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## Histology of gonads in *Oreochromis niloticus* (Linnaeus 1757) fed cottonseed meal-based diets

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### Abstract

This study was conducted to evaluate the effect of cottonseed meal-based diets on histology of testes and ovaries of *Oreochromis niloticus*. Two hundred (200) apparently healthy *Oreochromis niloticus*, average body weight of (10g ± 0.20) were used for the investigation. The Fish were randomly distributed into five groups each at a rate of twenty (20) fish per treatment. Ten male and ten female fish were stocked in ten (10) concrete tanks (2m x 2m x 1m), each treatment was replicated twice. Five isoproteic diets were formulated at 37.5% crude protein. In the experimental diets, 0%, 25%, 50%, 75%, and 100% of soybean meal protein were substituted with cottonseed meal. The fish were fed at 5% body weight twice daily for 90 days. At the end of the 90 days, the testes and ovaries were sectioned for histological examination. At the end of the experiment, it is only in the control diet (0g CSM/kg diet), that the histological section of the testes of *O. niloticus* showed normal sperm cell distribution and abundant spermatozoa in the seminiferous cavities with no visible lesion. In all the other cottonseed meal-based diets, there were numerous cavities where spermatocytes appeared scanty, scanty lumina of the seminiferous tubules, thick and reduced interstitium/sertoli cyst and reduced lumen size. Cottonseed in the diets was able to destroy the spermatocytes and therefore, there was no reproduction at all in the other cottonseed meal-based diets. Histological section of ovary from *O. niloticus* fed 0g/CSM/kg diet (control) showed normal ovary architecture and the vitellogenic stages, no visible lesion seen. In all the cottonseed meal-based diets, *O. niloticus* ovary showed distorted vitellogenic stages and other stages still in their early phases of development. The damage done to the ovaries could not allow them to complete the reproductive cycle, the eggs could not be hatched and there was no reproduction.

**Keywords:** Cottonseed meal, gossypol, *Oreochromis niloticus*, soyabean meal, crude protein

### 1. Introduction

Tilapia is one of the most productive and internationally traded food fish in the world and a major protein source in many of the developing countries Modadugu and Belen 2004 [26]. Tilapia is farmed in at least 85 countries of the world and is the most broadly reared of any farmed fish on the planet Burden 2014 [5]. Tilapia can be cultured in a wide range of system; this makes them suitable in most of the developing countries where they are cultured in ponds, concrete tanks, raceways, cages and rice fields Fagbenro 2002 [12]. *Oreochromis niloticus* is found in Africa and the coastal rivers of Israel. It is a maternal mouth brooder and becomes sexually matured in 4-5 months at small size often below market weight (10 cm; 20-50g) Balarin and Hatton 1979 [3]. Tilapia's major challenge is still in their early sexual maturity. Mair and Little 1991 [24] appraised several methods in combating this problem, though effective but still have their disadvantages and shortcomings. There is therefore the need to explore natural and indigenous reproductive plants in combating this undesirable trait. Cotton is a natural vegetable fiber obtained from the cotton plant of the genus *Gossypium* and belongs to Malvaceae family. The capsules (seeds) of this plant are formed as soon as the petals fall off and burst open into four parts upon maturity thereby revealing the cottonseeds after which they are harvested mechanically. Cotton and related species all contain gossypol, a polyphenolic compound that is an integral part of the cotton plant's self-defense system against insect pests and possibly some diseases Jodi and Gabriela 2008 [19]. It is a toxic, naturally occurring pigment in cotton plants. Gossypol, present in the stem, seeds and roots of cotton plant, is known to exert unique and selective effects upon reproduction in various species such as fish, rats, mice, hamsters, rabbits, monkeys and human beings Coutinho 2002 [8]. Fish can tolerate higher levels of dietary gossypol than terrestrial animals. [10]. Available information shows

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that the susceptibility of fish to dietary levels of gossypol varies between fish species. Several studies have been conducted on the utilization of cottonseed meal as substitute for fish meal and soyabean meal in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) Yue and Zhou 2008 [31]. Studies on fish have focused on cottonseed meal supplementation, reporting adverse effects on blood parameters (hematocrit and hemoglobin), growth, and development Blom *et al.*, 2001; Rinchar *et al.*, 2003b [4, 29]. Various studies have also been conducted on the nutritional value of cottonseed meal for tilapia Mbahinzireki *et al.*, 2001; El-Saidy and Gaber 2004 [25, 10]. Thus, this study is aimed at examining the effects of cottonseed on the testes and ovaries of *Oreochromis niloticus* as a way to control their reproduction towards a better yield.

## 2. Materials and Methods

### 2.1. Preparation of experimental diets

The study was carried out at the Research Farm and Research Laboratory of the Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, Ondo State. Raw cottonseed used in this study was obtained from Metrovet Veterinary shop Ado-Ekiti and was identified by plant Scientist at The Department of Plant Science, Ekiti State University, Ado-Ekiti as *Gossypium herbaceum*. The seeds were first cleaned in order to remove farm residues after which they were dehulled by manual machine to remove the staples and linters. They were oven dried in the laboratory at a temperature of 130°C, to a moisture content of 12%. The

seeds were then crushed into powder using local milling machine. The crushed sample was weighed and mixed with ethanol. The mixed sample was placed on a folded filter paper and inserted into the soxhlet apparatus. 300ml of the solvent extractant (ethanol, 60°C – 80°C) was measured using a measuring cylinder and then poured into 500ml round bottom flask of the soxhlet apparatus. This was heated at 60°C for 6 hours. As the solvent boiled, it evaporated into the reflux condenser and this hot solvent vapour was cooled by the surrounding water which flowed continuously through the soxhlet apparatus. The cooled solvent then condensed back into the portion of the soxhlet containing the folded sample and this was done until all the oil was extracted from the sample. The extracted sample left after the oil had been removed was subjected to hot pressing using hydraulic press to remove the bulk of the oil remaining and this was what produced the cottonseed meal. The total and free gossypol content of the cottonseed meal was determined according to the method of Botsoglou 1991 [4]. Five isoproteic diets were formulated to provide 37.5% crude protein using the method of Fagbenro & Adebayo 2005 [13]. In the experimental diets, 0%, 25%, 50%, 75%, and 100% of soybean meal protein were replaced by cottonseed meal. This was followed by thorough mixing in a Hobart A-200T pelleting and mixing machine. Hot water was added at intervals to gelatinize starch. The diets were pelletized using a 1.0 mm diameter die and air-dried at ambient temperature for 72 hours to constant moisture content. The dry diets were broken up, sieved, packed in covered plastic containers and labelled.

**Table 1:** Ingredient composition (g/kg) of diets (37.5% CP) used in Experiment.

	Control diet CD0	Gossypol Meal-based Diets			
		GSP1	GSP2	GSP3	GSP4
Fish meal (64.5%CP)	280	280	280	280	280
Soybean meal (47.5%CP)	370	370	370	370	370
Yellow maize (10.8%CP)	200	200	200	200	200
Cod liver oil	30	30	30	30	30
Vegetable oil	20	20	20	20	20
Vitamin-mineral mix <sup>1</sup>	30	30	30	30	30
Corn starch	70	70	70	70	70
Free gossypol mg/kg CSM	0	330	650	980	1310

### Proximate composition

Ingredients	% Moisture	% Ash	% Protein	% Fat	Crude fiber	NFE
Fish meal	12.00	12.00	61.95	14.00	0	0.05
Maize	13.67	2.00	9.63	2.00	2.40	70.30
Soyabean	10.67	8.00	57.93	8.00	4.55	10.85
Cottonseed	2.67	4.00	35.18	14.00	10.53	33.62

Vitamin premix – A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, K<sub>3</sub> = 2 mg, B<sub>12</sub> = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg

Key: CDO=control diet; GSP=gossypol; CSM=cottonseed meal.

### 2.2. Experimental fish and feeding trial

Two hundred (200) apparently healthy male and female *O. niloticus* (10g ± 0.20) were purchased from the Teaching and Research Farm, The Federal University of Technology Akure. After acclimation period, ten male and ten female fish with a mean weight of 20g were stocked in each of the 10 concrete tanks (2m x 2m x 1m) containing clean water, which was sourced from a borehole of the Federal University of Technology, Akure, Ondo State. Each treatment was replicated twice. The fish were fed at 5% body weight twice daily between 9h – 9h and 30 min and 17h- 17h and 30 min

for 90 days. At the end of the 90-day treatment, four male and four female fish were taken from each treatment, two fish from each replicate, sorted by sex and weighed. The fish were killed by decapitation and their testes and ovaries, removed for sectioning and histological examination. They were fixed for 24 hours in formalin-saline solution made of equal volumes of 10% formalin and 0.9% sodium chloride solution using Sharma *et al.*, 2011 [30] method. Histological sections of 8µ thickness were prepared following standard procedures (Histology Laboratory Manual 2011-2012). Photomicrographs were taken with Leitz (Ortholux II)

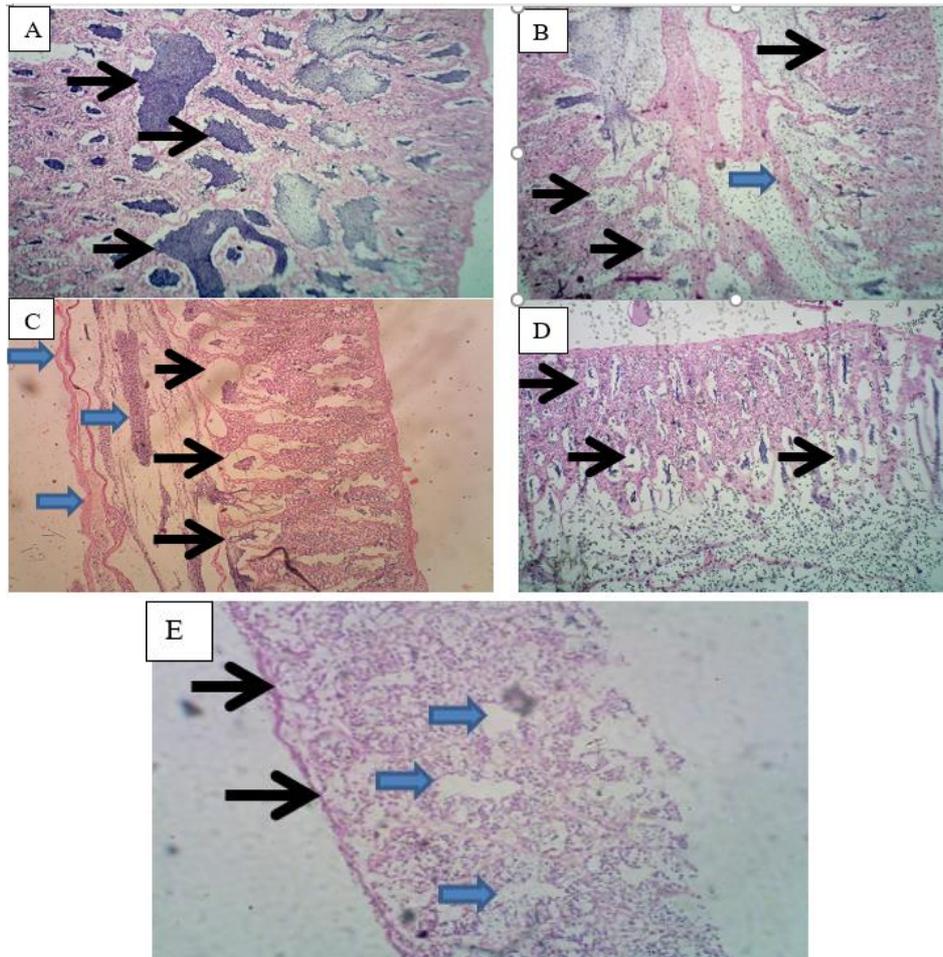
microscope and camera, standard model BHTU-11. The data resulting from the experiments were subjected to one-way analysis of variance (ANOVA) test using the SPSS version 11. Significant differences between the treatments were determined at 5% confidence limit using Duncan's multiple Range Test 1955 [9].

### 3. Results

#### 3.1. Histology of testes in *O. niloticus* fed cottonseed meal-based diet

Section of testes in *O. niloticus* fed the control diet 0% SBM replacement (0g CSM/kg) showed normal sperm cell distribution, abundant spermatozoa in the seminiferous cavities and no visible lesion (black arrows). Histology of

testes in *O. niloticus* fed 25%SBM replacement (109g CSM/kg) diet showed numerous cavities where spermatocytes appeared scanty (black arrows) and thick interstitium/sertoli cyst (blue arrows). Section of testes in *O. niloticus* fed 50%SBM replacement (218g CSM/kg) diet showed scanty lumina of the seminiferous tubules (black arrows); thick interstitium/sertoli cyst (blue arrows). At 75%SBM replacement (327g CSM/kg) diet, histology of testes in *O. niloticus* showed that the lumen is reduced in size, spermatocytes are scanty in the lumina (black arrows). Section of testes in *O. niloticus* fed 100%SBM replacement (436g CSM/kg) revealed greatly reduced interstitial cells (black arrows) and very scanty amount of spermatozoa in the cavities (blue arrows).

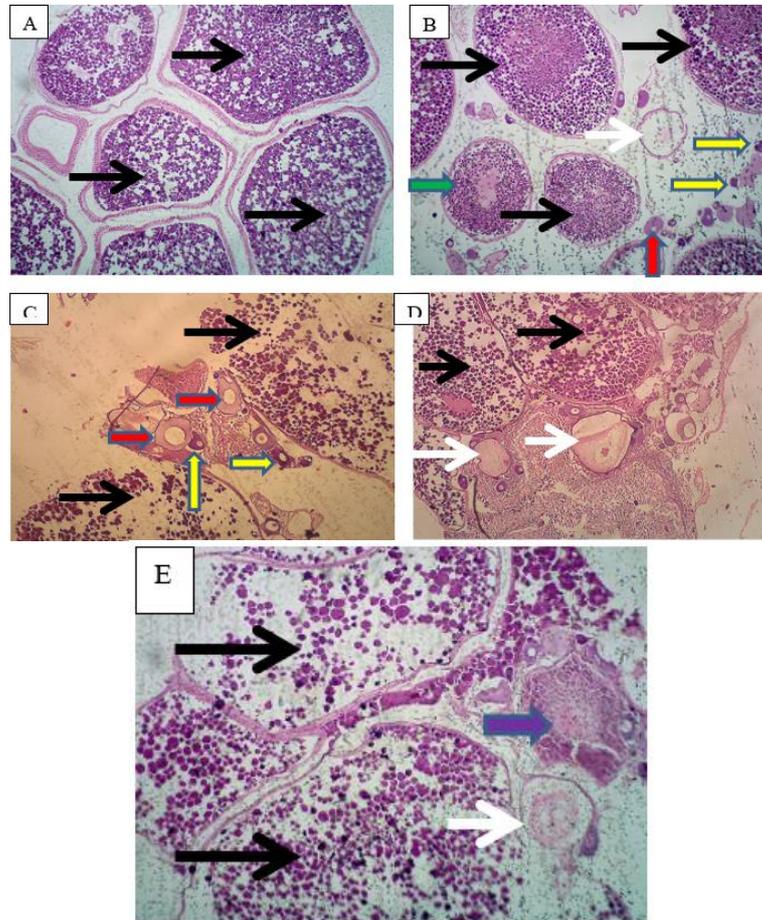


**Fig 1:** Histology of testes in *O. niloticus* fed cottonseed meal- based diet (A) abundant spermatozoa (black arrows). (B) scanty spermatocytes (black arrows), thick interstitium/sertoli cyst (blue arrows). (C) scanty lumina of the seminiferous tubules (black arrows); thick interstitium/sertoli cyst (blue arrows). (D) reduced lumen and scanty spermatocytes (black arrows). (E) reduced interstitial cells (black arrows), scanty spermatozoa (blue arrows). Mag. X40

#### 3.2. Histology of ovary in *O. niloticus* fed cottonseed meal-based diets

Section of ovary in *O. niloticus* fed control diet shows normal architecture of a mature ovary and the vitellogenic stages (black arrow). In *O. niloticus* fed (109g CSM/kg) diet shows the vitellogenic stage (black arrow), yolk granules and fat vacuoles in the ooplasm, basophilic cytoplasm and a conspicuous central nucleus with diffuse chromatin, oocytes breaking from the germinal epithelium and continuing maturation within the folds of the ovigerous lamellae and

atretic oocytes. Section of ovary in *O. niloticus* fed (218g CSM/kg) diet shows unrounded and distorted vitellogenic stage, stage II and III oocytes having a conspicuous central nucleus with diffuse chromatin oocytes breaking from the germinal epithelium. Distorted stage III oocytes alongside vitellogenic stage was observed in *O. niloticus* fed 75%SBM replacement (327g CSM/kg) while in *O. niloticus* fed 100%SBM replacement (436g CSM/kg) diet, histology shows unrounded and distorted vitellogenic stage, distorted stage V oocyte and atretic oocytes.



**Fig 2:** Histology of ovary in *O. niloticus* fed cottonseed meal- based diets (A) normal architecture of a mature ovary and the vitellogenic stage (black arrow). (B) vitellogenic stage (black arrow), stage IV oocytes - yolk granules and fat vacuoles in the ooplasm (green arrow), stage II oocytes - nucleus with diffuse chromatin (yellow arrows), stage III oocytes (red arrow) and atretic oocyte (white arrow). (C) Unrounded and distorted vitellogenic stage (black arrows), stage II oocytes - cytoplasm and a central nucleus with diffuse chromatin (yellow arrows) and stage III oocytes - oocytes breaking from the germinal epithelium and (red arrow). (D) vitellogenic stage V (black arrows) and distorted stage III oocytes breaking from the germinal epithelium (E) unrounded and distorted vitellogenic stage, (black arrow), distorted stage V oocyte (purple arrow) and atretic oocyte (white arrow). Mag. X40

## 4. Discussion

### 4.1. Histology of testes in *Oreochromis niloticus* fed with cottonseed meal-based diets

The results obtained at 1090g CSM/kg, 218 CSM/kg and 327 CSM/kg agrees with Adeyemo *et al.*, 2007<sup>[1]</sup> who fed cottonseed cake-based diets to breeder cocks for 23 weeks and the result showed that dietary treatments that had higher than 50% cottonseed cake replacement for soyabean cake had a significantly lower sertoli cells values than those with lower levels of cottonseed cake, The seminiferous tubule diameters for the different diets followed the proportion of sertoli cells in their tubules and the conclusion was that, any diet that has influence on the number of sertoli cells will influence the size of the seminiferous tubules. The reduced interstitium and scanty spermatozoa at 436g CSM/kg diet agrees with Adeyemo *et al.*, 2007<sup>[1]</sup> who reported that dietary treatments that had higher proportions of spermatozoa were also observed to have a high corresponding proportion of interstitium or sertoli cells. The implication of this is that any substance that limits the activity of sertoli cells will definitely affect sperm production. The relationship between interstitium or sertoli cells and spermatozoa can be explained in terms of the function of the sertoli cells which is known as the nurse cell, which provides nourishment during spermiogenesis. Any activity inhibiting this performance will definitely obstruct and subsequently lowers sperm production Adeyemo *et al.*, 2007<sup>[1]</sup>.

### 4.2. Histology of ovaries in *Oreochromis niloticus* fed with cottonseed meal-based diets

Histological sections of the ovary in *O. niloticus* fed with the control diet (0g CSM/kg diet) showed normal ovary histology, featuring stage 5 vitellogenic of a mature oocyte. This ovary histology in the control group is normal and expected as it is an untreated group according to Elham *et al.*, 2013<sup>[11]</sup> who worked on the effect of pawpaw (*Carica papaya*) seeds meal on the reproductive performance and histological characters of gonads in Nile tilapia (*Oreochromis niloticus*). Sections of the ovary in *O. niloticus* fed with 109g CSM/kg diet also showed the other oocyte stages along with the vitellogenic stages. Jirarach and Kingkaew 2001<sup>[18]</sup> worked on the histological structures of Nile tilapia and this finding is in line with his report that at 3 months and above, histological sections revealed vitellogenic (yolk) stages, increased oocyte size and small yolk granules visible as a ring of deep eosinophilic in the cytoplasm which indicates maturation and imminent spawning Elham *et al.*, 2013<sup>[11]</sup> also reported ovary of medium dose treated *O. niloticus* with *Carica papaya* showing oocyte containing nucleus and yolk granules. The result obtained at 218g CSM/kg diet and 327g CSM/kg diet, is in agreement with the findings of Jegede *et al.*, 2008a<sup>[17]</sup> who fed *O. niloticus* with different doses of *C. papaya* seeds meal for 60 days. They observed severe atretic follicle in ovaries of fish fed high dose (2.0g *C. papaya*

/kg). Similar histological effect was reported on ovaries of *O. niloticus* by using other medicinal plants; *Hibiscus rosa-sinensis* leaf meal Jegede 2010<sup>[15]</sup> and *Aloe Vera* latex Jegede 2011<sup>[16]</sup>. At 436g CSM/kg diet, histological section revealed distorted vitellogenic stages. This result is in line with Jegede 2010<sup>[15]</sup> who controlled the reproduction of *Oreochromis niloticus* using *Hibiscus rosa-sinensis* and reported that at high dose, stage 4 vitellogenesis was observed in which several oocytes that would have been laid were atretic because the physiological conditions were unfavourable for oocyte development and consequent spawning. This result is also in line with Luo *et al.*, 2006<sup>[23]</sup> who fed female rainbow trout with partial or total replacement of fishmeal by solvent-extracted cottonseed meal diets and concluded that female rainbow trout fertility was adversely affected by complete replacement of fishmeal with cottonseed meal. Same report was made by Elham *et al.*, 2013<sup>[11]</sup> of histological effects on the ovary of high dose treated *O. niloticus* fish showing necrosis, many vacuolated and fusion of ova in females of high dose treatment.

## 5. Conclusion

It is obvious from this study that inclusion of cottonseed in the diets of *O. niloticus* affected the tissues of both testes and ovaries as revealed by the histological sections. As the inclusion of cottonseed increased in the diets, more damage was recorded in terms of emptiness of the spermatocytes within seminiferous lumina of the testes and distortion in the ovaries, the eggs could not complete their reproductive cycles prior to hatching and fertilization and the spermatozoa could not fertilize the eggs either because of their emptiness. Thus, in combating precocious breeding and early reproduction in *O. niloticus*, cottonseed meal is a tested and promising remedy.

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