver showed normal hepatocyte in the control experiment and focal coagulative necrosis when fed 40g TBLM/kg diet. The histological observation and milt analysis revealed that Tragia benthani leaf meal is effective as fertility inhibitory agent in O.niloticus.

Introduction

Tilapia is the second most important group of farmed fish after carp, and the most widely grown of any farmed fish on the planet (Burden, 2014). Farmed tilapia production is about 1,500,000 tons (1,500,000 long tons; 1,700,000 short tons) annually with an estimated value of US$1.8 billion. It is almost equal to that of salmon and trout (De Silva *et.al*, 2004).

Tilapia is considered an economic resource and an important food source for man; hence it is farmed in at least 85 countries of the world (Burden, 2014). In Nigeria, tilapia culture production is predominantly an extensive land-based (earthen ponds) system practiced at subsistence levels (Fagbenro, 2002) while commercial tilapia culture is yet to become popular and widespread (Afolabi *et.al*, 2000).Tilapia has acquired notoriety as being among the most serious invasive species in many subtropical and tropical parts of the world. For example, *Oreochromis aureus, O. mossambicus, Sarotherodon melanotheron, Tilapia mariae,* and *T. zilli* have all become established in the southern United States, particularly in Florida and Texas. Cichlids being prolific breeders, when stocked in ponds or tanks will result in large population of small fish with little economic value, hence the need to control its reproduction rate. Fortes (2005), reported seven methods of controlling tilapia populations. They are periodic harvesting of fry and fingerlings, culture of single-sex fish obtained through: manual separation of sexes, hybridization, hormone augmentation and genetic manipulation methods (e.g. androgenesis, gynogenesis, polyploidy and transgenesis). Others are culture in cages, high density culture, and biological control, eradication using organic toxicants and/or other chemicals, and lastly, sterilization. All these methods are costly, the hormone are not accessible, skilled labour are required, predators are not easily obtained and the methods are tedious. The need arises therefore to examine less expensive and appropriate technology in addressing the problems of uncontrolled breeding and precocious maturity in tilapia using biological inhibitory agents (Akin-Obasola and Jegede, 2016). Modern medicine now tends to use the active ingredients of plants with known phytochemicals as traditional remedies for medical challenges although there are continuing research into the efficacy and possible adaptation of these herbal treatments.

Many plants contra-indicated in animals have been used to control the fertility rate of many animal models and according to Priya et.al, (2012), about 62 plants with antiestrogenic, anti-implantation, abortifacient, contraceptive and anti-ovulatory

activities were reported. *Tragia benthani* is usually a sprawling or climbing plant. . It often has it habitat in the floodplain thickets and termitaria in floodplain grassland pan margins in mopane woodland; at elevations from sea level to around 1000 metres. The plant, however, has no edible relevance and are hardly cultivated with a height range of 250m. Because the plant stimulates uterin contractions the roots are given to women with labour pain. The root extracts is therefore taken as an abortifacient, to ease child delivery and as cure for gonorrhoea. (Saganuwan, 2014).

The goal of the study is to evaluate the effects of feeding *Oreochromis niloticus* male and female on the histology of the gonads.
The specific objectives includes:

Assessing the nutrient utilisation and growth of *Oreochromis niloticus* post juvenile fed *Tragia benthani* leaf meal and determining the effects of feeding *Tragia benthani* leaf meal on its reproduction(milt count and fecundity) .

Materials and methods

Experimental fish

Oreochromis niloticus post juveniles (of the same genetic stock) used in this study was purchased from Federal Ministry of Agriculture, Ado – Ekiti, Ekiti State. One hundred and fifty (150) post juveniles of the mean weight 18±0.6 (2month old) were purchased and kept in aerated water and transported to Forestry Wildlife and Fisheries Management Laboratory, Ekiti State University, Ado – Ekiti. The fish was acclimatized in a rectangular tank (55x35x40cm) of 25litres capacity for 3days before the commencement of the experiment.

The tanks were filled with water pumped from a dug well. The fish were fed at 40% crude protein commercial feed during the experiment. The dietary ingredients were Menhaden fish meal, soya meal, yellow maize, cod liver oil, vegetable oil, vitamin - mineral premix and starch. The experimental diets were prepared by supplementing the fish diets with *Tragia benthani* leaf meal at 0g(control), 10g(Treatment 1), 20g(Treatment 2), 30g(Treatment 3) and 40g(Treatment 4)/kg inclusion levels respectively and formulated at 40% crude protein level required by tilapia. The ingredients for each diet were mixed thoroughly with hot water and the diets were pelletized using a pelleting machine with a die size of 4.00mm. The pellets were sun dried and packed into well labelled containers and stored in a cool and dry place.

The experiment was conducted under a standard and controlled environmental condition following the procedures of the Environmental Assessment and Restoration Bureau of laboratories, Florida to define the response of the test organism (*Oreochromis niloticus*) to *tragia benthani*.

The test material, *tragia benthani* leaf was procured from the forest with help of a Plant Scientist from the Department of Plant Science, Ekiti State University, Ado – Ekiti, This was air dried at room temperature and the leaf pounded to powder form. The experiment was replicated thrice. The fish (mean weight : 18.6 ± 0.6g) were picked at random and 10 fish were introduced into the rectangular tank of 25litres water capacity.

Table 1: Composition of diets using *Tragia benthani* leaf meal

Ingredients	T_1 (g/kg diet)	T_2 (g/kg diet)	T_3 (g/kg diet)	T_4 (g/ kg diet)	T_5 (g /kg diet)
Menhaden (Fish meal)	280	280	280	280	280
Soybean Meal	370	370	370	370	370
Corn Meal	250	250	250	250	250
Cod liver oil	30	30	30	30	30
Groundnut oil	20	20	20	20	20
Vit. - Mineral mix	30	30	30	30	30
Starch	20	20	20	20	20
Tragia benthani	-	10	20	30	40
Total	1000	1000	1000	1000	1000

The experiment lasted for 42days and five diets were fed to the fish in each corresponding tank within this period. The treatments were replicated thrice to minimise experimental errors, and all the treatments was subjected to the same environmental conditions. The fish were fed twice daily at 4% of their body weight at 09:00 - 09:30 hours and 17:00 - 17:30 hours for the entire period of the experiment. The feeding rate was adjusted fortnightly according to the increase in body weight. Uneaten feed and faeces was siphoned out of the pond every morning to avoid pollution. The Growth and reproduction data were recorded, and evaluated to know the performance of each diet and the effects of *tragia benthani* leaf meal on both male and female reproductive organs.

Tissue samples from liver, testes, ovaries were fixed in 10% Formalin for a minimum of 24hours. The samples were dehydrated by washing in ascending grades of ethanol cleared with xylene, embedded in paraffin wax, sectioned with a microtome, stained with Hematoxylin and Eosin (H and E) and mounted on Canada balsam. All the sections were examined under light microscope(x10 magnification). The photographs of the slides were taken with an Olympus photomicroscope for observation and documentation. The data collected were statistically analyzed using SPSS (version 21) and one way Analysis Of Variance (ANOVA) and Duncan New multiple range post Hoc test were used, mean differences at $P \leq 0.05$ were considered significant.The dissolved oxygen level, pH and temperature of water in the tank were determined using digital dissolved oxygen meter, pH meter and Mercury in the glass thermometer respectively at 9am and at 24hrs interval.

Results and discussion

Histological results were interpreted using Morrison et.al, 2006 method

TESTIS

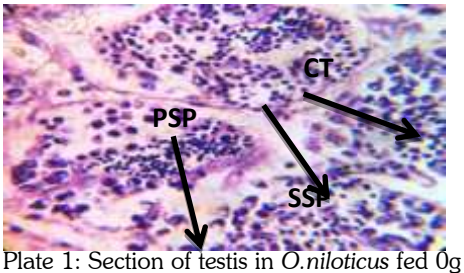

Plate 1: Section of testis in *O.niloticus* fed 0g of TBLM/kg diet showing primary (PSP) and secondary spermatocytes (SSP) in the lumen of the seminiferous tubule. Connective tissue (CT) was also prominent. Mag. X100.

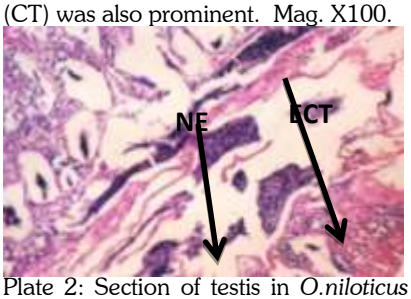

Plate 2: Section of testis in *O.niloticus* fed 10g of TBLM/kg diet showing eroded connective tissue (ECT) and necrosis (NE). Mag. X100

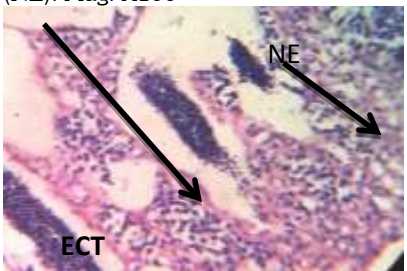

Plate 3: Section of testis in *O.niloticus* fed 20g of TBLM/kg diet showing severe erosion of connective tissue (ECT) and necrosis (NE). Mag. X100

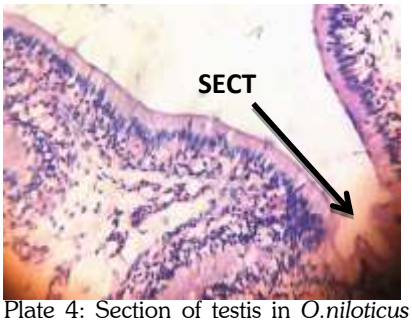

Plate 4: Section of testis in *O.niloticus* fed 30g of TBLM/kg diet showing severely eroded connective tissue (SECT) Mag. X100

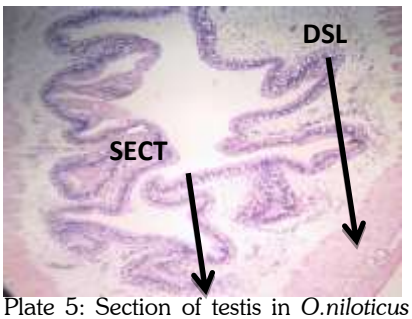

Plate 5: Section of testis in *O.niloticus* fed 40g of TBLM/kg diet showing severely eroded connective tissue (ECT) and disintegration in the seminiferous lobule (DSL) Mag. X100

OVARY

Plate 6: Section of ovary in *O.niloticus* fed 0g of TBLM/kg diet showing normal histology with lumen filled with yolk droplets (YD) that tends to coalesce and become larger. Mag.X100.

Plate 7: Section of ovary in *O.niloticus* fed 10g of TBLM/kg diet showing increase interstitial cell (IIC).

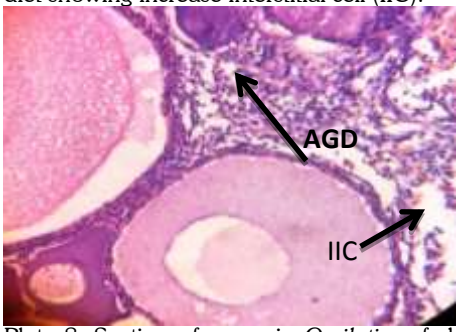

Plate 8: Section of ovary in *O.niloticus* fed 20g of TBLM/kg diet showing increase in interstitial cell (IIC) and abnormal gonadal development (AGD).

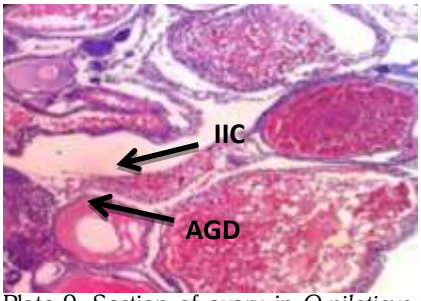

Plate 9: Section of ovary in *O.niloticus* fed 30g of TBLM/kg diet showing increase in interstitial cell (IIC) and abnormal gonadal development (AGD).

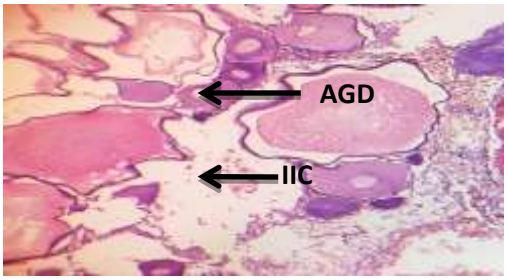

Plate 10: Section of ovary in *O.niloticus* fed 40g of TBLM/kg diet showing increase in interstitial cell (IIC) and abnormal gonadal development (AGD).

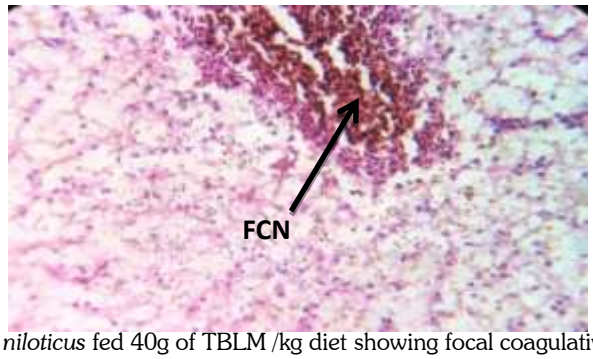

Plate 11: Section of liver in *O.niloticus* fed 40g of TBLM /kg diet showing focal coagulative necrosis (FCN) Mag. X100.

Table 2: Reproductive performance in female *Oreochromis niloticus* fed *Tragia benthani* leaf meal.

Parameters	0g	10g	20g	30g	40g
Fish Initial weight(g)	10.33 ± 0.20^{d}	7.29 ± 0.10^{c}	13.37 ± 0.1^{5e}	10.19 ± 0.00^{b}	10.00 ± 0.0^{a}
Fish Final weight(g)	19.15 ± 0.25^{c}	13.44 ± 0.00^{a}	20.77 ± 0.00^{d}	17.59 ± 0.00^{ab}	17.35 ± 0.00^{b}
Ovary weight	1.53 ± 0.00^{d}	1.16 ± 0.00^{c}	1.16 ± 0.00^{c}	1.09 ± 0.00^{b}	0.94 ± 0.00^{a}
Size of Egg	0.91 ± 0.00^{c}	0.93 ± 0.00^{d}	0.86 ± 0.00^{b}	0.85 ± 0.00^{b}	0.81 ± 0.00^{a}
Fecundity	142.34 ± 0.00^{d}	89.50 ± 0.00^{c}	88.17 ± 0.00^{c}	68.00 ± 0.00^{b}	56.00 ± 0.00^{a}

Table 3: Reproductive Performance of male *Oreochromis niloticus* fed *Tragia benthani* Leaf meal.

Parameters	0	10	20	30	40
Fish Initial Weight(g)	10.30 ± 0.20^{c}	7.20 ± 0.10^{b}	10.30 ± 0.10^{c}	10.15 ± 0.05^{c}	6.30 ± 0.10^{a}
Fish Final Weight(g)	19.15 ± 0.25^{c}	13.35 ± 0.15^{a}	17.70 ± 0.10^{b}	17.55 ± 0.15^{b}	13.65 ± 0.50^{a}
Milt Vol.(ml)	0.42 ± 0.02^{a}	0.35 ± 0.05^{a}	0.27 ± 0.07^{a}	0.25 ± 0.04^{a}	0.26±0.01a
Testes Weight (g)	0.54 ± 0.01^{a}	0.53 ± 0.01^{a}	0.51 ± 0.01^{a}	0.51 ± 0.01^{a}	0.50 ± 0.01^{a}
Motility (Min)	4.35 ± 0.15^{e}	3.84 ± 0.14^{d}	3.47 ± 0.05^{c}	1.92 ± 0.07^{b}	0.11 ± 0.01^{a}
Milt Count (S/Min)	2.81 ± 0.11^{c}	1.72 ± 0.10^{b}	1.47 ± 0.07^{b}	0.96 ± 0.06^{a}	1.00 ± 0.00^{a}

Table 4: Growth and nutrient utilization of female *O. niloticus*

Parameters	0	10	20	30	40
Growth Rate	0.21 ± 0.00^{c}	0.14 ± 0.00^{a}	0.17 ± 0.00^{b}	0.17 ± 0.00^{b}	0.17 ± 0.00^{b}
Feed Fed	17.21 ± 0.41^{c}	12.10 ± 0.20^{b}	17.39 ± 0.25^{c}	17.01 ± 0.21^{c}	10.56 ± 0.14^{a}
Weight Gained	8.85 ± 0.05^{c}	6.15 ± 0.05^{a}	7.40 ± 0.00^{b}	7.40 ± 0.10^{b}	7.35 ± 0.05^{b}
FCR	1.94 ± 0.03^{b}	1.96 ± 0.01^{b}	2.35 ± 0.05^{c}	2.29 ± 0.00^{c}	1.44 ± 0.03^{a}

Discussion

The testes of *Orechromis niloticus* in plate 1 fed 0g of (TBLM)/Kg diet showed primary and secondary spermatocytes in the lumen of the seminiferous tubules and connective tissue was prominent. This corroborate the work of Jegede (2011) who worked on the effects of Aloe vera (lilaceae) on the Gonad development in Nile Tilapia , *Oreochromis niloticus* (Linnaeus 1758) and reported severe tissue atrophy,

spermatids disintegration and necrosis in fish fed 20g aloe vera meal. As shown in plate 2, the section of testis in *O.niloticus* fed 10g of TBLM/kg diets showing eroded connective tissue and necrosis. While in plate 3 and 4 where the fish was fed 20g and 30g respectively, the testis section showed severe erosion of connective tissue and necrosis. This supports the report of Akin Obasola and Jegede (2013) where *Coptodon zilli* was *Mormodica charantia* leaf meal diet and reported necrosis, very few matured germinal cells in the lumen of proceeding diets and disintegration in sperm cells in fish fed 60g *Mormodica charantia* leaf meal.

Histological section of ovary in *O. niloticus* fed 0g of TBLM/ kg diet (plates 6) showed normal histology with lumens filled with yolk droplets(YD) that tends to coalesce and become larger. This corroborates the report of Jegede and Fagbenro (2008b) on the histology of gonads in *Tilapia zilli* (Gervais) fed Neem leaf meal diets.

It was noted that the control diet showed normal ovary histology and no pathological lesion was observed. Histological section of ovary in *O.niloticus* fed 10g of TBLM/ kg diet (plate7) was similar to fish fed 20g, 30g and 40g of TBLM/kg diet except for the observations of abnormal gonadal development (AGD) recorded in the latter treatments. They all had increased interstitial cells (IIC). It corroborates the report of Jegede (2010) in the control of reproduction in *Oreochromis niloticus* (linnaeus 1758) using *Hibiscus rosa- sinensis* (linn) leaf meal as a reproduction inhibitor with fish fed 1.0g of HLM/kg diet showed slight increase in Interstitial cell in testes, fish fed 2.0g HLM/kg diet showed swollen spermatids nuclei, increased interstitial cells and Focal necrosis in testes and hydropic degeneration and ruptured Follicles.

In plate 11 section of liver in *O. niloticus* fed 40g of TBLM/kg diet showed Focal coagulative necrosis (FCN). Akin- obasola and Jegede (2016) also reported Focal coagulative necrosis in the section of liver of *O. niloticus* fed 5g, 10g, 15g and 20g of *Gossypium herbaceum* root bark /kg diets.

The inclusion of *Tragia benthani* for 42days in the diets of *Oreochromis niloticus* at varying levels of 0,10,20,30 and 40g/kg diets revealed normal oval shape of egg. No harmful change was noticed in the shape of the eggs when observed under electronic.

This result is related to the inference of Obaroh and Nzeh (2013) who reported normal Oval shape of egg when *Azadirachta Indica* (0, 1.0,2.0,4.0 and 8.0g/kg) was fed to *Oreochromis niloticus*. It is evident in table 2 that size of egg reduced as the inclusion of *Tragia benthani* leaf meal increased (0.91±0.00, 0.93±0.00, 0.86±0.00, 0.85±0.00, 0.81± 0.00). There were no significant differences between the size of egg of fish fed 20, 30 and 40g TBLM diets. Likewise, the control and fish fed 10g of TBLM shared a similar significance at ($P<0.05$). Fecundity decreased (142.34±0.00, 89.50±0.00, 88.17± 0.00, 68.00±0.00 and 56.00±0.00) as the inclusion level of *Tragia benthani* increases. A similar result was obtained by Abdelhak et.al (2013) who reported the same reduction in fecundity when *Carica papaya* of 60, 90 and 120g/kg diets were fed to *Oreochromis niloticus.*

In table 3, milt volume was higher in control experiment (0.42 ±0.01, 0.35±0.05, 0.27±0.07,0. 15±0.04, 0.24±0.01) and decreased with increasing concentration of *Tragia benthani* in the diet($P<0.05$). Milt count was also higher in the control diet and reduced with increase in treatment level (2.81±0.11, 1.72±0.10, 1.47±0.07, 0.96±0.06 and 1.00±0.00 respectively). Motility duration also decreased with increase in the concentration TBLM (4.35±0.15, 3.84±0.14, 3.47±0.05, 1.92±0.07 and 0.11± 0.01 respectively). Jegede (2011) reported similar decrease in milt motility and milt count when *0.niloticus* was fed 1.0, 1.5 and 2.0ml/kg diets of Aloe Vera diets for 60days.

It was shown in table 4 that the best overall growth response was noticed in the control treatment which was fed basal diet. Weight gained, specific growth rate and Final weight of fish had high values in the control experiment. Nonetheless, feed conversion ratio, weight gain and Growth rate. In fish fed 20g TBLM diet is the next to the high values of the control diet. Hence, it corroborate the inferences of Akin-Obasola and Jegede, (2016) who reported that in animals, castration may be intended for favoring a given desired development and this may have improvement in weight added. There were no significant difference ($P<0.05$) in growth performance of fish fed 20,30 and 40g of TBLM diet. While 10g diet was significantly different. Weight gained was between 6.15±0.20 and 8.85±0.05, Feed conversion ratio was between 1.5 and 2.00 as recommended by Ofori et. al, (2009).

In conclusion, *Tragia benthani* leaf meal possesses anti-fertility agents capable of reducing motility duration, testes weight, relative fecundity, ovary weight, size of egg and milt count of *O. niloticus* at higher concentrations therefore, the use of TBLM diets to control reproduction in Tilapia is advocated.

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