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Research Article
Growth Performance and Reproduction Control of *Oreochromis niloticus* Fed *Calotropis procera* (Giant Milk) Leaf Meal as Fertility Inhibitor

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Abstract
Background and Objectives: The early maturation of *Oreochromis niloticus* results in overpopulation of tanks, poor growth and production rate hence the need to sterilize and control its reproduction for better harvest. The specific objectives of this study were to: assess the nutrient utilization of *Oreochromis niloticus* fed *Calotropis procera* leaf meal (CPLM), determine the effect of CPLM diets on the growth performance and reproduction control in *Oreochromis niloticus* (Nile Tilapia). **Materials and Methods:** This experiment was lasted for 62 days. Control treatments (T1) and treatments 2, 3, 4 and 5 were prepared adding 0, 6, 12, 18 and 24 g of milled *Calotropis procera* leaf kg^{-1} diets respectively to provide 40% crude protein required by tilapia. **Results:** The histology of ovary in *Oreochromis niloticus* fed CPLM diet at different levels revealed that the ovary was filled with developing oocytes at the level of 6 g kg^{-1} of diet, predominantly degenerating oocytes at level of 12 g and 18 g kg^{-1} of CPLM and alteration in ovary development, consisting of predominantly degenerating oocytes at the highest level (24 g). In the control experiment, the oocytes were separated by the stroma cells and interstitium was free from clustering. The histology of testis in *Oreochromis niloticus* fed CPLM diet at the level of 6 g kg^{-1} of diet showed spermatogenetic series at varying degree of maturation, increase in necrosis at the level of 12 g, great distention and loss of spermatogenic cells within the seminiferous tubules at levels of 18 and 24 g. However, the control treatment showed normal architecture. Histology of liver in *Oreochromis niloticus* fed CPLM showed infiltration of inflammatory cells at the level of 6 g kg^{-1} of diet, loss and degeneration of hepatocytes at the level of 12 g kg^{-1} of CPLM, loss and degeneration of hepatocytes and vascular congestion at the level of 18 g kg^{-1} of CPLM and severe loss and degeneration of hepatocytes at the level of 24 g kg^{-1} of CPLM. Histology of gills in *Oreochromis niloticus* fed CPLM diet at different levels revealed degenerative changes which were consistent with autolysis at the level of 6 g kg^{-1} of diet, necrosis and degenerative changes revealed at the level of 12 g kg^{-1} of diet, inflammatory cell infiltration, hyperplastic epithelia congestion and features suggestive of telangiectasis were revealed at 18 g while necrosis and inflammatory cell infiltration, hyperplastic epithelia (HPE) were revealed at the level of 24 g kg^{-1} of diet. However control treatment appeared normal. **Conclusion:** At the end of the experiment, there were significant differences in growth performance of fish at different treatments used. The best growth response was noticed in treatment 5 where the weight gain, feed conversion ratio and specific growth rate were the best. The results obtained showed that *Calotropis procera* could be used as anti-fertility agent in controlling *Oreochromis niloticus* reproduction and its growth performance.

Key words: *Calotropis procera*, fertility inhibitor, medicinal plants, *Oreochromis niloticus*, reproduction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The annual production of tilapia in Nigeria is over 1.5 million tonnes[1] and *Oreochromis niloticus* is one of the most popular tilapia fish cultured in Nigeria. Taxonomist has a special interest in this fish majorly for research purpose. Tilapia spawn and produce offspring with ease and these make them good fish to farm. However, several challenges are commonly associated with their massive reproductive ability that inhibit their full aquaculture potential, these include precocious maturity and frequent breeding behavior leading to increased competition for supplementary food, stunted somatic growth, crowding and stunting, competition between fries and fingerlings for food, space and dissolved oxygen[2,3].

The precocious maturity and prolific breeding nature of tilapia species in aquaculture presents numerous production challenges such as poor growth, feed utilization competition, health and welfare. As a result, various techniques for controlling tilapia reproduction have been developed. Such methods include, periodic harvesting of fry and fingerlings, monosex culture, culture in cages, high density culture, biological control and sterilization[4]. Among these methods, hormonal sex reversal using exogenous steroids, mainly 17 alpha methyl testosterone (MT), yields high success rate which is the most widely used method in producing all male tilapia, nevertheless, the carcinogenic potential of MT and the associated adverse effects on human health and aquatic ecosystem is of public health concern[5-7].

Because of these aforementioned challenges, plant extracts are becoming an integral part of fish culture, as alternatives to chemicals, drugs and hormones, in response to the increasing pressure to reduce the adverse impacts on aquaculture, human and environmental health as well as the increasing global demand for organically produced agricultural products, including fish[7,8]. The organic plant products are relatively safe, inexpensive and easy to prepare and are thus viewed as a means to achieve sustainable fish production[9,10].

Calotropis procera (giant milk) is a species of flowering plant in the family of *Apocynaceae* that is native to North Africa, tropical Africa, Western Asia, South Asia and Indochina, it is commonly known as giant milk and "*bomubomu*" in Yoruba. The green fruits contain a toxic milky sap that is extremely bitter and turns into a gluey coating which is resistant to soap. It is a multipurpose plant, which can be utilized for medicine, fodder and fuel purposes, timber and fiber production, phytoremediation and synthesis of nano particles. It has been widely used in traditional medicinal systems, it is being extensively explored for its potential pharmacological applications[11]. Water soluble compounds were more abundant in *Calotropis procera* than alcohol soluble compounds and contained many biological active chemical groups including, cardenolides, steroids, tannin, glycosides, phenols, tarpenoids, sugars, flavonoids, alkaloids and saponins which can inhibit fertility in fish and has been extensively used to control fertility in rats[12,13]. *Calotropis procera*, with anti-fertility properties, may likely offer possible solution to the precocious growth and uncontrolled reproduction in tilapia. This study was designed to determine the effect of *Calotropis procera* leaf meal (CPLM)diets on the growth performance and control of reproduction in *Oreochromis niloticus* (Nile Tilapia).

MATERIALS AND METHODS

Collection of plant: *Calotropis procera* (Giant milk) Plant was collected from the Department of Plant Science, Ekiti State University, Ado Ekiti. The leaf was air dried at room temperature, milled into fine powder and packaged in an air tight container until it was needed for the experiment.

Collection and selection of *Oreochromis niloticus*: Post juvenile (*Oreochromis niloticus*) (a total of 150) were purchased from the Ministry of Agriculture, Ado Ekiti, acclimated for 72 hrs and after weighing distributed into 15 labelled plastic tanks filled with clean borehole water.

Experimental design: Five isonitrogenous diets were formulated using: Menhaden fish meal (72%), soya bean meal (48%), yellow maize (8.8%), cod liver oil, vegetable oil, vitamin-mineral premix as shown in Table 1. The ingredients were milled and mixed together using hammer mill machine and it was gelatinized in a cool and dry place. The diets were prepared by including *Calotropis procera* leaf in the diets at different grams for each treatment as required, the ingredients for each diet were mixed together thoroughly with hot water and the mixture pelletized using manually made pelleting machine with a die size of 2.00 mm. The pellets were sun dried and packed into well labelled air tight containers and stored in cool and dry place. The treatments were T1 (diet with 0 g kg^{-1} of CPLM as a control), T2 (diet with 6 g kg^{-1} of CPLM), T3 (diet with 12 g kg^{-1} of CPLM), T4 (diet with 18 g kg^{-1} of CPLM) and T5 (diet with 24 g kg^{-1} of CPLM).

The experiment was performed at Fisheries and aquaculture departmental wet laboratory of Ekiti State University, Ado Ekiti under a standard and controlled environment condition using the procedure of Environmental Assessment and Restoration Bureau of Laboratories, Florida

Table 1: Composition (g kg^{-1}) of experimental diet using milled *Calotropis procera* leaf

Ingredient	T_1 (g kg^{-1} diet)	T_2 (g kg^{-1} diet)	T_3 (g kg^{-1} diet)	T_4 (g kg^{-1} diet)	T_5 (g kg^{-1} diet)
Menhaden	275	275	275	275	275
Soya meal	380	380	380	380	380
Yellow maize	235	235	235	235	235
Cod liver oil	30	30	30	30	30
Vegetable oil	20	20	20	20	20
Vitamin-mineral (premix)	30	30	30	30	30
Starch	30	30	30	30	30
Calotropis procera leaf (CPL)	0	6	12	18	24
Total	1000	1000	1000	1000	1000

(The Florida Department of Environmental Protection, Assessment and Restoration content) to be able to define the response of the test organism: *Oreochromis niloticus* to the plant: *Calotropis procera*.

Each treatment was replicated thrice. Feeding of fish commenced after acclimation and it lasted for 70 days. The fish were fed at 4% body weight and twice per day at 08:00-09:00 AM and 18:00-18:30 PM, respectively. Fish were weighed every 2 weeks and this lasted till the end of the experiment.

Water quality, temperature, dissolve oxygen and pH were monitored using mercury in glass thermometer, dissolve oxygen meter (model number: Mettle Toledo 320) and pH meter respectively, water quality parameters were determined on the first and the last day of the experiment.

Milt production and quality were analysed at the end of the experiment. Two male fish, randomly selected from each treatment were used. Milt was obtained by putting the fish in water containing 2-phenoxyethanol (400 mg L^{-1})[14] to make milt collection easier. Drummond microcaps disposable micropipettes were used to collect the milt by torching the papilla opening with the pipette. Milt volume was measured using syringe of 1.0 mL capacity calibrated in 0.1 mL. Motility duration was determined by placing 1 µL of milt from each male on a Neubauer haemocytometer, a drop of distilled water was added and covered with a slip.

The sperm activity was viewed under Olympus BH2 microscope at 100X magnification and motility was determined by the progressive and no progressive movements of the sperms observed[15]. Milt count was determined by counting the number of spermatozoa in diluted sample (10 µL sperm in 90 µL MFR to make 100) (dilution 1), 10 µL dilution in 90 µL MFR to make 100 (Dilution 2). Aout 0.1 mm of the dilute was dropped on Neubauer haemocytometer and sperm counted under the microscope at 400X magnification using Kumar *et al.*[4] method.

Histological examination of the female and male reproductive organs, gills and liver were carried out at the end of the experiment. Two male and female fishes were taken from each tank, weighed and killed by decapitation. Both the testes, ovary, gills and liver tissues were removed for sectioning and histological examination. The organs were fixed in formal-saline solution made of equal volumes of 10% formalin and 0.9% sodium chloride solution. The organs were removed, put in cassettes and processed in Shandon Citadel 2000 machine after which they were embedded in wax, trimmed and sectioned. Using microtone, histological sections of 5 µm thickness were prepared following standard procedures (Histology Laboratory Manual 2011-2012). Sections were fixed on clean slides and stained with haematoxylin and eosin. Photomicrographs at 100X objective were taken with Leitz (Ortholux II) microscope and camera standard model BHTU-111.

Statistical analysis: Data were analyzed using one-way Analysis of Variance (ANOVA); milt volume, milt count, motility duration and testes weight at different treatment levels were checked. Turkey test was used to compare differences among individual means at $p<0.05$.

Other growth parameters were evaluated as follows:

$$\text{Weight gain} = \text{Final weight } (W_2)\text{-initial weight } (W_1)$$

Specific growth rate was obtained as cited by Ivoke[16]:

$$\text{Specific growth rate (SRG)} = \frac{100 \times \text{logefinal weight } (w_2)\text{-logeinitial weight } (w_1)}{T_1\text{-}T_2}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Weight of feed (g)}}{\text{Fish weight gain (g)}}$$

RESULTS

Histology of *Oreochromis niloticus* fed different levels of *Calotropis procera* leaf meal (CPLM): Figure 1-4 show the histopathology of ovary, testes, liver as well as the gill of *Oreochromis niloticus* fed different levels of CPLM.

Histology of the ovary of *Oreochromis niloticus* fed CPLM diet at different levels: Figure 1a (Section of ovary in treatment 1) Shows histoarchitecture of the ovarian tissue

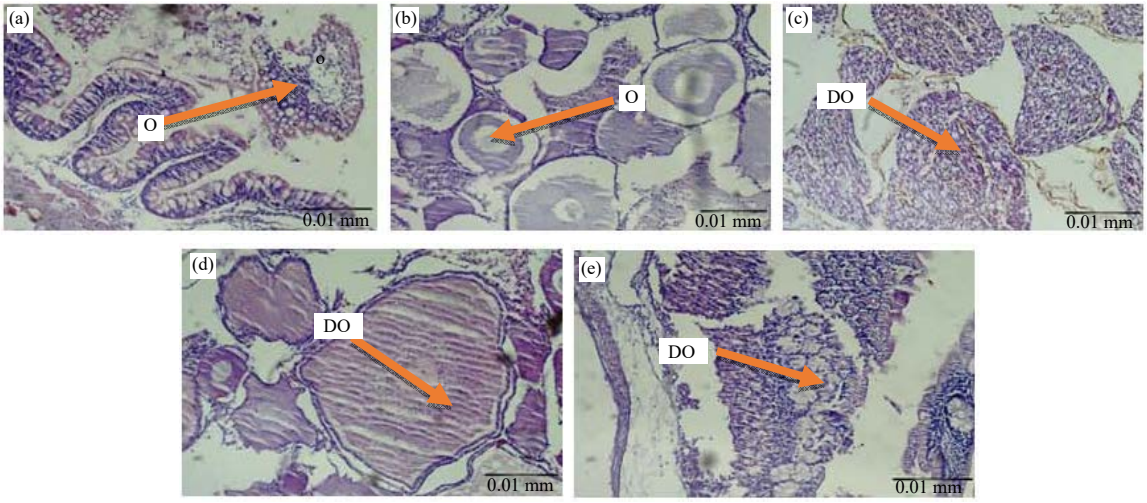

Fig. 1(a-e): Section of ovary in *Oreochromis niloticus* (Mag. ×100) (a) T_1 (b) T_2 (c) T_3 (d) T_4 and (e) T_5

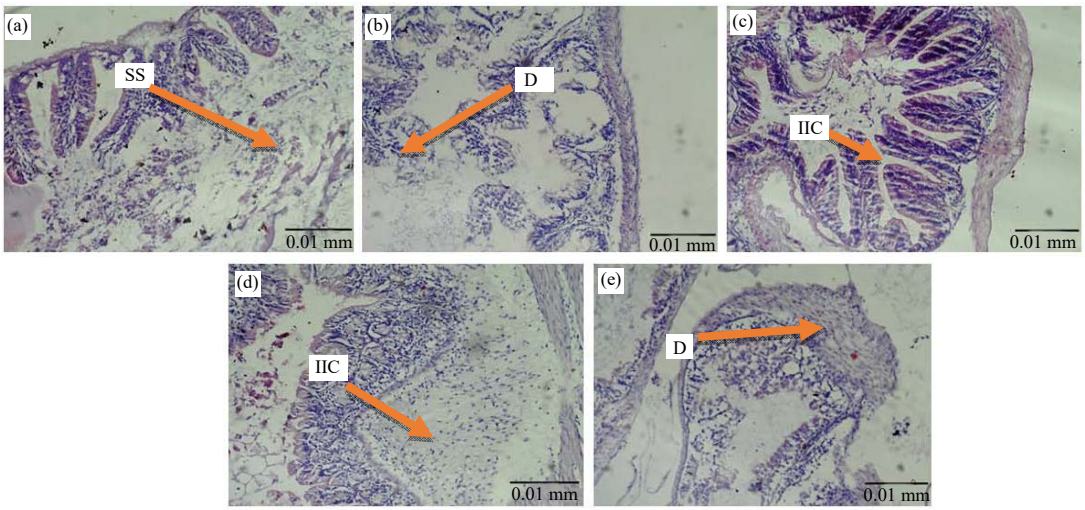

Fig. 2(a-e): Section of testes in *Oreochromis niloticus* (Mag. ×100) (a) T_1 (b) T_2 (c) T_3 (d) T_4 and (e) T_5

which contain the oocytes (O) at varying degree of maturation, the oocytes are separated by the stroma cells and interstitium free from collections. Morphological alterations are consistent with autolysis, while Fig. 1b (Section of ovary in treatment 2) Shows the histoarchitecture of the ovarian tissue composed of the oocytes (O) at varying degree of maturation, the oocytes are separated by the stroma cells and interstitium free from collections. Morphological alterations are consistent with autolysis. Figure 1c (the section of ovary in treatment 3) Consist of predominantly degenerating oocytes (DO). Figure 1d (Section of ovary in treatment 4) Shows the histoarchitecture of the ovarian tissue containing the oocytes at varying degree of maturation, the oocytes are separated by the stroma cells and interstitium free from collections. Figure 1e (Section of ovary in treatment 5) Consist of predominantly degenerating oocytes (DO).

Histology of the testes of *Oreochromis niloticus* fed CPLM diet: Figure 2a shows the histoarchitecture of the testicular tissue which consist of the germinal epithelium containing spermatogenetic series (SS) at varying degree of maturation. The seminiferous tubules are separated by the interstitium and free from collection. Figure 2b (section of testes in treatment 2) Shows distention (D) and loss of spermatogenic cells within the seminiferous tubules. Figure 2c (section of testes in treatment 3) shows testicular tissue comprising of the

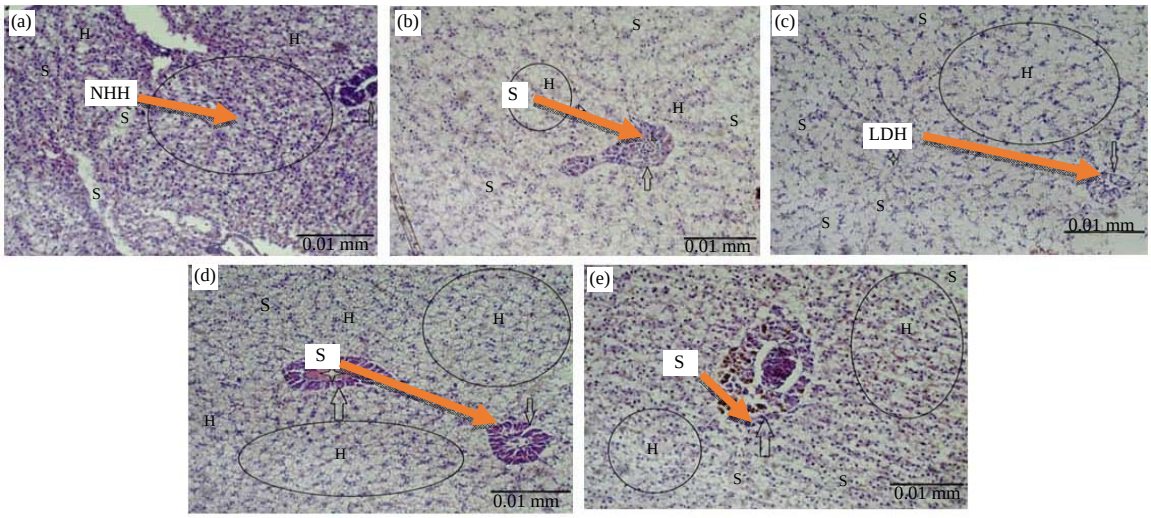

Fig. 3(a-e): Section of liver in *Oreochromis niloticus* (Mag. ×100) (a) T_1 (b) T_2 (c) T_3 (d) T_4 and (e) T_5

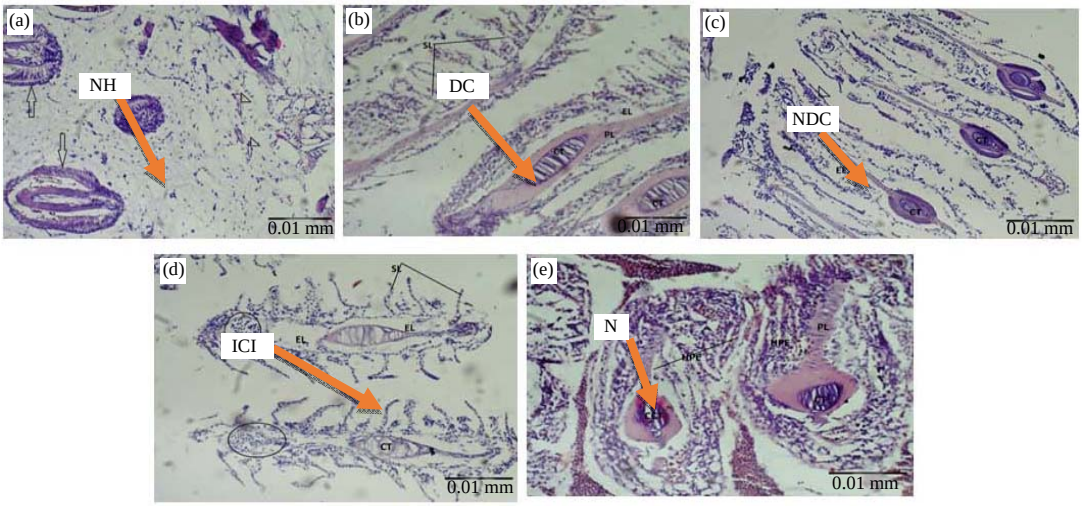

Fig. 4(a-e): Section of gills in *Oreochromis niloticus* (Mag. ×100) (a) T_1 (b) T_2 (c) T_3 (d) T_4 and (e) T_5

germinal epithelium consisting of the spermatogenetic series at varying degree of maturation and increase in interstitial cell (IIC) was noticed. Figure 2d (section of testes in treatment 4) Shows the testicular tissue containing the germinal epithelium. The section revealed increase in interstitial cell (IIC) and necrosis (N). Figure 2e, (the section of testes in treatment 5) Shows the spermatogenetic series with varying degree of maturation and degeneration (D).

Histology of the liver of *Oreochromis niloticus* fed CPLM diet at different levels: Figure 3a (section of liver in treatment 1) Shows predominantly hepatic parenchymal and portal regions. The hepatocytes (H) appear polygonal and are disposed in sheet (Circle) with a well outlined nucleus the hepatocytes are separated by the sinusoids (S) with thin endothelial lining and free from collections. The portal regions consist of hepatic portal vessels (arrow) ducts. The section was consistent and have normal hepatic histoarchitecture (NHH). Figure 3b (Section of the liver in treatment 2) Shows predominantly hepatic parenchymal and portal regions. The hepatocytes (H) appear polygonal and are disposed in sheet (Circle) with a well outlined nucleus the hepatocytes are separated by the sinusoids (S) with thin endothelial lining and free from collections. The portal regions consist of hepatic portal vessels (arrow) ducts. Treatment 3 (Fig. 3c) shows loss and degeneration of hepatocytes (LDH) while Fig. 3d

(treatment 4) shows hepatocytes (H) which appeared polygonal and are disposed in sheet (Circle) with a well outlined nucleus and the hepatocytes was separated by the sinusoids (S) with thin endothelial lining and free from collections. Figure 3e also shows the hepatocytes (H) appearing polygonal and are disposed in sheet (Circle) with a well outlined nucleus. The hepatocytes are separated by the sinusoids (S) with thin endothelial lining and free from collections.

Section of the gill of *O. niloticus* fed with 0 g kg^{-1} of CPLM appeared normal without any form of Hyperplasia (NH), epithelial lifting's and telangiectasia (Fig. 4a). The section was consistent with normal gill histomorphology. Section of the gill of *O. niloticus* fed with 6 g kg^{-1} of CPLM shows degenerative changes (DC) consistent with autolysis (Fig. 4b). Section of the gill of *O. niloticus* fed with 12 g kg^{-1} of CPLM shows necrosis and degenerative changes (NDC) consistent with autolysis (Fig. 4c). Section of the gill of *O. niloticus* fed 18 g kg^{-1} of CPLM shows inflammatory cell (circle) infiltration (ICI), hyperplastic epithelia congestion and features suggestive of telangiectasis (Fig. 4d). Section of the gill of *O. niloticus* fed with 24 g kg^{-1} of CPLM shows necrosis (N) and inflammatory cell (Star) infiltration, hyperplastic epithelia (HPE) (Fig. 4e).

Table 2 shows that the weight gain in *O. niloticus* fed with 0 g kg^{-1} of CPLM (6.99±0.53%) was lower than the groups fed with 6, 12, 18 and 24 g kg^{-1} of CPLM with weight gain 8.24±0.78%, 7.85±0.33%, 8.19±0.46% and 8.54±0.48%, respectively. Feed conversion ratio in the *O. niloticus* fed with CPLM was significantly ($p<0.05$) higher than that of the control group (fed with 0 g kg^{-1} of CPLM). Specific growth rate was not significantly different in groups fed with 6, 12 and 18 g kg^{-1} of CPLM however, there were significant ($p<0.05$) difference in the groups fed with 0 and 24 g kg^{-1} of CPLM.

Reproduction performance of *Oreochromis niloticus* fed CPLM: Table 3 shows that the weight of ovary and testes reduced significantly ($p<0.05$) as the concentration of CPLM increased. Fecundity was higher in the group fed with 0 g kg^{-1} of CPLM (154) compared to those fed with 24 g kg^{-1} of CPLM (90). Also, milt counts reduced significantly as the concentration of CPLM increased [0 (2.82 sec min^{-1}), 6 (2.01 sec min^{-1}), 12 (1.68 ses min^{-1}), 18 (1.42 sec min^{-1}) and 24 (0.94 sec min^{-1})]. The same trend was noted for size of egg.

Physicochemical analysis of water used at the initial and the final week: Table 4 shows that there were no significant changes in the initial (22.65±0.10°C) and final (23.23±0.34°C) temperature, pH (6.47±0.01-initial, 6.51±0.05-final) of control group (T1). The initial dissolve oxygen (DO) of T1, T2, T3, T4 and T5 were 5.17±0.03, 4.81±0.33, 4.22±0.02, 3.39±0.25 and 3.11±0.01 Mg L^{-1} respectively with a reduced DO in the final week.

DISCUSSION

Histology of the ovary of *Oreochromis niloticus* (Plate 1) showed the histoarchitecture of the ovarian tissue which contain the oocytes at varying degree of maturation. In *O. niloticus* fed with 6 g kg^{-1} of CPLM, the ovary was filled with developing oocytes. This result agrees with a previous study conducted by Akin-Obasola *et al.*[17] where *Coptodon zillii* fed

Table 2: Growth and nutrient utilization of *Oreochromis niloticus* fed *Calotropis procera* leaf meal

	Concentrations (g kg^{-1})				
Parameters	0	6	12	18	24
Initial weight (g)	10.34±0.44^{a}	10.67±0.48^{a}	9.67±0.23^{a}	9.34±0.42^{a}	11.99±0.44^{a}
Final weight (g)	17.33±0.97^{c}	18.92±0.16^{a}	17.52±0.56^{c}	17.53±0.88^{c}	20.53±0.92^{b}
Weight gain (g)	6.99±0.53^{b}	8.24±0.78^{a}	7.85±0.33^{c}	8.19±0.46^{a}	8.54±0.48^{a}
Feed fed (g)	10.00±0.27^{a}	15.24±0.20^{a}	15.15±0.21^{a}	15.80±0.13^{a}	15.46±0.42^{a}
Feed conversion ratio	1.43±0.50^{a}	1.85±0.32^{d}	1.93±0.12^{b}	1.93±0.17^{c}	1.81±0.41^{d}
Specific growth rate	1.08±0.28^{b}	0.81±0.12^{a}	0.79±0.27^{a}	0.84±0.07^{a}	0.91±0.04^{c}

Means with the same subscripts are not significantly different at 5% probability

Table 3: Reproduction performance of *Oreochromis niloticus* fed *Calotropis procera* leaf meal

	Concentrations (g kg^{-1})				
Parameters	0	6	12	18	24
Initial weight (g)	8.77±0.22^{a}	9.42±0.44^{a}	8.52±0.15^{a}	8.20±0.27^{a}	10.72±0.35^{a}
Final weight (g)	17.33±0.97^{c}	18.92±0.16^{a}	17.52±0.56^{c}	17.53±0.88^{c}	20.53±0.92^{b}
Ovary weight (g)	1.42±0.05^{a}	1.25±0.03^{b}	1.19±0.03^{c}	1.13±0.04^{d}	1.00±0.07^{e}
Testes weight (g)	0.53±0.02^{a}	0.50±0.01^{a}	0.49±0.04^{a}	0.49±0.02^{a}	0.49±0.03^{a}
Fecundity	154.00^{a}	100.00^{b}	98.00^{c}	93.00^{d}	90.00^{e}
Milt count (sec min^{-1})	2.82^{a}	2.01^{b}	1.68^{d}	1.42^{d}	0.94^{c}
Size of egg (g)	0.93^{a}	0.91^{b}	0.87^{c}	0.84^{c}	0.81^{c}

Means with the same subscripts are not significantly different at 5% probability

Table 4: Physicochemical analysis of water used at the initial and the final weeks

	Initial			Final		
	Temperature (°C)	pH	DO (Mg L^{-1})	Temperature (°C)	pH	DO (Mg L^{-1})
T_1	22.65±0.10[a]	6.47±0.01[a]	5.17±0.03[a]	23.23±0.34[a]	6.51±0.05[a]	5.12±0.07[d]
T_2	22.60±0.35$_a$	6.27±0.02[b]	4.81±0.33[b]	22.21±0.34[a]	6.42±0.31[b]	4.20±0.00[d]
T_3	21.17±0.33[b]	6.21±0.02[c]	4.22±0.02[c]	22.29±0.27[a]	6.24±0.11[c]	3.47±0.03[a]
T_4	22.50±0.45[a]	6.11±0.25[c]	3.39±0.25[d]	22.44±0.22[a]	5.40±0.19[d]	3.21±0.00[b]
T_5	22.67±0.27[a]	5.80±0.02[c]	3.11±0.01[d]	23.09±0.03[a]	5.20±0.07[d]	3.03±0.01[c]

Means with the same subscripts are not significantly different at 5% probability
Key; Treatment 1: 0 g kg^{-1} of *Calotropis procera* leaf meal (CPLM), Treatment 2: 6 g kg^{-1} of CPLM, Treatment 3: 12 g kg^{-1} of CPLM, Treatment 4: 18 g kg^{-1} of CPLM and Treatment 5: 24 g kg^{-1} of CPLM

with 0 g (*Abrus precatorius* Root Bark Diets) APRB kg^{-1} diet. APRB kg^{-1} showed developing oocytes surrounded by a thin wall. *O. niloticus* fed with 6 and 12 g kg^{-1} of CPLM showed predominantly degenerating oocytes. This result corroborates with the findings of Jegede[18] who used the *Hibiscus rosa-sinensis* (linn) leaf meal as a reproduction inhibitor to control reproduction of *Oreochromis niloticus* (linnaeus 1758), fish fed 2.0 g HLM kg^{-1} diet showed swollen spermatids nuclei, hydropic degeneration and ruptured Follicles. Section of the ovary of *O. niloticus* fed with 18 and 24 g kg^{-1} of CPLM showed predominantly deteriorating oocytes. This result agrees with a previous study conducted by Jegede[18] where reproduction of *O. niloticus* was controlled using Hibiscus *(Rosa-sinensis)* leaf meal at concentration of 1.0 g. *O. niloticus* fed with 24 g kg^{-1} of CPLM revealed no oocyte in the lumen and alteration in ovary development. Also, Padhy *et al*.[19] reported that the extract of *Calotropis procera* is potent contraceptive in rats and the ethanolic extract of the plant reduced estrogen, uterus weight and prevent pregnancy in rat.

Histology of the testes of *Oreochromis niloticus* fed CPLM diet (section of testes in Plate 1) (fed with 0 g kg^{-1} of CPLM) showed the histoarchitecture of the testicular tissue consist of the germinal epithelium consisting of the spermatogenetic series at varying degree of maturation. The seminiferous tubules were separated by the interstitium and was free from collection while degenerative changes were consistent with autolysis. This result agrees with Akin-Obasola and Jegede[20] who used *Mormodica charantia* leaf meal as fertility inhibitor in *T. zilli*, the histological analysis revealed necrosis, severe atrophy with very few matured germinal cell in the lumen of preceding ducts and disintegration in sperm cells in fish fed 40, 60 and 80 g of the diet; and similar results were reported by Fang[21] who fed 2 g of pawpaw seed powder per kg diet to *O. niloticus* for 15 and 30 days. The section of testes in *O. niloticus* fed with 6 g kg^{-1} of CPLM, showed distention and loss of spermatogenic cells within the seminiferous tubules. While the section of the testes of *O. niloticus* fed with 12, 18 and 24 g kg^{-1} of CPLM showed increase in necrosis and great distention and loss of spermatogenic cells within the seminiferous tubules, this result agrees with Akin-Obasola[3] who observed an alteration in the testicular architecture and lystic seminiferous tubules, sever tissue atrophy, spermatids disintegration and necrosis were seen in fish fed 6, 12 and 18 g (Abrus precatorius Root Bark Diets) APRB kg^{-1} diet. Abdelgader and Elsheikh[13] reported that the extracts and latex of *Calotropis procera* reduced the testicular and epididymal weights in rats.

According to the histology of the liver of *Oreochromis niloticus* fed 0 g of CPLM kg^{-1} diet, the portal regions show normal hepatic histoarchitecture. This result agrees with the findings of Akin-Obasola and Jegede[22] who recorded normal hepatocellular architecture of *O. niloticus* fed 0 g of (*Gossypium herbaceum* root bark) GHRB kg^{-1} diet. Section of the liver of *O. niloticus* fed with 6 g kg^{-1} of CPLM showed infiltration of inflammatory cells. *O. niloticus* fed with 12 g kg^{-1} of CPLM showed loss and degeneration of hepatocytes while those fed with 18 g kg^{-1} of CPLM showed loss and degeneration of hepatocytes and vascular congestion. *Oreochromis niloticus* fed with 24 g kg^{-1} of CPLM showed severe loss and degeneration of hepatocytes. Similar results were reported by Obaroh and Nzeh[23] who recorded histological changes in *O. niloticus* fed *Momordica charantia* leaf meal at the higher concentrations.

Oreochromis niloticus fed CPLM diet showed the normal histology of the gills without any form of Hyperplasia, epithelial lifting's and telangiectasia. This agrees with Akin-Obasola *et al*.[17], who recorded normal architecture of gill lamellae and gill ray in *O. niloticus* fed 0 g of (*Martynia annua* leaf meal) MALM kg^{-1} diet.

Growth performance of *O. niloticus* fed CPLM diet changed with time. *Oreochromis niloticus* fed with 24 g kg^{-1} of CPLM showed the best growth performance and weight gain. Significant differences were observed in growth performance of fish fed 6, 12, 18 and 24 g of CPLM diet in each of the weeks.

The pH level decreased as the treatment level increased. There were significant differences in temperature at different weeks. Also the dissolved oxygen had significant difference for all the weeks. Physico-chemical parameters measured confirm the FAO recommendations for tilapia production[1].

CONCLUSION

The use of plant as an anti-fertility agent for animal is earning wide range acceptance in many part of the world today. Medicinal plants exist abundantly in many thick forest or vegetative environment, making their accessibility easier, faster and cost effective. However, medicinal plants often possess several traits which include contraceptive, inhibitory, involuntary, abortifacient and other traits that are capable of altering the histological arrangement and reproductive performances of fish sexuality. Hence, the result of the experiment has shown that CPLM possess anti-fertility agents capable of reducing fecundity and milt count of *Oreochromis niloticus* at higher concentrations, therefore the use of CPLM diet should be advocated.

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