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RESEARCH PAPER

Evaluating the Use of Crude and Synthetic Gossypol as Reproduction Bio-control Agents in *Coptodon zillii* and *Oreochromis niloticus*

Oluwatosin Helen Tope Jegede[1,*]

[1]Federal University Oye, Faculty name, Department of Fisheries and Aquaculture, Oye Ekiti/ Ekiti State, Nigeria

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Corresponding Author
E-mail:
oluwatosin.topejegede@fuoye.edu.ng

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Abstract

Select this text, right click and click Merge Formatting (B) from the paste options. Two hundred (200) *Coptodon zillii* and *Oreochromis niloticus* (10g ±0.13) were subjected to an investigation to uncover the efficiency of crude and synthetic gossypol as reproduction bio-control agents. The treatment groups were five (5) altogether with twenty (20) fish per treatment (male and female 1:1). Each treatment was replicated twice and subjected to the same condition. The fish were fed with five isoproteic experimental diets of 37.5% crude protein for 90 days. In the diets, cottonseed meal (CSM) and synthetic gossypol (GSP) were used to replace soyabean meal (SBM) at 0%, 25%, 50%, 75% and 100% respectively. Histological sections of testes in *O. niloticus* and *C. zillii* showed a decline in spermatozoa with increasing inclusion of crude CSM and synthetic gossypol. Diets four and five which has the highest inclusion of both crude and synthetic gossypol (327g CSM/Kg diet, 436g CSM/Kg diet) exhibited spent and empty seminiferous lumina. Ovarian section in *C. zillii* and *O. niloticus* showed distortion with increasing inclusion rates of crude CSM and synthetic gossypol; to the point that most of the eggs were still in their early phases of embryonic formation after 90 days trial with prominent vitellogenic stages for 0% 0g (CSM/kg diet) and 25% (109 CSM/kg diet); atretic oocytes for 75% (327g CSM/Kg diet) and 100% (436g CSM/Kg diet). Milt and egg production of both *O. niloticus* and *C. zillii* all declined as the dietary inclusion of crude and synthetic gossypol increased. Overall results showed that dietary crude and synthetic gossypol destroyed the spermatocytes and oocytes; lumina was devoid of spermatozoa, subsequent reproduction was inhibited; the eggs were unable to finish their cytological cycles which would have led to fertilization. There were fewer offspring in crude gossypol-based diet treatments and there was no reproduction at all in all the synthetic gossypol-based diet treatments.

Introduction

After carps, tilapia has become widely accepted as a fish for production. According to data from the UN Food and Agriculture Organization (FAO), tilapia production increased from 3.1 million tonnes in 2010 to 5.6 million tonnes in 2018 (FAO, 2020). Tilapia is one of the most widely traded food fish in the world and serves as a primary source of protein for many developing nations (Modadugu and Belen, 2014). Relatively speaking, tilapia are raised in 85 nations worldwide, making them the most commonly reared fish in aquaculture (Burden, 2014). Because of their ease of raising in a wide range of environments, tilapia are suitable and acceptable for raising in cages, concrete tanks, raceways, ponds, and rice fields in many developing countries (Fagbenro, 2002). The popular name for the Cichlidae family, which includes three genera—Sarotherodon, Oreochromis, and Tilapia—comprising over seventy species, is Tilapia (Meyer, 2002). Numerous published literatures exist that discuss reproduction in *C. zillii* (El-Sawy, 2006; Negassa and

Getahun, 2003). Having children in C. Meyer (2002) states that zillii is a substrate that is highly euryhaline, allowing for a wide range of salinity. Maternal mouth brooder *Oreochromis niloticus* is well suited for aquaculture due to its broad range of trophic and environmental acclimation, enabling it to live in a variety of tropical and sub-tropical freshwater alcoves (Trewavas, 1983). Both C. The top ten most often cultivated fish species worldwide are *O. niloticus* and zillii, which together account for 99.5% of the world's tilapia production (FAO, 2010). Despite possessing these advantageous characteristics, tilapias procreate at a young age, frequently below market weight. This leads to an adverse impact on their development rates, known as premature maturity, and is frequently seen as a significant setback in the tilapia farming industry (Obaroh and Nzeh, 2013). Due to their early maturation and reproduction, tilapia often overcrowd culture systems, especially clay ponds (Kapinga et al., 2019). many approaches to managing the tilapia population have been reevaluated by many writers (Kulwa et al., 2022, Jegede 2010, Fagbenro, 2002, Mair & Little, 1991). Nevertheless, there are technological drawbacks to several of these techniques. Therefore, it is necessary to investigate additional biological control strategies that are known to have antifertility properties. Certain plants, including Momordica charantia (Akin-Obasola and Jegede, 2014), Aloe vera (Jegede, 2011), Azadirachta indica (Jegede et al., 2008a), and Hibiscus rosa-sinensis (Jegede, 2010), have been shown to have the ability to limit reproduction. Because cottonseed is an ancient plant protein source used to feed both terrestrial and aquatic animals and is thought to have reproductive inhibitory properties, crude and synthesized gossypol were selected for this study (Lim and Lee, 2008, Gatlin et al., 2007, Li and Robinson, 2006). After soybean meal and rapeseed meal, cotton is the third-largest oil-seed meal produced worldwide (Lee et al., 2006). Cotton is a rich source of arginine, an amino acid that is vital for aquatic animals, and has more of it than soybean meal and fish meal combined. Due to its 40–45% crude protein content, cottonseed meal—a by-product obtained from the oil extraction process of cottonseed—is fed to aquatic animals (Kumar et al., 2021). Free gossypol is the main anti-nutritional component preventing fish from using cottonseed meal (Bian et al., 2017). Free gossypol is a polyphenolic substance that has hydroxyl and aldehyde groups that are brisk in color. Reproductive inhibition is one of the negative effects of free gossypol (Blom et al., 2001). However, a variety of processing techniques have been tried to eliminate the gossypol-related anti-nutritional elements from oilseed by-products, such as soybean meal, cottonseed meal, and rapeseed meal, in light of their potential use as animal feed sources of protein (Duodu et al., 2018).

Researcher recommendations for the usage of cottonseed meal vary; most suggest levels not to exceed fifty percent (Mbahinzireki et al., 2001); nevertheless, only a small number have suggested that cottonseed meal might completely replace animal protein in tilapia diets (El- Sayed, 1999). Reports on the quantity of synthetic and crude gossypol required to regulate reproduction in *C. elegans* are scarce. *Oreochromis niloticus* and zillii. Therefore, the purpose of this study is to evaluate the effects of both synthetic and crude gossypol in *C. zillii* and *O.niloticus* as a bio-control agent for reproduction to increase yield.

Materials and Methods

Crude Cottonseed Harvesting and Extraction of Oil

Under the ethical permission number ORDI/AD/EAC/ 23/159, the research was carried out in the Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, Ondo State, Nigeria. The source of the raw cottonseed utilized was the Metrovet Veterinary Store in Ado-Ekiti, Ekiti state. A plant analyst from the Department of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, determined that the raw seed was Gossypium herbaceum. The seeds were first cleaned to remove farm debris before being de-cased using a hand tool to peel out the linter and staples. They were then dried in an oven at 1300C until their moisture content was 12%. Following this, a nearby milling machine was used to help grind the seeds. A rotary magnetic shaker was used to agitate 100 grams of the milled fragment for 72 hours while it was macerated in 100 milliliters of methanol, acetone, ethanol, chloroform, pet ether, and hot water within conical flasks. Subsequently, the material was passed through a No. 42 what man folded filter paper before being inserted into the Soxhlet device.

300 ml of the solvent extractant (600–800°C) was weighed using a measuring cylinder before being added to a 500 ml round-bottom flask in the Soxhlet apparatus and heated for six hours at 60°C. The solvent keeps evaporating and bristling as it flows steadily through the soxhlet device and into the reflux condenser, which is kept cool by the surrounding water. The folded sample was then placed in the soxhlet apparatus, and the cooled solvent condensed back into the ration. This procedure is repeated until all of the oil has been extracted from the sample. The extracted material is then placed in an airtight bottle with a label and chilled at 4˚C before being used again and used for basic phytochemical testing. After the oil was extracted, the extracted portion that was left was hot pressed using a hydraulic press to extract the residual oil from the pressed cake. It was then dried for a whole night at 50°C in a convection oven. After cooling in a desiccator, the sample was weighed. We measured and determined the dry mass yield and moisture content. A byproduct is meal made from cottonseed. The technique of (Botsoglou, 1991) was used to identify the cottonseed meal's free and total gossypol content.

Identification of Phytochemicals at an Early Stage

To identify the phytochemicals found in the sample, various analyses were conducted using the techniques outlined by Thamilmrlai et al. (2011) and Edeogal and Mbaebie (2005).

To test for the presence of alkaloids, 5 milliliters of 2N HCL were added to 5 milliliters of boiling and filtered cottonseed meal extract. When small drops of Mayer's reagent were added to the filtrate, a cream-colored precipitate that indicated the presence of alkaloids was immediately generated.

In order to extract saponins, 5 milliliters of cottonseed meal extract were cooked in 10 milliliters of distilled water in a test tube. The test tube was then vigorously shaken for 30 seconds, and then allowed to settle for 30 minutes. The formation of foam indicates the presence of saponins. 5 milliliters of cottonseed meal extract were mixed with tiny drops of 1% lead acetate to produce tannins. The appearance of yellow precipitate indicates that tannins are present. A solution of ferric chloride (2 ml) was added to 2 ml of cottonseed meal extract for the phenols. Phenols are present when a bluish green color solution appears. To make steroids, 1 milliliter of cottonseed meal extract was diluted in 10 milliliters of chloroform, and the equivalent amount of strong sulfuric acid was added. Steroids are present when the uppermost layer begins to turn red and when yellow fluorescence turns green.

The presence of glycosides was determined by adding strong sulfuric acid and a few drops of ferric chloride to one milliliter of cottonseed meal extract containing glacial acetic acid. The presence of cardiac glycosides is indicated by the appearance of bluish green color in the uppermost layer and reddish brown color at the intersection of two layers. Anthraquinones were examined by heating 5 milliliters of cottonseed meal extract and 10 milliliters of sulfuric acid to a boil, then distilling the resulting filtrate while still hot and flushing it with 5 milliliters of chloroform. The chloroform layer was pipetted out into a second test tube after 5 milliliters of diluted ammonia was added. Anthraquinones are indicated by a change in color. One milliliter of the plant extract was tested for the presence of flavonoids by adding a few drops of diluted sodium hydroxide. This generated a strong yellow color in the extract, which turned colorless when a few drops of diluted acid were added. This suggests that flavonoids are present. One milliliter of cottonseed meal extract, one milliliter of acetic anhydride, and two milliliters of strong sulfuric acid were added to one milliliter of chloroform to test for terpenoids. The appearance of a reddish hue indicates the existence of terpenoids. A milliliter of cottonseed extract was mixed with a few drops of the Ninhydrin reagent to evaluate the amino acids. Amino acid content is indicated by the appearance of purple color. In order to test reducing sugars, 1 milliliter of cottonseed extract was added to 10 drops of Fehling's solution, which was then heated for 15 minutes. Reducing sugars are present when brick-red precipitate appears. To test monosaccharides, 1 ml of Barfoed's reagent was added to 1 ml of cottonseed extract and heated in a water bath. The appearance of brown precipitate indicates that monosaccharides are present.

Detecting Gossypol

A 25 ml conical flask filled with ethanol was filled with 5 ml of the extract in an attempt to find the gossypol content in the crude cottonseed. In a test tube, two milliliters of solid antimony chloride and two milliliters of sample solution were added and thoroughly mixed. Using Ventalchalam et al.'s (1980) approach, thin layer chromatography (TLC) was chosen for the qualitative analysis of gossypol in the seed extract sample. In order to prepare the TLC cover, silica gel F254 was used as an absorptive agent. Following the diffusion of 1 g of the sample in 5 ml of diethyl ether, the cover had a density of 0.02 mm. Subsequently, a suitable quantity of the dispersed sample and cottonseed ether extract were applied to the cover using a fine capillary jet. The ratio of 91:10:4 was used for the materialization of the chromatoplate using ethyl acetate, pet ether, and acetic acid. The cover was then taken off the chamber and carefully dried. Pb $(CH_3COO)_2$ was used in a chemical test to find gossypol in the cottonseed extract. After 20 minutes, the presence of a brownish precipitate containing lead acetate provided proof. The most abundant source of gossypol is cottonseed, which is why color potency increased over time.

Gossypol: A Quantitative Analysis

According to (Botsoglou, 1991), the recording in chloroform for the absorption spectra of cottonseed distillation and sample was carried out in 250–350 nm areas in quartz cell using UV-visible spectrometer. A one-gram sample was dispersed in 100 milliliters of chloroform. At 254 nm, the sample was precisely absorbed. At the UV region, a spectrophotometric measurement of the sample's cottonseed extract was created. According to observations, a spectral maxima were present in cottonseed extract at 270 nm (Harborne, 1985). For the sample, two spectral maxima readings were obtained: 285 nm and 273-274 nm. The maximum extermination coefficient was 285 nm, which allowed for an accurate quantification of gossypol. The higher extermination coefficient at 273-274 nm was comparable to that of pure gossypol reported in literature. This UV light region's manifestation of spectral maxima is what revealed the existence of gossypol in the sample. At precisely 290 nm, the spectrophotometric analysis was carried out. This was done to prevent interference with other aromatic compounds, which typically show absorbance at a wavelength of around 270 nm.

Preparing Trial Meals Using Both Artificial Gossypol and Crude Cottonseed Meal

According to Fagbenro & Adebayo (2005), 37.5% crude protein isocaloric diets were created, with cottonseed meal being substituted for soyabean meat at 0%, 25%, 50%, 75%, tableand 100%, respectively (Table 1). Yellow maize, fish meal, soybean meal, cod liver oil, vegetable oil, and vitamin-mineral premix were the dietary items that were bought at Adedom Agricultural Input Supply in Akure, Ondo State, Nigeria. The method described by (Botsoglou, 1991) was used to determine the amount of free gossypol in the cottonseed meal. Between 0 mg/kg CSM in the control diet, 330 mg/kg CSM in diet 2, 650 mg/kg CSM in diet 3, 980 mg/kg CSM in diet 4, and 1310 mg/kg CSM in diet 5, the free gossypol in each diet varied. The diets included varying amounts of synthetic gossypol, which was acquired from Adooq Bioscience in California, U.S.A. for use in the experiment. There were variations in the amount of free gossypol in each diet: 0 mg/kg in the control diet, 330 mg/kg in GSP1, 650 mg/kg in GSP2, 980 mg/kg in GSP3, and 1310 mg/kg in GSP4. Each nutritional ingredient was ground to a separate size and weighed using a Metler top loading balance (PB-8001 Model). Then, a Hobart A-200T pelleting and mixing machine with a 1.0 mm diameter die was used to carefully blend and pellet the ingredients. For seventy-two hours, the diets were allowed to air dry at room temperature with a consistent moisture content. Plastic containers with labels were used to store dry diets.

Fish Stocking and Feeding

A total of four hundred (400) male and female *Coptodon zillii* and *Oreochromis niloticus*, both weighing around (10 g), were obtained from The Federal University of Technology Akure. They spent seven days getting used to ten (2m x 2m x 1 m) concrete tanks filled with clean water. Ten females and ten males with a mean weight of 10 grams were stocked. There were five treatments in total, and each was duplicated. For ninety days, the fish were fed at 5% body weight twice a day, from (09:00 to 09:30 and 17:00 to 17:30h). Both *C. zillii* and *O. niloticus* were subjected to varying degrees of crude and synthetic gossypol-based meals during this operation.

The Gonads of *C. zillii* and *O. niloticus* Examined Histologically after Being Fed Diets Containing Various Grades of Crude Cottonseed Meal and Synthetic Gossypol

Fish were taken out of the experiment, weighed, and arranged according to gender. Sex assurance was verified by gonad vision examination. In order to extract the gonads for histological cross-examination, two male and two female subjects from each treatment were slaughtered and decapitated. For 24 hours, gonad fixation (testes and ovaries) was performed in a solution containing equal parts 10% formalin and 0.9% distilled water. After that, they were dried out in different proportions of alcohol: 50% for half an hour, 70% for ninety minutes, 95% for two hours, and 100% for two hours. Two repetitions of the 100% alcohol were made. In order to get rid of extra fixative, the specimens were put on a rotator and rotated occasionally in water that had been washed. Following desiccation, gonads (testes and ovary) were immersed in a solution of 50:50 absolute alcohol and xylene for approximately three hours, or until the tissue was fully brown and translucent. Afterward, the mixture was drained off. For one hour, xylene was poured into test bottles containing the test organs. Three incremental runs of the action, each lasting an hour, were completed. A large amount of paraffin was added to a fixing block split so that the bottom may be covered while fixing tissues into paraffin blocks. After that, the mold was set on the embedding block's hot plate. Melted paraffin was poured from the embedder dispenser into the mold until it was

Table 1. Experimental diets composition (g/kg) (37.5% CP)

(%SBM replacement by CSM)	Control diet CDO 0%	CSM-based diets				Gossypol-based diets			
		CSM1 25%	CSM2 50%	CSM3 75%	CSM4 100%	GSP1 25%	GSP2 50%	GSP3 75%	GSP4 100%
Yellow maize(10.8%CP)	200.0	200.0	200.0	200	200.0	200.0	200.0	200.0	200.0
Vegetable oil	20.0	20.0	20.0	20	20.0	20.0	20.0	20.0	20.0
Corn starch	70.0	53.5.0	37.0	20.5	4.0	70.0	70.0	70.0	70.0
Cod liver oil	30.0	30.0	30.0	30	30.0	30.0	30.0	30.0	30.0
Vitamin-mineral mix[1]	30.0	30.0	30.0	30	30.0	30.0	30.0	30.0	30.0
Cottonseed meal g/kg (crude)	0	109.0	218.0	327	436.0	-	-	-	-
Synthetic gossypol (free gossypol g/kg)	-	-	-	-	-	0.33	0.65	0.98	1.31

Dietary Ingredients	% protein	% Fat	% moisture	% Ash	Crude fiber	NFE
Fish meal	61.95	14.00	12.00	12.00	0	0.05
Maize	9.63	2.00	13.67	2.00	2.40	70.30
Soyabean	57.93	8.00	10.67	8.00	4.55	10.85
Cottonseed	35.18	14.00	2.67	4.00	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_{3} = 2 mg, panthothenic acid - 5 mg, biotin - 0.04 mg, nicotinic acid = 14 mg, selenium-2.2 mg, folic acid - 0.4 mg, choline - 150 mg, cobalt - 0.2 mg, B_{12} = 0.015 mg, copper - 4.5 mg, iron - 21 mg, manganese - 20 mg, iodine - 0.6 mg, zinc - 20 mg, antioxidant - 2 mg

Key: g/kg-gram per kilogram, CSM-cottonseed meal, CDO-control diet; GSP-gosssypol

completely full. After being taken out of the paraffin bath, a tape was put on a hot plate. The tissue sample was then carefully moved from the cassette to the mold after the cassette was opened and the lid was removed with forceps. In order to facilitate better ribbon well cutting, the tissue was aciculated to make cutting easier and maintained away from the mold's edge with enough wax surrounding the tissue. The coagulated wax covering the test organ was sliced with a hot knife into a square shape and secured on a wooden block for mounting, sectioning, and staining.

Using a rotary microtome, a light section of (8 µm) was created in order to fix the tissues. The samples underwent a series of steps including dehydration, staining with Harris hematoxylin-eosin (H&E) using a microtome, clearing by immersion in lukewarm water (38oC), selection with a perfect slide, and oven drying at 58oC for half an hour to dissolve the wax. After sectioning the tissues on the slides and clearing them with xylene and various alcohol concentrations (50, 70%, 90%, 95%, and 100%) for two minutes, the tissues were stained for ten minutes with hematoxylin-eosin. The stained slides were seen under a light microscope at various magnifications. Using the Histology Laboratory Manual 2011-2012, sections with an 8µ thickness were created. Leitz Ortholux II microscopes and camera standard type BHTU-11 were used to take photomicrographs. The experiment was conducted on *C. zillii* and *O. niloticus* fed diets containing different amounts of synthetic gossypol and crude cottonseed meal. Everything was done in accordance with Sharma et al. (2011).

Milt Growth of *C. zillii* and *O. niloticus* Fed Meals that Varied in the Amount of Synthetic Gossypol and Crude Cottonseed Meal

The treatment tanks were emptied and their outputs screened when the ninety-day trial came to an end. Testes of two male *C. zillii* and *O. niloticus* were removed after they were selected from treatment tanks and dissected. They were preserved right away by being placed into clearly labeled sample bottles filled with 0.9% saline solution. After taking the milt out of the sample bottles, it was put on a dry, clean slide and 5 milliliters of purified water were added to start the milt activation process. This was examined with an (Olympus CX40 light microscope) in order to count and determine motility. The testicular lobes were dissected, and the milt was then extracted and placed in a petri dish to determine the volume of the milt. A syringe with a 1.0 ml volume and a 0.1 ml calibration was used for the measurement. A single drop of purified water was added to 1µl of milt from each sample, which was then placed on a Neubaeur haemocytometer and covered with a slip to measure the duration of motility. Sperm activity was measured at 100X magnification using an Olympus BH2 microscope. According to (Mims, 1991), motility was determined by the sperms' continuous and non-continuous motion. Milt sample was measured for percentage motility using a light microscope at 400X magnification after 20 µl of filtered water was added as an activating solvent. Immobile sperm cells (ISC) were numbered as soon as spermatozoa activation began, and whole sperm cells (WSC) were numbered as well when the activation stopped (Canyurt and Akan, 2008). The formula for calculating mobile sperm cells (MC) was

$$MC=WSC – ISC$$

$$\%MC=MC/WSC\times100.$$

The number of spermatozoa in diluted samples (10µl sperm in 90µl MFR to create 100) (dilution 1) and 10µl dilution in 90µl MFR to produce 100) (Dilution 2) was counted in order to determine the sperm count. Using the approach of Rainis et al. (2003), 0.1 mm of the diluted was placed on a Neubauer haemocytometer, and sperm were counted under a microscope at 400X Magnification.

Production of Eggs by *C. zillii* and *O. niloticus* Fed Diets that Varied in the Amount of Synthetic Gossypol and Crude Cottonseed Meal

Using a light microscope with a calibrated eye piece graticule, the short and long axes of two egg samples from each procreate (treatment) were measured to determine the size of the egg. Next, the formula for mean egg diameter (mm) was determined:

$$Mean\ egg\ diameter(mm) = \frac{length\ of\ long\ axis + length\ of\ short\ axis}{2}$$

At the conclusion of the ninety-days period, two female *C. zillii* and *O. niloticus* were taken out of their treatment tanks, weighed, measured, and had their ovaries removed in order to determine fecundity. These ovaries were kept fresh in sample vials with obvious labels that contained 0.9% saline solution. To count the eggs in grams, one gram (one ounce) of the ovary was taken out, weighed, and then put on a Neubaeur counting chamber to be examined under an electronic microscope (Olympus Light Microscope, model CX 40). The number of eggs/g of the excised section of the ovary multiplied by the ovary's entire weight equals the total number of eggs in the ovary. The formula for both absolute and relative fecundity was derived from (Bhujel, 2000):

$$Absolute\ fecundity = Total\ weight\ of\ eggs\ per\ female(g) \times Numberof\ eegs\ in\ one\ gram$$

$$Relative\ fecundity = \frac{Absolute\ fecundity}{Body\ weight(g)}$$

SPSS version 11's one-way analysis of variance (ANOVA) test was used to examine the research's data. Duncan's Multiple Range Test (Duncan, 1955) was utilized to determine the significant difference between the treatments at a 5% confidence level. Both *C. zillii* and *O. niloticus* were given varying amounts of crude and synthetic gossypol-based diets before the aforementioned operation was performed on them.

Results

Testicular Histology in *C. Zillii* Fed A Diet Based Primarily on Crude Cottonseed Meal

Testes in a portion of *C. zillii* given the control diet 0g of CSM/kg of feed With 0% SBM replacement, had no apparent lesion, a normal distribution of sperm cells, and an abundance of spermatozoa in the seminiferous lumina were observed. The histology of the testes in *C. zillii* fed the diet containing 25% SBM replacement (109g CSM/kg) also revealed the presence of reduced interstitium/sertoli cysts and spermatocytes (black and blue, respectively). The histological slice showed a large sertoli cyst (arrow in blue) and limited lumina of the seminiferous tubules (arrow in black) at 218g CSM/kg diet. The testes from *C. zillii* fed diets based on crude cottonseed meal at 327 and 436 grams of CSM/kg showed empty spermatocytes (black arrows), a shrunken interstitium (blue arrows), and an empty lumen (black arrows) (Figure 1).

Ovarian Histology in *C. zillii* Fed a Diet Based Primarily on Crude Cottonseed Meal

The ovarian section of *C. zillii* fed the control diet (0g CSM/kg) showed stage V oocytes (black-colored arrow) and typical ovarian histology. It showed deformed vitellogenic oocytes at 109g CSM/kg diet. The results showed atretic oocytes (arrow in red) and late stage V oocytes (arrow in blue) in *C. zillii* fed a diet containing 218g CSM/kg. The ovarian section of *C. zillii* fed a diet containing 327g CSM/kg showed late stage V

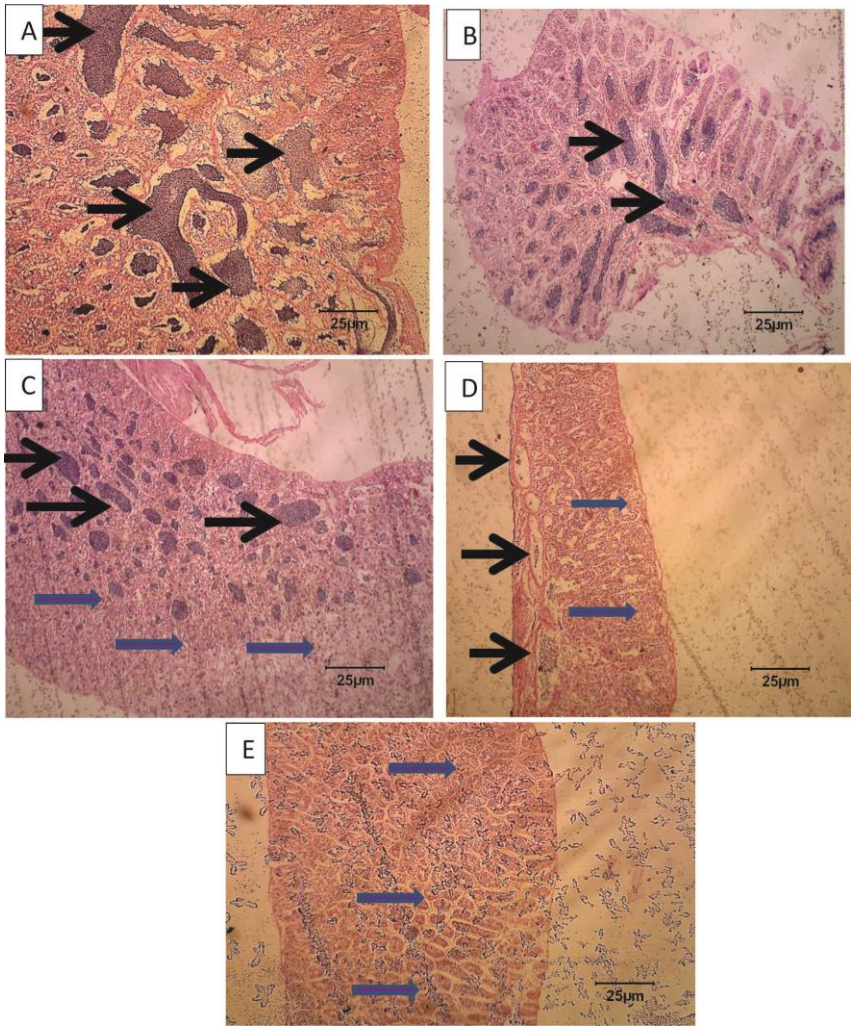

Figure 1. A section of the testes in *C. zillii* fed a diet based on crude cottonseed meal (A) control diet Showing usual sperm cell dispersal, generous spermatozoa (arrow in black colour); (B) 109g CSM/kg diet revealing spermatocytes (arrow in black colour), shrunken interstitium/sertoli cyst (arrow in blue colour); (C) 218g CSM/kg diet revealing limited lumina of the seminiferous tubules (black arrow), thick sertoli cyst (blue arrows); (D) 327g CSM/kg diet revealing empty spermatocytes (black arrow), shrunken interstitium (blue arrows); (E) 436g CSM/kg diet revealing empty lumen (black arrow). The normal dispersal of sperm cells is shown by these two observations.

oocytes and deformed vitellogenic oocytes (blue arrow). Diet showed malformed and unrounded oocytes at 436g CSM/kg (Figure 2).

Histology of the Testes In *O. niloticus* on Diets Based on Crude Cottonseed Meal

A section of the testes in an *O. niloticus* fed a control diet containing 0% SBM replacement (0g CSM/kg) revealed no apparent lesions, a normal distribution of sperm cells, and an abundance of spermatozoa in the seminiferous cavities. Testicular histology in *O. niloticus* on a meal containing 25% SBM replacement (109g CSM/kg) revealed several cavities with sparse spermatocytes (black arrows) and a thick interstitium/sertoli cyst (blue arrows). A section of the testes of *O. niloticus*, which was fed a diet containing 50% SBM replacement (218g CSM/kg), revealed a thick interstitium/sertoli cyst (blue arrows) and sparse lumina of the seminiferous tubules (black arrows). The histopathology of the testes in *O. niloticus* at 75%SBM replacement (327g CSM/kg) diet revealed that the lumen is smaller and that there are less spermatocytes in the lumina (black arrows). When *O. niloticus* was fed 100% SBM replacement (436g CSM/kg), a section of the testes showed much fewer interstitial cells (black arrows) and very little spermatozoa in the cavities (blue arrows) (Figure 3).

Ovarian Histology in *O. niloticus* Fed Diets Based on Crude Cottonseed Meal

O. niloticus section given a control diet displays the typical architecture of a fully developed ovary together with the stages of vitelline development (black arrow). The diet of *O. niloticus* fed at 109g CSM/kg displays the vitelline stage (black arrow), stage two oocytes (yellow arrow), stage four oocytes (green arrow), stage three oocytes (red arrow), and atrophied oocyte (white arrow). *O. niloticus* section of the ovary fed (218g CSM/kg) reveals stage two oocytes (arrow in yellow), stage three oocytes (arrow in red), and unglobular and

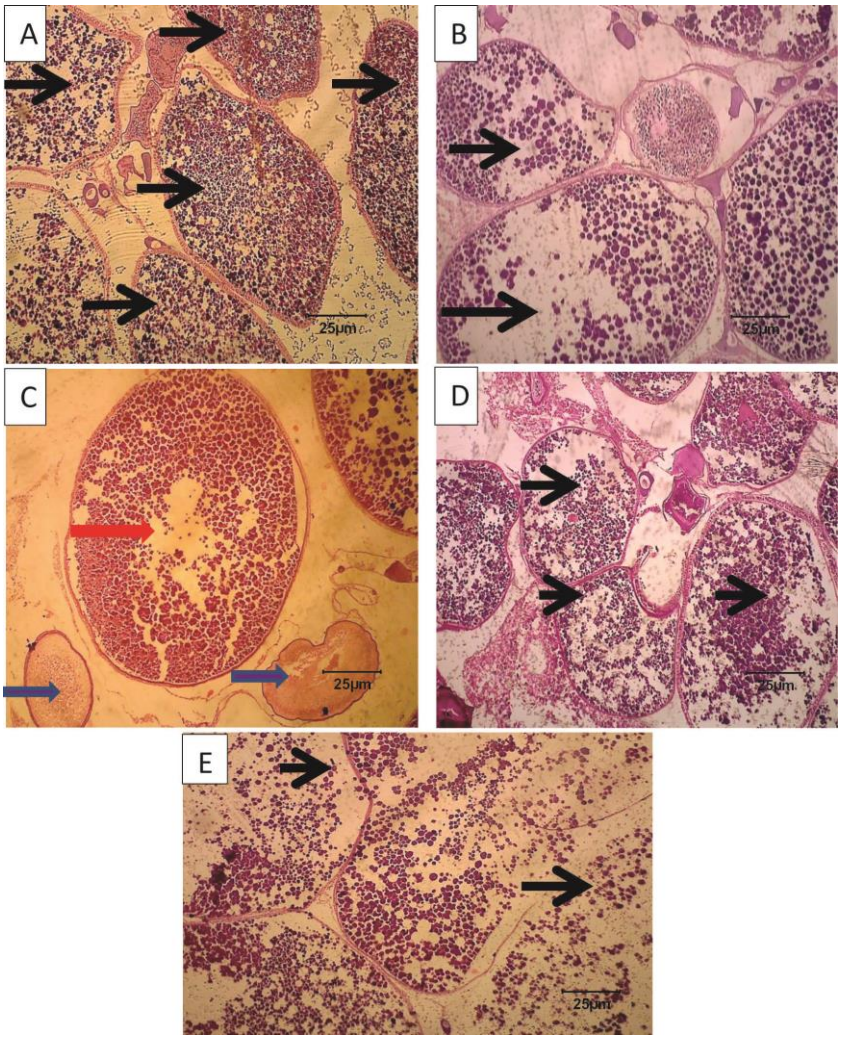

Figure 2. Ovarian section in *C. zillii* fed on crude cottonseed meal-based diets (A) showing normal ovarian histology and stage V oocytes (black arrow), (B) showing deformed vitellogenic oocytes from the 109g CSM/kg diet. (C) 218g CSM/kg diet displaying atretic oocytes (red arrow) and late stage V oocytes (blue arrow). (D) A diet of 327g CSM/kg displaying distorted vitellogenic oocytes, namely late stage V oocytes (shown by the blue arrow); (E) A diet of 436g CSM/kg displaying oocytes that are twisted and not round.

deformed vitelline stage (arrow in black). Histology of *O. niloticus* fed 100%SBM replacement (436g CSM/kg) diet reveals unglobular and disfigured vitellogenic stage (arrow in black), disfigured stage five oocyte (arrow in purple), and atrophied oocyte (arrow in arrow in black color). Stage five oocytes (arrow in black color) and disfigured stage three oocytes were observed in *O. niloticus* fed 75%SBM replacement (327g CSM/kg) (Figure 4).

Histology of the Testes in *C. zilli* fed a Diet Based on Synthetic Gossypol Meals

A section of the testes from *C. zillii* fed the control diet (0 mgFG/kg diet) revealed a large number of spermatozoa in both the general pool and the seminiferous lumina. Spermatocytes in *C. zillii* fed 330 mgFG/kg diet were still encased in germinal cysts, which were maturing. Spermatocytes were still encased in germinal cysts (maturing) in *C. zillii* fed a meal containing 650 mgFG/kg. While *C. zillii* provided 1310 mgFG/kg diet showed germinal cells remaining mostly wrapped in the germinal cysts (maturing), *C. zillii* fed 980 mgFG/kg diet likewise revealed the spermatocytes still enclosed within the germinal cysts (Figure 5).

Ovary Histology in *C. zillii* Fed Diets Including Synthetic Gossypol Meal

An ovarian section taken from 0 mgFG/kg diet (control) shows a clearly defined vitellogenic stage and normal ovary histology. The diet of *C. zillii* fed 330 mgFG/kg likewise showed developed, but twisted and empty, vitelline stages. When stage two oocytes with basophilic cytoplasm and a prominent central nucleus with diffuse chromatin were examined in the ovarian

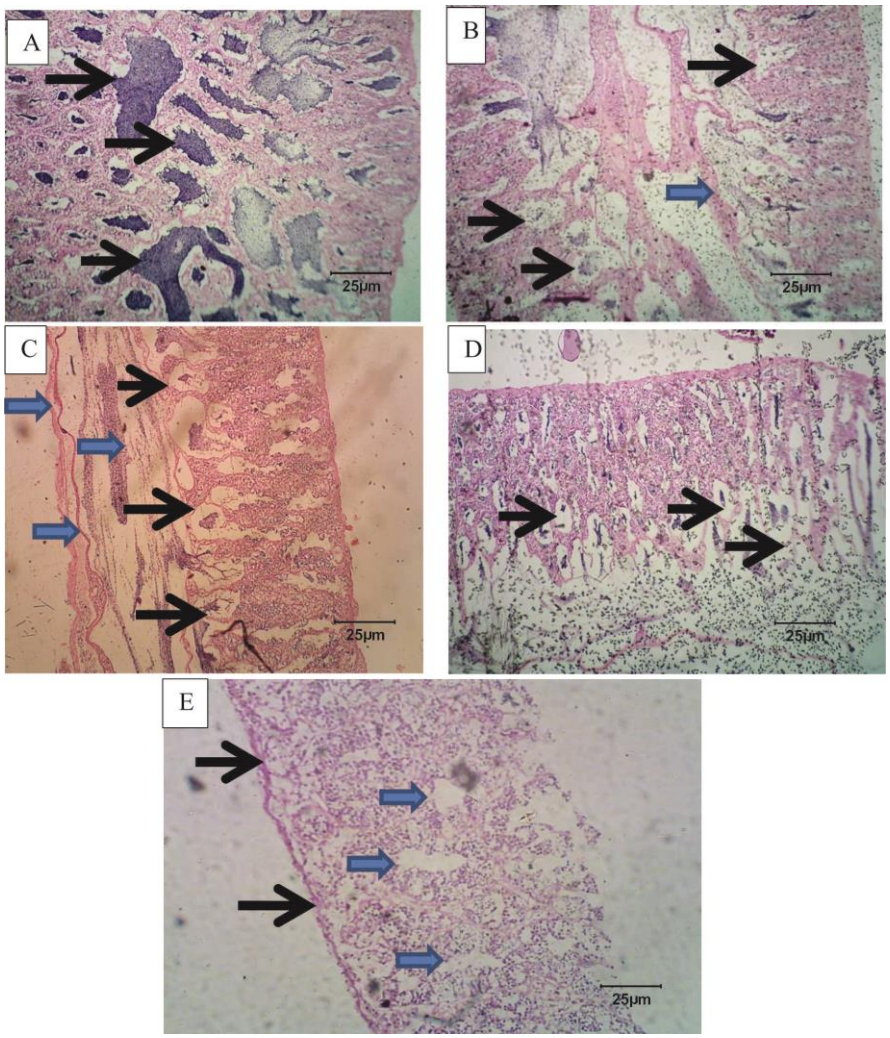

Figure 3. A section of the testes in *O. niloticus* fed a crude diet based on cottonseed meal (A) demonstrates the normal distribution of sperm cells, enough spermatozoa in the seminiferous canals, and the absence of any visible lesions (black arrow). (B) A 109g CSM/kg diet that showed several spermatocyte-free cavities (arrow in black), a thick sertoli cyst (arrow in blue). (C) 218g CSM/kg reveals a thick sertoli cyst and scantiness in the lumina of the seminiferous tubules (black arrow). (D) 327g CSM/kg), which shows a smaller lumen and fewer spermatocytes (black arrow). (E) 436g CSM/kg, which shows significantly less interstitial cells (black arrow) and an extremely uncommon amount of spermatozoa in the cavities (blue arrow).

section of *C. zillii* fed 650 mgFG/kg diet, stage three oocytes were seen to be rupturing from the germinal epithelium and continuing to mature within the folds of the ovigerous lamellae. At this point, the ova are encased in a basic squamous follicular epithelium, and provitelline nucleoli are visible in the karyoplasm (white arrows), deformed vitelline stage (black arrow), and late stage five oocytes (blue arrow). Section of Ovary in *C. zillii* fed 980mgFG/kg diet revealed more of stages three and four oocytes (arrow in white) and late stage five oocytes (arrow in blue). In contrast, section of Ovary in *C. zillii* fed 1310mgFG/kg diet revealed more of stage two and three oocytes—squamous follicular epithelium that envelopes the ova, evident provitelline nucleoli in the karyoplasm (white arrows), and stage five oocytes (blue arrows) (Figure 6).

Histology of the Testes in *O. niloticus* Fed Diets Based on Synthetic Gossypol Meal

In *O. niloticus* fed 0 mgFG/kg diet (control), there was no apparent damage in the section of the testes. Spermatozoa were packed densely inside the seminiferous tubules. *O. niloticus* fed 330 mg FG/kg diet shows a significantly shrunken or diminished germinal cyst with very little spermatozoa in the lumen. The testicular pouches of *O. niloticus* fed a 650 mgFG/kg diet showed very little spermatozoa (arrow in red) and significantly decreased or diminished germinal cyst lumens (arrows in black). *O. niloticus* administered a meal containing 980 mgFG/kg showed several spent cavities that were shrinking in size and lacking spermatozoa. In addition to having many spent cavities

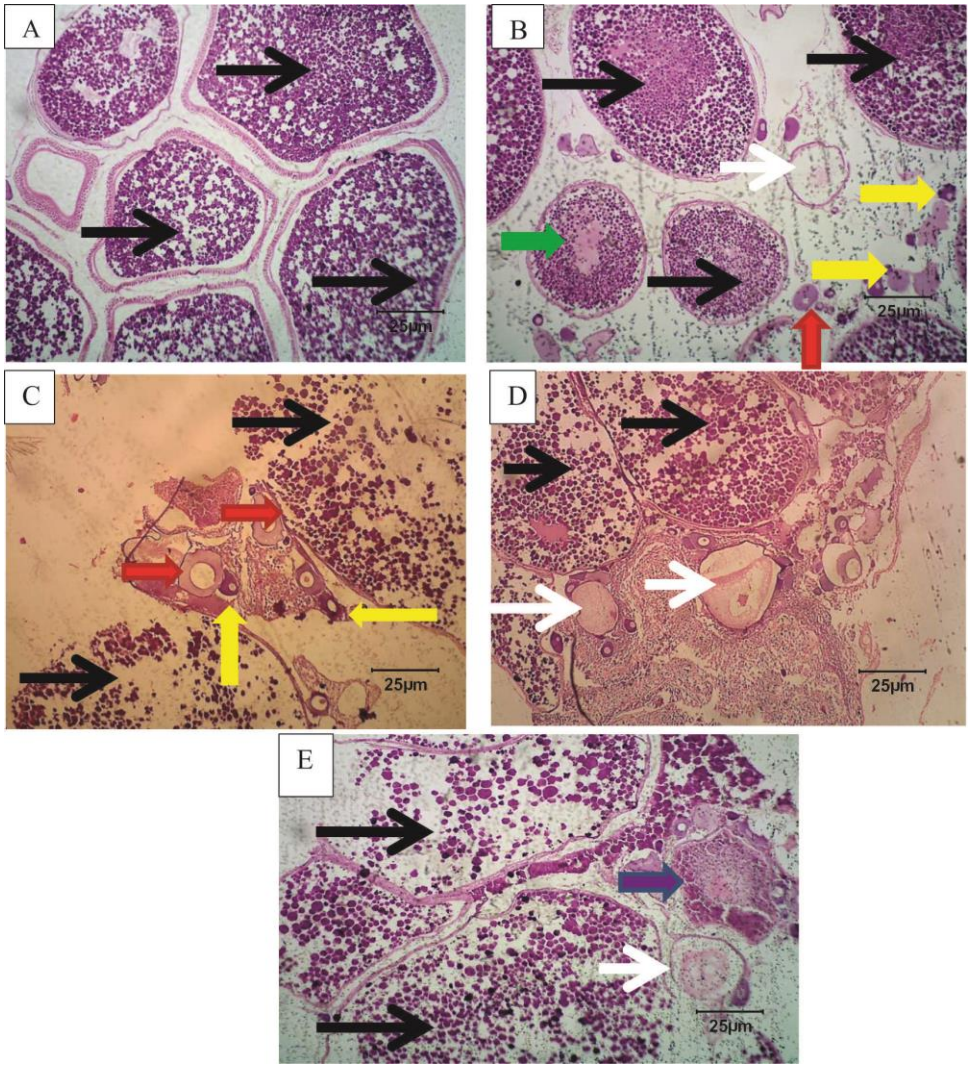

Figure 4. A section of the ovary in *O. niloticus* fed a diet based primarily on crude cottonseed meal (A) Control diet displaying the typical architecture of a fully developed ovary and the phases of the vitelline (black arrow). (B) the vitelline stage (black arrow), the stage two oocytes (yellow arrow), the stage four oocytes (green arrow), the stage three oocytes (red arrow), and the atrophied oocyte (white arrow). (C) The unglobular and deformed vitelline stage (shown by a black arrow), the stage two and three oocytes (shown by a yellow and red arrow, respectively). (E) unglobular and distorted vitellogenic stage, (D) stage five oocytes (arrow in black color), disfigured stage three oocytes (arrow in black color), and atrophied oocyte (arrow in arrow in black color).

empty of spermatozoa, *O. niloticus* on a diet containing 1310 mgFG/kg also had shrinking cavities (Figure 7).

Ovarian Histology in *O. niloticus* Fed Meals Based on Synthetic Gossypol Meal

Ovarian section of *O. niloticus* fed 0 mgFG/kg diet (control) showed typical stages of vitellogenic development and ovarian architecture. *O. niloticus* on a diet containing 330 mgFG/kg showed early stage five (arrows in white) and deformed and unrounded vitelline oocytes (arrows in black). When *O. niloticus* was fed a diet containing 650 mg FG/kg, clear evidence of stage three oocytes—which broke out from the germinal epithelium—and stage four oocytes—which showed yolk granules and fat vacuoles in the oviplasm—was seen (white arrows).

In *O. niloticus* fed a diet containing 980 mgFG/kg, a section of the ovary showed few early stages (three and four) and late stage five oocytes (arrow in black). Stage six oocytes were atretic. Section of the ovary in *O. niloticus* fed 1310 mgFG/kg diet showing deformed vitelline oocytes, few early stages (red arrow) and stage six at locations of atresia (white arrow) (E) Stage six at locations of atresia (arrow in white) and a few early stages (arrow in red) of deformed vitelline oocytes were revealed with a diet of 1310 mgFG/kg (Figure 8).

Milt Production of *C. zillii* and *O. niloticus* Nourished with Crude Cottonseed Meal-based Diets

The initial milt count of *C. zillii* in the control diet was 121,500 ml and diminished to 94,000 ml in fish fed with 436 g CSM/kg diet. Also, in the control group of *O. niloticus*, the milt count dropped from 162,500 ml in the control diet to 96,500 ml in fish administered with 436 g CSM/kg diet. In contrast to a milt sample with a lower mass of spermatozoa, dense milt containing more

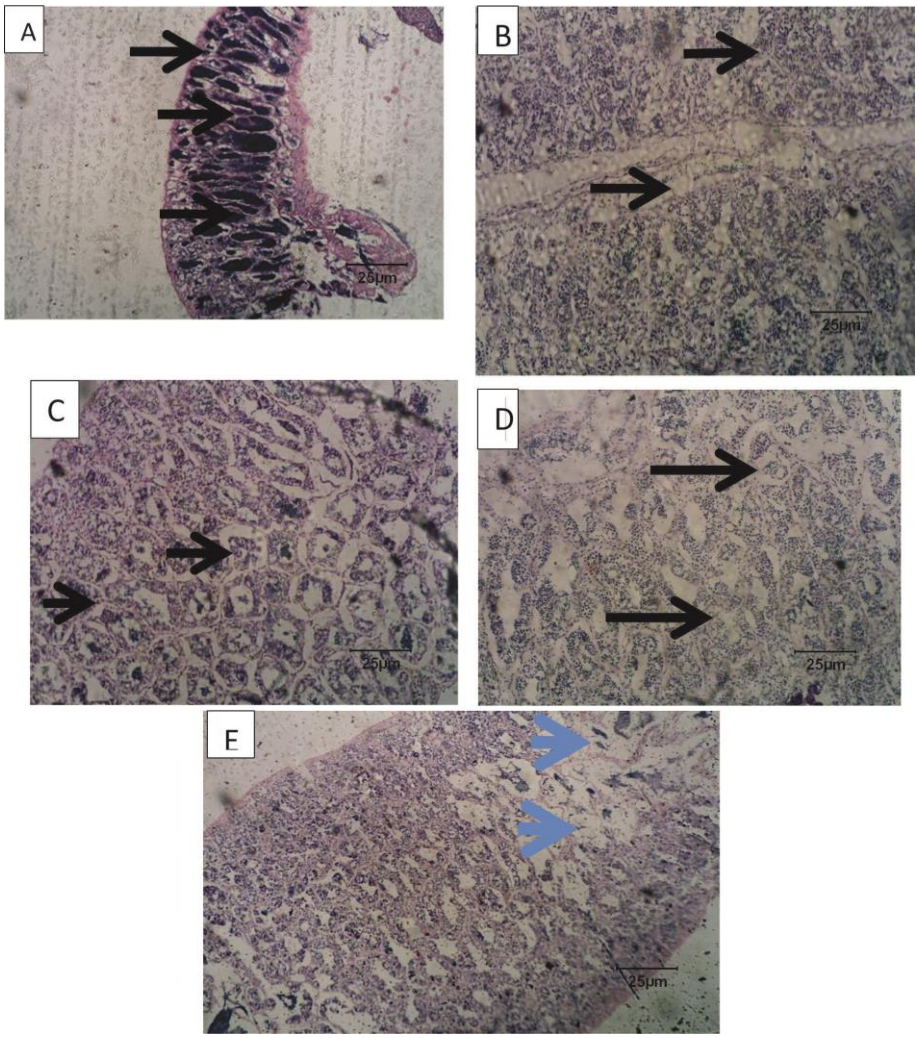

Figure 5. A section of the testes in *C. zilli* fed diets based on synthetic gossypol meal. (A) 0 mgFG/kg diet, which shows a large amount of spermatozoa in the general pool and seminiferous lumina (black arrow). (B) 330 mgFG/kg diet, which shows maturing germinal cysts enclosing spermatocytes. (C) A diet of 650 mg FG/kg similarly showed maturing germinal cysts including contained spermatocytes. (D) 980 mgFG/kg diet, which shows mature germinal cysts including spermatocytes still contained. (E) 1310 milligrams FG/kg diet demonstrating that most spermatocytes are empty and devoid of spermatozoa, with germinal cells still encased in germinal cysts (blue arrows).

counts of sperm cells sample is more likely to result in fertilization, as evidenced by the significant difference and variance in the milt count of the control group and those nourished with cottonseed meal at high inclusion. Motility duration in the control group of *C. zillii* nourished with cottonseed meal-based diet declined from 46 seconds to 13 seconds in fish given 436g CSM/kg diet. Similarly, when *O. niloticus* fish were nourished with 436g CSM/kg diet, their motility duration diminished from 51 seconds to 15 seconds. The fall in the milt duration mirrored the same trend as in milt motility, the more the inclusion of cottonseed meal in the diets, the less the duration of the milt. When *C. zillii* was given a cottonseed meal-based diet, the initial milt motility in the control group was 77.77%; however, when *O. niloticus* was given the same diet, the initial milt motility was 81.24%; however, it declined to 37.04% when the fish was given 436g CSM/kg diet. Milt quality is a measure of the ability of sperm to successfully fertilize an egg which such ability mostly depends on qualitative parameters of milts. Given that the quality of the milt has a significant impact on the number of offspring, the very poor milt motility observed in diets supplemented with high dosages of cottonseed meal demonstrated the antifertility activity of cottonseed meal (Table 2).

Egg Production of *C. zillii and O. niloticus* Nourished with Crude Cottonseed Meal-based Diets

The mean egg diameter of *C. zillii* nourished with diets based on cottonseed meal did not change significantly ($p>0.05$). In the diets, the mean egg diameter spanned 1.31mm ±0.01 to 1.39mm ±0.01, whereas in the cottonseed meal-based diets administered to *O. niloticus*, the mean egg diameter varied from 2.22mm ±0.01 in the control diet to 1.94±0.34 in fish fed 436g CSM/kg diet. The degree of cottonseed meal inclusion in the diets was found to have an impact on the sizes of the organisms, with the control diet having the lowest mean egg diameter and *O. niloticus* fed 436g CSM/kg diet having the highest mean egg diameter, despite the fact that there was no significant difference ($p>0.05$) between them. The study's findings for *C. zillii* revealed that when cottonseed was added to diets, absolute fecundity

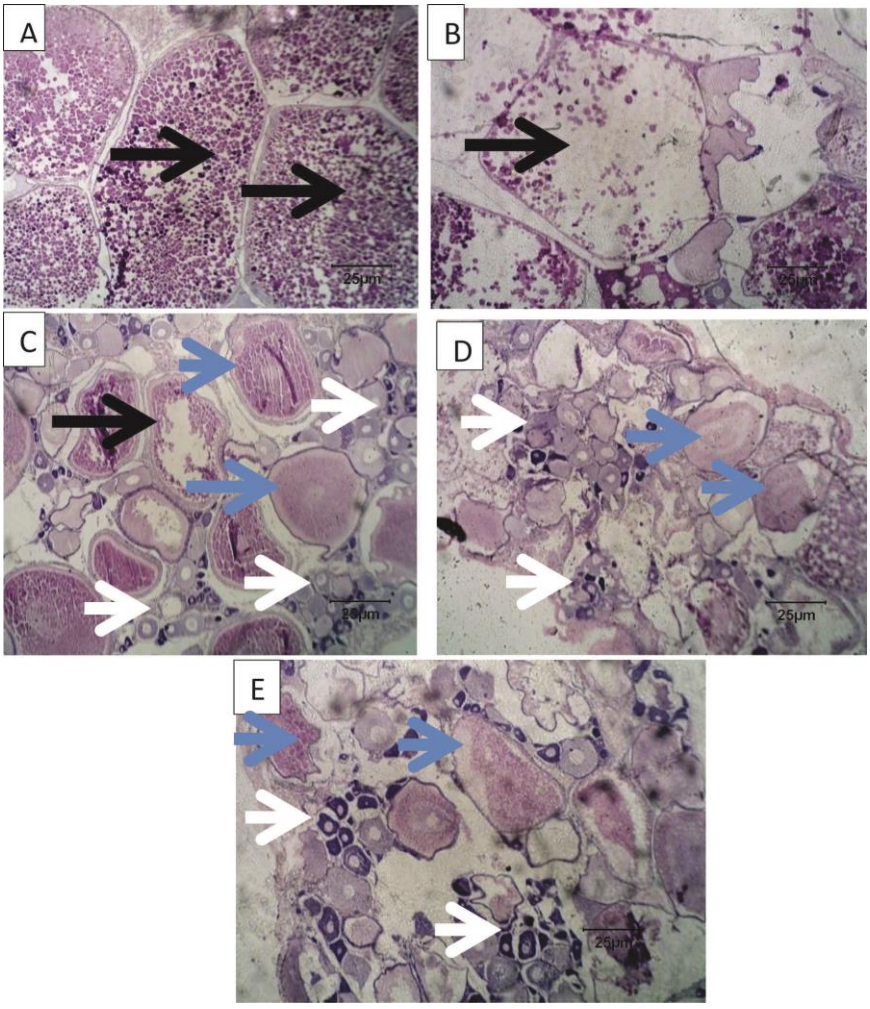

Figure 6. A section of the ovary in *C. zillii* fed diets based on synthetic gossypol meal (A) Normal ovarian histology with a clearly visible vitellogenic stage (0 mgFG/kg diet). (B) Mature vitelline stages are seen but they are twisted and empty in the 330 mgFG/kg diet. (C) 650 mgFG/kg diet exposing oocytes in stages two and three (white arrow), seven late-stage oocytes (blue arrow), and deformed vitelline stage (black arrow). (D) 980 mg FG/kg of food Oocytes in stages two and three (white arrows); oocytes in stages five (blue arrows). (E) 1310 mgFG/kg diet) late stage five oocytes (blue arrow) and stages three and four oocytes (white arrow).

dramatically ($p<0.05$) reduced from 685.00±33.00 to 148.50±16.50. The fish receiving CSM addition to their feed showed the lowest absolute fecundity, at 436g CSM/kg, while the control diet showed the greatest value. A comparable pattern was seen when *O. niloticus* were given diets based on cottonseed meal at varying inclusion levels, with 335.00±10.00 in the control diet and 125.50±0.50 in fish receiving CSM added to the diet at 436g CSM/kg. Because *C. zillii* is a guarder genus and both parents protect the eggs, the reproduction approach employed by the specie ensures that the young receive proper care from the parents By ensuring that the majority of the eggs mature into fry and subsequently adult fishes, *C. zillii's* reproductive technique maintained a large population of fish. Because the eggs were raised by a parent and might not have had enough oxygen to develop into young, the reproductive strategy of the maternal mouth brooder does not favour an increase in fish population, as evidenced by the significantly ($p<0.05$) lower result for *O. niloticus* compared to *C. zillii.* For *C. zillii* and *O. niloticus* fed with CSM-based diets at different inclusion levels, relative fecundity declined considerably as CSM inclusion rose ($p<0.05$), from 21.50±2.50 to 4.50.50±0.50 and from 7.97±0.00 to 2.55±0.10, respectively. The gonadosomatic indices for both *O. niloticus* and *C. zillii* decreased as cottonseed meal was added to the diets, going from 4.69±0.46 in the control diet to 3.96±0.03 in diet 5. The values of diets 4 and 5 did not differ significantly ($p<0.05$). *O. niloticus* fed diets containing increasing amounts of cottonseed meal exhibit the same pattern as *C. zillii*, going from 5.03±0.05 in the control diet to 3.67 to ±0.21 in diet 5 (Table 3).

Milt Production of *C. zillii* and *O. niloticus* Nourished with Synthetic Gossypol Meal-based Diets

The group of fish fed synthetic gossypol meal-based diets did not differ in milt volume from the control group of *C. zillii* and *O. niloticus* in a statistically significant way ($p>0.05$).

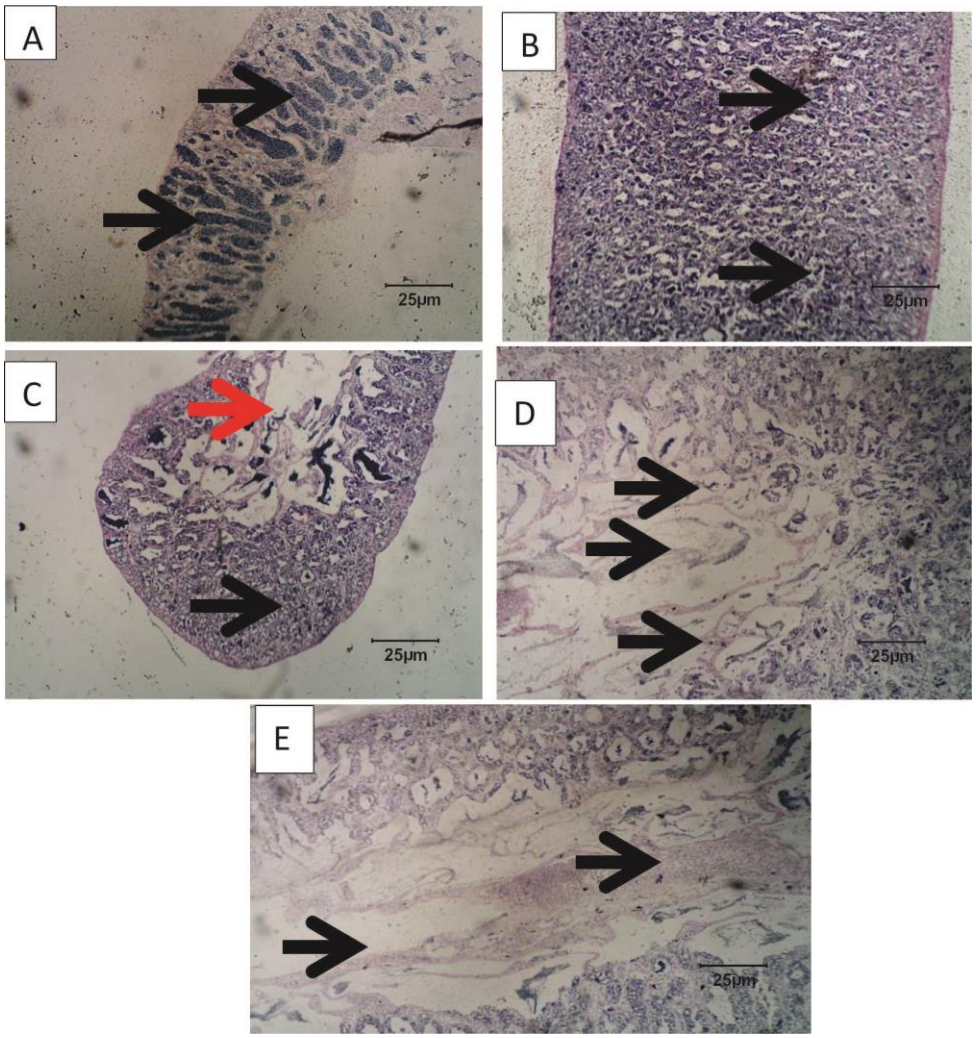

Figure 7. A portion of the testes of *O. niloticus* fed a diet consisting of synthetic gossypol meal (A) Spermatozoa are numerous and contained in seminiferous tubules when fed a 0 mgFG/kg diet. (B) A meal containing 330 mg FG/kg shows a significantly shrunken or diminished germinal cyst with very few spermatozoa in the lumen. (C) A diet of 650 mgFG/kg shows that the germinal cyst lumen has significantly shrunk or decreased (black arrows), and there is very little spermatozoa in the testicular pouches (red arrow). (D) The semiferous cavities are shrunken and contain no spermatozoa, with 980 mgFG/kg diet spent cavities (black arrows). Shrunken cavities with no spermatozoa were observed at 1310 mgFG/kg diet (black arrows).

The initial milt count of *C. zillii* fed the control diet (0mgFG/kg diet) was 131,000ml and declined to 51,000ml in GSP4 (1310mgFG /kg diet) while in the control group of *O. niloticus*, milt count declined from 168,500ml to 39,000ml in (1310mgFG /kg diet). The spermatozoa's capacity to fertilize eggs may have been hampered by the extremely low sperm count in all of the gossypol-treated fish, as evidenced by the fact that fertilization did not occur in any of the tanks except the control group. Motility duration in the control group of *C. zillii* nourished with synthetic gossypol meal-based diet declined from 43.50 seconds to 15 seconds in GSP4 while in the control group of *O. niloticus*, motility duration declined from 56 seconds in the control diet to 13 seconds in GSP4. The control group of *C. zillii* nourished with synthetic gossypol meal-based diet had initial milt motility of 82.82%; however, this subsequently dropped to 54.12% in GSP1, 36.81% in GSP2, 22.79% in GSP3, and ultimately 18.61% in GSP4. The initial milt motility of *O. niloticus* fed a synthetic diet based on gossypol was 78.93%; however, it also rapidly decreased to 56.90% in GSP1, 26.67% in GSP2, 16.47% in GSP3, and ultimately to 15.39% in GSP4. The inhibition of spermatogenesis by gossypol, which was shown in the histology information, is also pointing towards the direction of the spermatozoid motility in these results (Table 4).

Egg Production of *C. zillii* and *O. niloticus* Nourished Synthetic Gossypol Meal-based Diets

The mean egg diameter of *C. zillii* fed with synthetic gossypol meal-based diets varied significantly ($p>0.05$). The order of mean egg diameter was between 1.32mm ±0.00 in the control diet and 1.25mm ±0.01 at (1310mgFG /kg diet) while the order of mean egg diameter for *O. niloticus* fed synthetic gossypol meal-based diets was 2.32mm ±0.00 in the control diet to 1.58±0.00 at (1310mgFG /kg diet). It was observed that the level of inclusion of synthetic gossypol in the diets

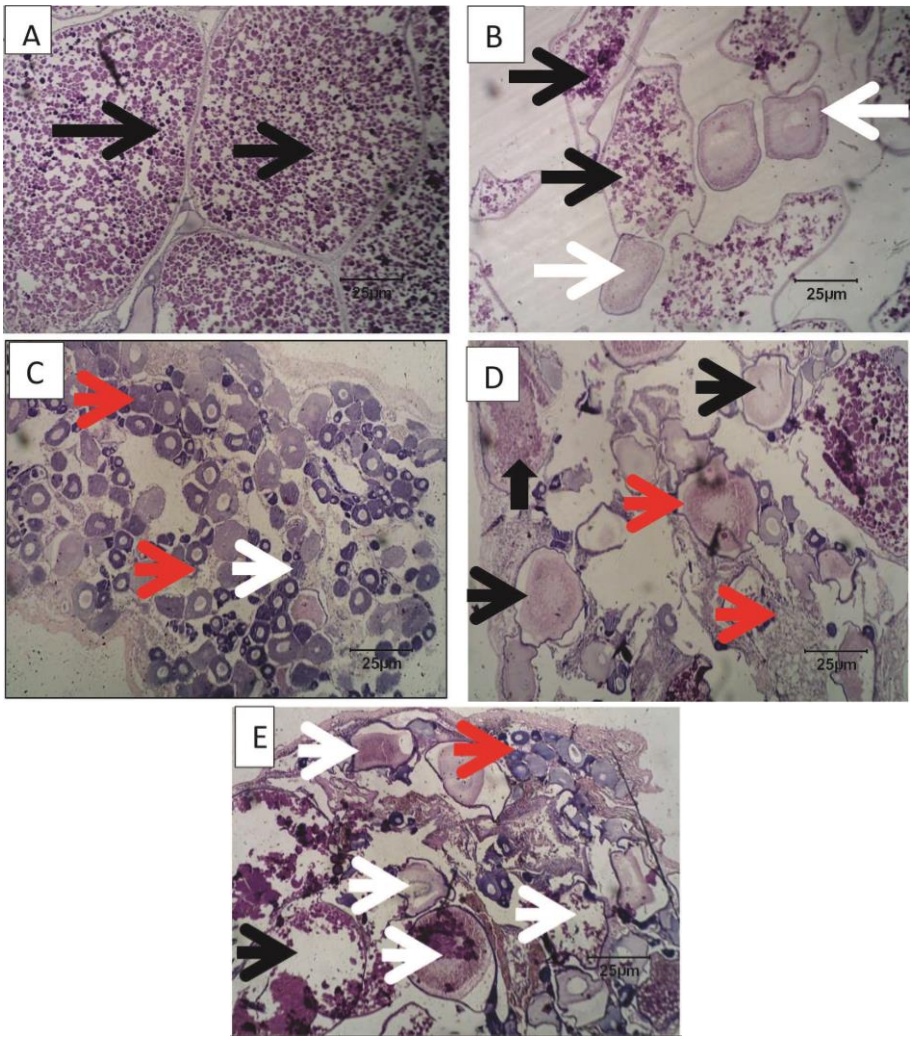

Figure 8. Ovarian section in *O. niloticus* fed meals based on synthetic gossypol meal (A) A diet of 0 mgFG/kg displaying the typical ovarian structure and vitellogenic phases. (B) Early stage five vitelline oocytes (white arrows) with distortion and roundness (330 mgFG/kg diet). (C) A meal containing 650 mgFG/kg makes stage three and stage four oocytes visible (red arrows). (D) A meal containing 980 mgFG/kg shows late stage five oocytes (black), stage six atretic oocytes (red), and only a small number of early stages (three and four). (E) A meal containing 1310 mgFG/kg revealed deformed vitelline oocytes at stage six at atresia points (white arrow), with a few early stages (red arrow).

affected the size of the eggs with control diet having the highest mean egg diameter for both *C. zillii and O. niloticus*. The results obtained from this study for *C. zillii* showed that absolute fecundity decreased significantly ($p<0.05$) as the addition of synthetic gossypol increased in the diets from 713.00±8.00 to 91.50±1.50. The highest absolute fecundity was recorded in the control diet while the lowest value was recorded in fish having synthetic gossypol added to the diet at (1310mgFG /kg diet). The same trend was recorded for *O. niloticus* fed synthetic gossypol meal-based diets at different inclusion with 356.00±6.00 in control diet and 55.00±1.0 in fish having synthetic gossypol added to the diet at (1310mgFG /kg diet). Relative fecundity in both *C. zillii* and *O. niloticus* fed with synthetic gossypol-based diets at various inclusion levels decreased significantly as the inclusion of synthetic gossypol increased ($p<0.05$) from 22.00±0.00 to 2.00.50±0.00 and from 7.49±0.18 to 0.68±0.01 respectively. The gonadosomatic indices recorded for both *C. zillii* and *O. niloticus* showed reduction as the inclusion of synthetic gossypol rose in the diets. For *C. zillii*, GSI ranged from 4.75±0.05 in the control diet to 2.70±0.00 in GSP4 and they all differed significantly ($p<0.05$). *O. niloticus* nourished with synthetic gossypol meal-based diets also follow the same trend as obtained in *C. zillii* from 4.55±0.04 in control diet to 1.97 to ±0.00 in GSP4 (Table 5).

Table 2. Milt production of *C. zillii* and *O. niloticus* nourished with crude cottonseed meal-based diets

	Control	CSM diet treatments (g/kg)			
	CDO	CSM1	CSM2	CSM3	CSM4
	0%SBM	25%SBM	50%SBM	75%SBM	100%SBM
Milt volume					
C. zillii	0.30^{c}±0.00	0.20^{abc}±0.00	0.25^{bc}±0.05	0.10^{a}±0.00	0.15^{ab}±0.05
O. niloticus	0.30^{c}±0.05	0.20^{abc}±0.05	0.25^{bc}±0.05	0.10^{a}±0.15	0.15^{ab}±0.05
Milt count					
C. zillii	121.50^{c}±2.50	122.00^{c}±2.00	116.50^{c}±0.50	105.50^{b}±1.50	94.500a±0.50
O. niloticus	162.500^{c}±1.50	130.500^{b}±7.50	119.000^{b}±1.00	98.000^{a}±4.00	96.500^{a}±4.50
Milt duration					
C. zillii	46.50^{c}±4.50	36.50^{bc}±3.50	33.00^{b}±3.00	25.00^{ab}±3.00	13.50^{a}±1.50
O. niloticus	51.00^{e}±2.00	43.50^{d}±0.50	37.00^{c}±1.00	22.50^{b}±1.50	15.50^{a}±1.50
Motility (%)					
C. zillii	77.77^{d}±0.46	76.62^{d}±0.79	70.38^{c}±0.55	59.19^{b}±3.42	37.04^{a}±1.25
O. niloticus	81.24^{e}±075	72.77^{d}±0.41	55.46^{c}±0.46	35.69^{b}±0.58	26.90^{a}±0.81

CDO = Control diet; CSM = Cottonseed meal.
The mean difference is significant at 0.05 level
Means for groups in homogenous subsets are displayed
Uses harmonic mean sample size = 2.00

Table 3. Egg production of C*. zillii* and *O. niloticus* nourished with crude cottonseed meal-based diets

	Control	CSM diet treatments (g/kg)			
	CDO	CSM1	CSM2	CSM3	CSM4
	0%SBM	25%SBM	50%SBM	75%SBM	100%SBM
Egg sizes (mm)					
C. zillii	1.31^{a}±0.01	1.34^{a}±0.04	1.35^{a}±0.03	1.39^{a}±0.01	1.34^{a}±0.04
O. niloticus	2.22^{a}±0.01	2.21^{a}±0.00	2.01^{a}±0.09	2.13^{a}±0.15	1.94^{a}±0.34
C. zillii					
Absolute fecundity	685.00^{d}33.00	719.00^{d}±6.00	539.00^{c}±4.00	303.50^{b}±38.50	148.50^{a}±16.50
Relative fecundity	21.50^{c}±2.50	22.00^{c}±1.00	16.00^{b}±0.00	8.00^{a}±0.00	4.50^{a}±0.50
O. niloticus					
Absolute fecundity	335.00^{c}±10.00	330.00^{c}±7.00	215.00^{b}±37.00	138.50^{ab}±31.00	125.50^{a}±0.50
Relative fecundity	7.97^{c}±0.00	7.29^{c}±0.20	5.62^{b}±0.58	3.21^{a}±0.48	2.55^{a}±010
Gonadosomatic indices					
C. zillii	4.69^{ab}±0.46	5.11^{b}±0.17	4.83^{ab}±0.19	3.96^{a}±0.15	3.96^{a}±0.03
O. niloticus	5.03^{a}±0.05	4.77^{a}±0.05	4.30^{a}±0.62	3.85^{a}±0.48	3.67^{a}±0.21

CDO = Control diet; CSM = Cottonseed meal.
The mean difference is significant at 0.05 level
Means for groups in homogenous subsets are displayed
Uses harmonic mean sample size = 2.00

Table 4. Milt production of *C. zillii* and *O. niloticus* nourished with synthetic gossypol meal-based diets

	Control	GSP diet treatments			
	CDO	GSP1	GSP2	GSP3	GSP4
Milt volume					
C. zillii	0.40^{b}±0.00	0.40^{b}±0.00	0.35^{ab}±0.05	0.30^{a}±0.00	0.30^{a}±0.00
O. niloticus	0.45^{a}±0.05	0.45^{a}±0.05	0.45^{a}±0.05	0.35^{a}±0.05	0.35^{a}±0.05
Milt count					
C. zillii	131.00^{a}±1.00	127.50^{b}±0.50	100.50^{c}±0.50	96.50^{d}±0.50	51.00^{e}±1.00
O. niloticus	168.5^{d}±0.50	94.00^{c}±4.00	52.50^{b}±0.50	42.50^{a}±0.50	39.00^{a}±1.00
Milt duration					
C. zillii	43.50^{c}±0.50	42.00^{c}±0.00	27.00^{b}±1.00	26.00^{b}±0.00	15.00^{a}±0.00
O. niloticus	56.50^{d}±0.50	33.50^{c}±3.50	19.00^{b}±1.00	12.50^{a}±0.50	13.00^{ab}±1.00
Motility (%)					
C. zillii	82.82^{e}±0.51	54.12^{d}±0.21	36.81^{c}±0.18	22.79^{b}±0.91	18.61^{a}±0.61
O. niloticus	78.93^{d}±0.66	56.90^{c}±0.23	26.67^{b}±0.25	16.47^{a}±0.19	15.39^{a}±0.39

CDO = Control diet; GSP = Gossypol.
The mean difference is significant at 0.05 level
Means for groups in homogenous subsets are displayed
Uses harmonic mean sample size = 2.00

Table 5. Egg production of *C. zillii* and *O. niloticus* nourished with synthetic gossypol meal-based diets

	Control	Gossypol diets			
	CDO	GSP1	GSP2	GSP3	GSP4
Egg sizes (mm)					
C. zillii	1.32^{b}±0.00	1.31^{b}±0.01	1.24^{a}±0.02	1.23^{a}±0.01	1.25^{a}±0.01
O. niloticus	2.32^{d}±0.00	2.01^{c}±0.00	1.81^{b}±0.11	1.65^{ab}±0.03	1.58^{a}±0.00
C. zillii					
Absolute fecundity	713.00^{e}±8.00	201.50^{d}±1.50	142.00^{c}±1.00	118.50^{b}±4.50	91.50^{a}±1.50
Relative fecundity	22.00^{d}±0.00	6.00^{c}±0.00	4.00^{b}±0.00	3.50^{b}±0.50	2.00^{a}±0.00
O. niloticus					
Absolute fecundity	356.00±6.00	211.00±5.00	105.50±1.50	51.50±2.50	55.00±1.00
Relative fecundity	7.49±0.18	3.44±0.03	1.57±0.01	0.69±0.01	0.68±0.01
Gonadosomatic indices					
C. zillii	4.75^{d}±0.05	4.56^{d}±0.05	3.69^{c}±0.04	3.29^{b}±0.14	2.70^{a}±0.00
O. niloticus	4.55^{c}±0.04	2.84^{b}±0.00	1.98^{a}±0.00	1.96^{a}±0.01	1.97^{a}±0.00

CDO = Control diet; GSP=Gossypol.
The mean difference is significant at 0.05 level
Means for groups in homogenous subsets are displayed
Uses harmonic mean sample size = 2.00

Discussion

Testicular Histology in *C. zillii* Fed a Diet Based Primarily on Crude Cottonseed Meal

The experimental diets utilized in the research contain a crude protein content of 37.5%, aligning with the recommended range of 35-40% dietary protein requirement for Tilapia as specified by the National Research Council (NRC, 1993). The high abundance of spermatozoa observed in the seminiferous lumina of *C. zillii* testes, nourished with the control diet containing 0g CSM/kg, is consistent with the findings of Ekanem and Okoronkwo (2003). They utilized seed as a reproductive control agent in male *O. niloticus* and reported that the testis under the control group exhibited a normal distribution of sperm cells. Reduced and shrunken interstitium/sertoli cyst in the group of fish fed 109g CSM/kg diet as well as sparse lumina of the seminiferous tubules in fish fed 218g CSM/kg diet is in line with (Irm *et. al.,* 2022) who assessed the effect of cottonseed meal fed to Hybrid Grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) and reported that cottonseed impaired reproductive development regardless of the gossypol concentration in the diet. In *C. zillii* fed 327g CSM/kg diet, testis showed scanty spermatozoa, some lumina were spent or emptied. This result is in accordance with (Elham *et. al.,* 2013) where *C. papaya* seed was fed to *O. niloticus* and distortion was reported in both seminiferous lobule and interlobular tissues of testes in males administered with high dose. Also, in a related research by (Akin-Obasola and Jegede, 2016), *Gossypium herbaceum* was fed as reproduction control to male *O. niloticus*, histological result of the testis showed that there was disunion in the seminiferous lobule at 15g of *Gossypium herbaceum* /kg diet. At 436g CSM/kg diet, section of testes manifested thickened interstitium and reduced lumina which were

devoid of spermatozoa. This result is in conformity with (Akin-Obasola and Jegede, 2016) who fed (*Gossypium herbaceum*) as reproduction control in male *O. niloticus* and at a high dose, section of testis revealed hydropic decadence and increased interstitium cells.

Section of Ovaries in C*. zillii* Nourished with Crude Cottonseed Meal-based Diets

Normal ovary histology and vitellogenic stages in *C. zillii* fed with the control diet (0g CSM/kg is in unison with (Elham *et al.,* 2013) with report of normal histological structure of ovaries tissues in negative control group of fish treated with *C. papaya* as reproduction inhibitor. At 109g CSM/kg diet which is the lowest inclusion of cottonseed meal, there were distorted oocytes. This is in line with (Irm *et. al.,* 2022) who assessed the effect of CSM in the diets of (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) and reported that cottonseed impaired reproductive development regardless of the gossypol concentration in the diet. At 218g CSM/kg diet, histological sections showed few atretic oocytes along with the vitellogenic stage. The report corresponds with (Jegede *et. al.,* 2008a) where pawpaw seed meal and *Azadirachta indica* leaf meal diet were fed to *Tilapia zillii* to control their population and result revealed that the ovaries were moderately damaged at lower dietary PSM or NLM levels (0.5-1.0 g/kg diet), while at greater PSM or NLM inclusion levels, fragmentation of many more cells was reported, leaving the ovaries empty without oocytes. The result also agrees with (El-Sayed *et. al.,* 2007) who carried out a research on the reproductive biology of *Tilapia zillii* and reported spent ovary with atretic oocytes. At 327g CSM/kg diet and 436g CSM/kg diet, histological sections of the ovary showed deformed vitelline phases. The result is further substantiated by (Jegede, 2010) when *Hibiscus rosa-sinensis* was fed to control reproduction in *Oreochromis niloticus* and at high dose, phase four vitelline was reported where numerous oocytes which would have been brought were atrophied because the physical parameters needed for the oocyte maturity and resultant spawning were absent. (Elham *et. al.,* 2013) also reported similar results when pawpaw seed meal was fed to *Oreochromis niloticus* to control its reproduction.

Section of Testes in *O. niloticus* Nourished wit Crude Cottonseed Meal-based Diets

Testicular section in *O. niloticus* when fed a control diet, displayed normal sperm cell organization and abundant spermatozoa in seminiferous lumina. This finding is consistent with the findings of (Adeyemo et al., 2007), who noted a correlation between spermatozoa quantity and sertoli or interstitium cells. The sertoli cells or interstitium will undoubtedly benefit from an excess of spermatozoa, and vice versa. Several empty holes with few spermatocytes and a thicker intestinal wall were found at 109g CSM/kg diet. This outcome aligns with the findings of Shengli et al., (2021), who fed juvenile golden pompano (*Trachinotus ovatus*) with low-gossypol cottonseed meal instead of fish meal. They observed a significant decrease in intestinal wall thickness and microvilli length as gossypol inclusion increased. Thickened sertoli cysts, compressed and shortened lumen, and inadequate lumina of the seminiferous tubules observed at 218g and 327g CSM/kg diets are consistent with the findings of study by (Adeyemo et al., 2007) in which breeder cocks were fed cottonseed cake in place of soyabean cake for 23 weeks. The results indicated that treatments with more than 50% substitution had significantly fewer sertoli cells than those with lower levels. In turn, this resulted in a shortage of spermatozoa in the testicular cavities of *O. niloticus* due to a significant reduction in the interstitium or sertoli cells. While at 436g CSM/kg diet, section showed deformed vitelline phases. In a similar research by (Jegede, 2010), *Hibiscus rosa-sinensis* was fed to *O. niloticus* at a high dose as reproduction control measure, resultant effect was phase four vitelline featuring atrophied oocytes which could have been brought forth. Similar report was put forward by (Elham *et. al.,* 2013) in sections of ovary of *O. niloticus* fed high dose of pawpaw seeds.

Section of Ovaries in *O. niloticus* Nourished with Crude Cottonseed Meal-based Diets

Ovarian section of *C. zillii* nourished with control diet (0mgFG/kg diet) exhibited usual ovary histology, together with vitelline stages. This result is in accordance with (Wang *et. al.,* 2014) who stated that usual follicles and well-organized granulosa cells, without being atrophied is expected of a control diet because it is an untreated group. In *C. zillii* fed with all the synthetic gossypol supplemented diets, histological sections showed mature vitelline stages but distorted and empty. This means that this group of fish managed to pass through the cytological stages up to the last stage where they would have been released but were distorted and destroyed. At (330mgFG/kg diet) 650mgFG/kg diet, 980mgFG/kg and 1310mgFG/kg diets showed different stages of oocyte and vitelline development but were distorted. In general, all the ovaries of synthetic gossypol supplemented diets were either distorted or empty or their maturation delayed. This submission is corroborated by (Dabrowski *et. al.,* 2000) having worked on gossypol containing diets fed to O*ncorhynchus mykiss* and delayed gonadal maturation was reported. Also (El-Saidy and Gaber, 2004) documented convincing delay in gonad maturation in *Oreochromis niloticus* when fish meal was replaced with cottonseed meal for detoxification of gossypol.

Section of Testes in C*. zillii* Nourished with Synthetic Gossypol Meal-based Diets

The abundant spermatozoa in the seminiferous lumina and general pool found in testes of *Coptodon zillii* nourished with the control diet (0mg FG/kg diet) is a representation of the matured state of the testes, as it is expected of a control sample after keeping them together (male and female) for 90 days. In a study conducted by Tiago et al., (2016), male lambs in the control group, nourished with cottonseed meal over a 24-month period, exhibited elevated testosterone levels, more mass movements, and superior scores for seminiferous epithelium compared to groups subjected to progressively higher dietary levels. In all *C. zillii* fed synthetic gossypol supplemented diets, (330mgFG/kg diet, 650mgFG/kg diet, 980mgFG/kg diet and 1310mgFG/kg diet); histological results followed the same trend as spermatocytes were all still enclosed within the germinal cysts. This shows that they are still maturing because spermatozoa are formed normally within germinal cysts. In an investigation by (Chris-Otolo *et. al.,* 2018) cottonseed oil was fed to male rats and histological results showed detrimental effect on the testis by disorganizing spermatogenesis and histological structure of the testis.

Section of Ovaries in *C. zillii* Nourished with Synthetic Gossypol Meal-based Diets

Ovarian histology in *C. zillii* When fed a control diet containing zero milligrams of folic acid per kilogram, zillii showed typical ovarian histology and vitellogenic phases. This conclusion is consistent with the findings of Wang et al. (2014), who noted that as the control diet group was not given any treatment, normal follicles and well-organized granulosa cells that are not atrophied are predicted. In histological sections of *C. zillii* fed with all the synthetic diets supplemented with gossypol revealed mature vitelline stages, although they were deformed and empty. This indicates that the fish in question made it through all of the cytological stages, including the final one where they were supposed to be released but were instead deformed and perished. Different stages of oocyte and vitelline development were observed at (330mgFG/kg diet), 650mgFG/kg diet, 980mgFG/kg diet, and 1310mgFG/kg diet, but they were distorted. All of the ovaries in diets fed with synthetic gossypol were, in general, either malformed, empty, or had delayed maturation. The work of Dabrowski et al. (2000), who investigated diets containing gossypol given to Oncorhynchus mykiss and showed delayed gonadal development, supports this argument. Additionally, El-Saidy and Gaber (2004) showed a significant delay in the maturity of the gonads in *Oreochromis niloticus* when cottonseed meal was substituted for fish meal in order to detoxify gossypol.

Section of Testes in O*. niloticus* Nourished with Synthetic Gossypol Meal-based Diets

Testicular section of *O. niloticus* nourished with the control diet (0mg FG/kg diet) showed abundant spermatozoa within the seminiferous tubules. At 330mg FG/kg diet and 650mgFG/kg diet, lumen of the germinal cyst appeared greatly shrunken with scanty amount of spermatozoa in the testicular pouches. In a similar study by (Chris-Otolo *et. al.,* 2018) cottonseed oil was fed to male rats and histological results exhibited increase in the luminal diameter of seminiferous tubules, a reduction in Leydig cell population, dispositioning of spermatogenic series and disengagement from the germinal epithelium; but in *O. niloticus* fed 980mgFG/kg diet and 1320mgFG/kg diet showed spent cavities devoid of spermatozoa and shrunken seminiferous cavities. In corroboration, (Hammami *et. al.,* 2009) fed crude extract of (*Allium sativum*) to male rats for 30 days and report showed rise in percentage of bare seminiferous tubules at 5%, 10%, 15% and 30%

Section of Ovaries in *O. niloticus* Nourished with Synthetic Gossypol Meal-based Diets

An ovarian section in *O. niloticus* fed the control (0mgFG/kg diet) showed typical vitellogenic stages and ovarian architecture. This outcome concurs with the findings of (Elham et al., 2013), which showed that the ovaries' tissues had a normal histological structure in the fish in the negative control group that received *Carica papaya* treatment as a suppressor of reproduction. Histological sections at *O. niloticus* fed 330mg FG/kg diet, 650mg FG/kg diet, 980mgFG/kg diet and 1310mg FG/kg diets all revealed early vitelline stages and atretic oocytes even at 90 days. This means that the ovaries were still in their early stages of development and could not complete their cytological phases while some of them were atretic. Corresponding submission was mad on section of ovaries of *O. niloticus* fed *Aloe Vera* latex as reproduction inhibitor (Jegede, 2011). Additional corroborating findings were reported by Luo et al. (2006), who conducted a study involving the partial or complete replacement of fishmeal with solvent-extracted cottonseed meal in the diet of female rainbow trout. Their findings indicated a detrimental impact on fertility in response to the dietary alterations.

Milt Production in *C. zillii* and *O. niloticus* Fed Diets Primarily Composed of Crude Cottonseed Meal

The initial milt count of *C .zillii* fed the control diet was 121,500ml and declined to 94,000ml at 436g CSM/kg diet; also in control group of *O. niloticus*, milt count declined from 162,500ml to 96,500ml in fish fed with 436g CSM/kg diet. The significance difference and variance in the milt count of the control group and those fed cottonseed meal at high inclusion showed that fertilization is more likely to happen when sample with

dense milt having bounteous counts of sperm cells is used for fertilization, compared to milt sample having smaller mass of spermatozoa (Saeed *et al.,* 2010) investigated fish milt quality and primary determinants affecting milt quality parameters and reported that once motility is activated, spermatozoa progress towards micropyles at the surface of eggs after which fertilization occurs. The initial milt motility in the control group of *C. zillii* fed cottonseed meal-based diet was 77.77%, and it declined to 37.04% in fish nourished with 436g CSM/kg diet whilst in the control group of *O. niloticus* nourished with cottonseed meal-based diet, initial milt motility was 81.24% and declined to 26.90 in fish fed with 436g CSM/kg diet. In a similar study by (Joy 2020), graded levels of whole cottonseed was used to feed red Sokoto bucks; in the result, lower gross sperm mobility was recorded in batch fed the highest percentage (45%) at 60, 75 and 90 days respectively compared to lower cottonseed fed bucks. Denseness and mobility of sperm are the main parameters that determine fertilization of spermatozoa frequently used in appraising the quality of milt (Krol *et al.,* 2006).

The milt motility was extremely low in diets supplemented with cottonseed meal at high doses, this showed the antifertility efficacy of cottonseed meal because the no of offspring depends largely on the quality of milt. Motility duration in the control group of *C. zillii* fed cottonseed meal-based diet declined from 46 seconds to 13 seconds in fish nourished with 436g CSM/kg diet whilst in the control group of *O. niloticus*, motility duration declined from 51 seconds to 15 seconds in fish fed with 436g CSM/kg diet. The reduction in the milt duration followed the same trend as in milt motility, as the inclusion of cottonseed meal increased in the diets, milt duration decreased. (Akin-Obasola and Jegede, 2016) in a similar study reported same flow in decrease of milt volume when *O. niloticus* was fed with *Gossypium herbaceum* root bark. In a similar investigation by (Singla and Meenu, 2007), pure gossypol was fed orally to *B. bengalensis*, results of those fed at 0.05% pure gossypol for 4 days and 0.01 and 0.02% pure gossypol for 16 days revealed substantial reduction in sperm mobility and viability in the caudal epidymal fluid.

Egg Production of *C. zillii* and *O. niloticus* Nourished with Crude Cottonseed Meal-based Diets

Absolute fecundity in *C. zillii* nourished with crude cottonseed meal decreased significantly with rise in the inclusion of cottonseed in the experimental diets from 685.00±33.00 to 148.50±16.50. The highest absolute fecundity was documented in the control diet while the least value was recorded in fish having CSM added to the diet at 436g CSM/kg. The same trend was documented for *O. niloticus* fed cottonseed meal-based diets at different inclusion with 335.00±10.00 in control diet to 125.50±0.50 in fish having CSM added to the diet at 436g CSM/kg. Observation showed that in *O. niloticus,* result was significantly ($p<0.05$) low compared to that of *C. zillii* because the breeding approach of *O. niloticus* happens in the mouth of the mother hence, it does not support high population because the eggs were brooded by a parent and may not receive enough oxygen to nurture them to fry unlike *C. zillii* whose both parents participate in the process and ensure that a good number of the eggs develops in to fry (Achionye-Nzeh, 2011). The result showed that maternal parent brooded lesser eggs which is in conformity with the findings of (Kariman and Hanan, 2008) who observed that in cavity brooding cichlids, fecundity is markedly lesser on the account of parents brooding less but assuring the survival of their young ones consequently, less mortality. The work of Blom et al., (2001) on the efficacy of reproduction and the distribution of gossypol from mothers to offspring in rainbow trout lends additional credence to this. The result was explicit on the effects of feeding high cottonseed meal diets on their reproductive effectiveness at 50%. Relative fecundity in both *C. zillii* and *O. niloticus* fed with CSM-based diets at various inclusion levels decreased significantly as the inclusion of CSM increased ($p<0.05$) from 21.50±2.50 to 4.50.50±0.50 and from 7.97±0.00 to 2.55±0.10 respectively.

Jaspe & Caipang (2011) did a research on selective breeding on O. mossambicus and submitted that relative fecundity reduced as size of female broodstocks advanced. Values of mean egg diameter of *C. zillii* nourished with cottonseed meal-based diets did not differ significantly ($p>0.05$). Mean egg diameter ranged between 1.31mm ±0.01 and 1.39mm ±0.01 in the diets whilst for *O. niloticus*, it ranged between 2.22mm ±0.01 in the control diet to 1.94±0.34 in fish fed 436g CSM/kg diet. It was observed that as the level of cottonseed increased in the diets, their sizes were also affected with control diet having the lowest while the highest mean egg diameter was recorded with *O. niloticus* fed 436g CSM/kg diet. The significant decline recorded in the egg diameter of all fish nourished with crude cottonseed meal is in conformity with (Jegede, 2011 and Elham *et al.,* 2013) who fed *O. niloticus* with *Aloe vera* latex and reported that egg diameter was within the range of (2mm-2.25mm). The gonadosomatic indices recorded for both *C. zillii* and *O. niloticus* reduced as cottonseed meal elevated in the diets from 4.69±0.46 in control diet to 3.96±0.03 in diet 5 with no convincing difference ($p<0.05$) in the values of diet 4 and diet 5. *O. niloticus fed* varying inclusions of cottonseed meal-based diets also follow the same trend as obtained in *C. zillii* from 5.03±0.05 in control diet to 3.67 to ±0.21 in diet 5. The obtained result can be linked to the damage already done to the spermatozoa and oocytes, evident in the sections of testis and ovary tissues which eventually led to production of few offspring in fish fed with medium to high dose of cottonseed meal. This report is supported by (Jegede and Fagbenro, 2008) who fed *Azadirachta indica* to *Tilapia zillii* for reproduction control and reported that the gonadosomatic indices

reduced significantly as *Azadirachta indica* increased in the diets. Similar result was documented by (Jegede, 2010, Akin-Obasola & Jegede, 2014).

Milt Production of *C. zillii* and O*. niloticus* Nourished with Synthetic Gossypol Meal-based Diets

The initial milt count of *C. zillii* control diet (0mgFG/kg diet) was 131,000ml and declined to 51,000ml in GSP4 (1310mgFG /kg diet) while in the control group of *O. niloticus*, milt count declined from 168,500ml to 39,000ml in (1310mgFG /kg diet). The effect of this absolutely low sperm count in all the gossypol treated fish may have limited the ability of the spermatozoa to fertilize eggs; which was evident as fertilization did not take place at all in all the tanks except in the control groups. The results are in agreement with (Babashami *et. al.,* 2015) where cottonseed cake and whole cottonseed cake were fed to rams and reported that at 10 and 12 weeks, ratio of dead spermatozoa considerably elevated at weeks ten and twelve for rams that were fed whole cottonseed cake as well as elevated dead spermatozoa at ten to fourteen weeks of those fed cottonseed cake. The result is also in corroboration with (Taha *et. al.,* 2006 and Akinola *et. al.,* 2006) who established that gossypol inhibits spermatogenesis by decreasing the sperm count of male rabbits. The initial milt motility in the control group of *C. zillii* fed synthetic gossypol meal- based diet was 82.82%, which gradually decreased to 54.12% in GSP1, 36.81% in GSP2, 22.79% in GSP3 and finally to 18.61% in GSP4. While in the control group of *O. niloticus* fed synthetic gossypol- based diet, the initial milt motility was 78.93% which also gradually declined to 56.90% in GSP1, 26.67% in GSP2, 16.47% in GSP3 and finally to 15.39% in GSP4. The low and reduced sperm count and spermatozoid motility in these results is as a result of the inhibition of spermatogenesis by gossypol. In a similar study, (Roychoudhury *et. al.,* 2009) established that gossypol hinders spermatogenesis in male rabbits by reducing spermatozoid mobility, viability and sperm count. Motility duration in the control group of *C. zillii* fed synthetic gossypol meal-based diet declined from 43.50 seconds to 15 seconds in GSP4 while in the control group of *O. niloticus*, motility duration declined from 56 seconds in the control diet to 13 seconds in GSP4. This result agrees with (El-Sharaky *et. al.,* 2010) who reported gossypol toxicity for male reproduction through inhibition of spermatogenesis, consequently, decrease in sperm motility, viability and sperm.

Egg Production of *C. zillii* and O*. niloticus* Nourished with Synthetic Gossypol Meal-based Diets

Mean egg diameter ranged between 1.32mm ±0.00 in the control diet and 1.25mm ±0.01 at (1310mgFG /kg diet) while the order of mean egg diameter for *O. niloticus* fed synthetic gossypol meal-based diets was 2.32mm ±0.00 in the control diet to 1.58±0.00 at (1310mgFG /kg diet). It was observed that rise in inclusion of synthetic gossypol consequently affected the size of the eggs with control diet having the highest mean egg diameter for both *C. zillii and O. niloticus*. This result is in reach of the range of egg diameter (2mm-2.25mm) documented in previous investigation by (Jegede, 2011) for *O. niloticus* fed with *Aloe vera* latex to control their reproduction. Obtained result from this study for *C. zillii* showed that absolute fecundity decreased as synthetic gossypol rose in the diets from 713.00±8.00 to 91.50±1.50. The highest absolute fecundity was documented in the control diet while the least value was documented in fish where synthetic gossypol was added to their diets at (1310mgFG /kg diet). The same trend was recorded for *O. niloticus* fed synthetic gossypol meal-based diets at different inclusion with 356.00±6.00 in control diet and 55.00±1.0 in fish having synthetic gossypol added to the diet at (1310mgFG /kg diet). Relative fecundity in both *C. zillii* and *O. niloticus* fed with synthetic gossypol-based diets at various inclusion levels decreased significantly as the inclusion of synthetic gossypol increased ($p<0.05$) from 22.00±0.00 to 2.00.50±0.00 and from 7.49±0.18 to 0.68±0.01 respectively. The gonadosomatic indices recorded for both *C. zillii* and *O. niloticus* reduced as synthetic gossypol rose in the diets. For *C. zillii*, GSI ranged from 4.75±0.05 in the control diet to 2.70±0.00 in GSP4 and they all differed significantly ($p<0.05$). *O. niloticus* fed synthetic gossypol meal-based diets also follow the same trend as obtained in *C. zillii* from 4.55±0.04 in control diet to 1.97±0.00 in GSP4. In contrast, there was no difference ($p>0.05$) by (Meric *et. al.,* 2011) in the gonadosomatic indices for both male and female fed with cottonseed cake in replacement of fishmeal but the values obtained in this study is in consonance with (Jegede and Fagbenro, 2008) who fed *Azadirachta indica* to retard reproduction in *Tilapia zillii* and result showed that gonadosomatic values reduced significantly as the quantity of *Azadirachta indica* rose in the diets. Similar result was reported by (Akin-Obasola & Jegede, 2014).

Water Quality

The average water quality parameter for temperature in the current study was 27.00±0.00°C, dissolved oxygen was 7.60±0.12 mg/L, and pH was 7.30±0.05. These values fall inside the acceptable range in C for the best possible development. *O. niloticus* and *C. zillii*. According to Ross (2000). The quality of the water was unaffected by the experimental treatments.

Conclusion

Gossypol, both synthetic and raw, was added to *C. zillii* and *O. niloticus* had a significant impact on their ability to reproduce. Their ability to reproduce decreased as the amount of synthetic and crude gossypol in their meals rose. The histological sections

demonstrated how it impacted egg quality and milt output. More harm was observed even at the lowest dosage of gossypol, both natural and synthetic, as their presence in the diets increased. Consequently, while addressing *C. zillii* and *O. niloticus* early development and reproduction, which consequently have detrimental effects on their size at maturity, crude and artificial gossypol is a potentially effective treatment.

Ethical Statement

The manuscript have been read and approved by the author.

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Author Contribution

First Author: Conceptualization, Writing -review and editing; Second Author: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft.

Conflict of Interest

The author declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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