Journal of Researches in Agricultural Sciences

http://faculty.eksu.edu.ng/agric.office/published-volumes
Vol. 6 (1), March, 2018. Pp 1-10

Histological Changes in Gonads of *Oreochromis niloticus* (L) Fed Bitter Melon (*Momordica charantia*) Leaf Diets.

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Abstract

The histology of gonads in *Oreochromis niloticus* fed bitter melon (*Momordica charantia)* leaf meal (MCLM) diets for 80 days was studied. Shade-dried bitter melon leaves were milled into fine particle sizes and used to prepare five is nitrogenous rations of 0, 30, 60, 90 and 120 g MCLM / kg diets to provide 35% crude protein using menhaden fish meal (65% crude protein), yellow maize, vegetable oil, vitamin-mineral premix, cod-liver oil and soybean. The paste formed after adding 10 ml hot water at 90 – 100 °C at intervals to gelatinize the starch content was pelletized and air-dried at ambient temperature (28°C) for 72 hours to constant moisture content. Juvenile of *Oreochromis niloticus* (male and female) of 30.36±0.13 g were stocked in plastic tanks (1 x1 x 1 m) containing 400 litres clean borehole water. The experiment was replicated thrice and the fish were fed twice at 4% body weight/day. Proximate analysis, histological analysis, fish milt and fecundity count were done using standard methods. Analysis of Variance (ANOVA) was used to test for significant differences in means and Turkey test (P>0.05) for post-hoc test. The hhistological section of *O. niloticus* ovary fed 30, 60, 90 and 120 g MCLM/kg diet showed increase in interstitial cells, few oocytes in the ovary, abnormal gonadal development and necrosis while male testes showed increase in interstitial cells, disintegration in connective tissues and seminiferous lobules. As the concentration of MCLM increases, the fecundity and milt count also decreased. These results showed that *Momordica charantia* can be used to control the fertility of tilapia.

Keywords: Histology, *Oreochromis niloticus*, *Mormordica charatia*, gonadal development.

Introduction

Tilapia is farmed in at least 85 countries of the world because it is the second most important group of farmed fish after carp and the most widely grown of any farmed fish on the planet (Burden,2014). The World Aquaculture Production and Principal Species Report listed Nile Tilapia (*Oreochromisniloticus*) world production at 3,197,330 metric tons, valued at $5,260,695,000 USD (FAO, 2012).The rapid growth rate of tilapia and its ability to reach the usual market size of 250-450 g in six months, palatability and hardiness has made it the focus of major aquaculture efforts in the world (Josupeit, 2005). Tilapia possess the attributes that include simplicity of rearing, hardness, undemanding feed requirements with minimal dependence on fish and oil resources, firm flesh texture and natural flavour. These features have made tilapia a desirable fish all over the world (Malcolm and Brendon, 2000). Tilapias attain sexual maturity at the age of 2-3 months and at a small size, around 8-10 cm body length (Chapman, 2012). The longevity of adults is six to eight years while some have been reported to reach eleven to twelve years of age (Rosagast, 2008). Tilapia spawns and produces offspring with ease which make it a good species to farm but this trait also creates problems. The survival of fries is high and

grow-out ponds often become over-populated and the cultured fish have stunted growth, as the natural food supplies in the pond is depleted (Fagbenro, 2002).Tilapia species and hybrids are yet to reach their full potential in aquaculture because of the problems of early sexual maturity, uncontrolled reproduction and overpopulation of production ponds with stunted fish (Adesulu, 1997).

Fortes (2005) reported that tilapia populations in grow-out ponds have been controlled by the periodic harvesting of fry and fingerlings (Mair, 2002), monosex culture (McGinty and Rakocy,1989), hybridization, hormone augmentation and genetic manipulation methods,which include androgenesis, gynogenesis, polyploidy and transgenesis, culture in cages, high density culture, biological control, eradication using organic toxicants and sterilization using medicinal plants (McGinty and Rakocy,1989), Plants possess antifertility properties which have been exploited in sperm demobilization in some animal models including tilapia (Jegede and Fagbenro, 2008). The plants with animal fertility-modulating properties are (Papaya) *Carica papaya* (Jegede and Fagbenro, 2008), Neem tree (*Azadirachta indica)* (Jegede and Fagbenro, 2007), Wild carrot (*Daucus carota),* Tumeric *(Carum carvi),* Mukajhuri *(Acalypha indica),* Betel *pepper (Piper betel),* and Sour Chinese date (*Zizyphus jujube) (*Priya *et. al.* 2012).

The use of the active ingredients in these plants rather than the crude extracts is likely to be more effective. Thus, phytochemicals may be synthesized once the active component is identified, compounded or otherwise transformed to make pharmaceuticals. Kumar *et.al.* (2012) reviewed potential anti-fertility agents from 577 plant species belonging to 122 families and reported that 298 of them are abortifacients (42%), 188 are contraceptives (31%) while149 are emmenagogues (24%) and 17 are sterilizers. The bitter melon, (*Momordica charantia)* Linn (Cucurbitaceae) grows in tropical areas, including parts of the Amazon, east Africa, Asia, and the Caribbean. It is cultivated throughout South America for food and medicine (Gunn and Fansworth, 2013). It is a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. All parts of the plant, including the fruit, taste very bitter (Tropical Plants Data Base, 2013). Bitter melon is used traditionally as an abortive and has been associated with weak uterine stimulant activity and therefore, contra-indicated during reproduction (Kumar *et.al*, 2010).

Materials and Methods

Source of *Momordica charantia* and formulation of experimental diets

Bitter melon (Momordica charantia) leaves (Plate 1) were obtained from Ifaki- Ekiti (7º 48´ 0" North, 5º 14´ 0" East) in Ido-Osi Local government area of Ekiti State, Nigeria where the plant grow naturally. The *Momordica charantia* leaf meal (MCLM) were spread and air-dried at ambient temperature (28ºC) for fourteen days and turned into powder using mortar and pestle. This leaf powder was stored in sealable polythene bag and refrigerated at 18°C for two months in. Five is nitrogenous diets were formulated to provide 35% crude protein using menhaden fish meal (65% crude protein), yellow maize, vegetable oil, vitamin-mineral premix, cod-liver oil and soybean. The feedstuffs were purchased from retail outlets in Ekiti State, Nigeria. All the ingredients were milled and reduced to 10 µ particle size. The feed stuffs were weighed on a MetlerTop Loading Balance (Model PB-800I) and mixed in a Hobart A-200T pelleting and mixing machine. 10 ml hot water at 90 - 100°C was added at intervals to gelatinize the starch content.

Plate 1:*Momordica charantia* leaves (SR)

Table 1: Composition of ingredients (g/kg) of experimental diet.

Ingredients	Menhaden	Soya meal	Yellow maize	Cod liver oil	Corn oil	Vit-Mineral premix(1:1)	Corn starch
(g/kg)	270	385	245	30	20	30	20

The MCLM portion at 0, 30, 60, 90 and 120 g were added to the five treatments used (except in treatment 1) as shown in Table 2.

Table 2: The treatments used for the experiment

Amount of MCLM in feed (g)	Description of feed sample
0	DIET A (Control)
30	DIET B
60	DIET C
90	DIET D
120	DIET E

The five diets were pelletized using 0.8 mm diameter die and air-dried at ambient temperature of 28°C for 72 hours on a raised concrete platform to constant moisture content. The dried pellets were crushed in a blender, sieved into 2 mm particle size, packed in polythene bags, labelled and stored at in a refrigerator (-18ºC).

Source of *O. niloticus* and maintenance

One hundred and fifty juvenile of *Oreochromis niloticus* (average weight = 30.36±0.13 g), consisting equal number of male and female were purchased from Ekiti State Ministry of Agriculture and Rural Development Fish Farm, Ado Ekiti and transported inside aerated polythene bags to the experimental site. Ten fish samples were kept in plastic tanks measuring 1 x 1 x 1m and 1.45 inches thickness to acclimatize for 14 days, during which the fishes were fed with commercial feed (Coppens) of 2 mm diameter, containing 30% crude protein. After acclimation, five males and females *O. niloticus* juvenile were selected and stocked in each of the plastic tanks containing 400 litres of borehole water.

Experimental design and feeding regimes

The treatments were replicated thrice. Feeding commenced 24 hours after stocking and the experiment lasted for 80 days. The fish were fed at 4% body weight per day in two instalments: at 0900-0930 h and 1700-1730 h. Kakabans were

placed at the corners of each tank in case of spawning by the female fishes.

Proximate and milt quality analysis

After feeding for 80 days the fish samples were weighed. Proximate analysis was performed for moisture, crude protein, crude lipid, crude fibre, ash and nitrogen free extract using AOAC (1995) procedures. Milt volume and quality were determined at the end of the experiment. Two male fish, randomly selected from each treatment were used. Milt was obtained by keeping the fish in anaesthetic water containing 2-phenoxyethanol (400 mgL^{-1}) (SRAC, 2004) to make milt collection easier. Drummond microcaps disposable micropipettes were used to collect the milt by torching the papilla opening with the pipette. Milt volume was measured using a syringe of 1.0 ml capacity with 0.1 ml calibration. Motility duration was determined by adding a drop of water to the milt collected to activate the sperm and placing 1µl of milt from each male on a Neubauer haemocytometer already covered with microscope slip. The sperm activity was viewed under microscope (Model: Olympus BH2) at X100 magnification and motility was determined by timing the progressive and non-progressive movements of the sperms observed (Mims, 1991). Milt count was determined by the number of spermatozoa in 0.1 ml diluted sample (10 µl sperm in 90 µl MFR to make 100 µl (dilution 1), 10 µl dilution1 was diluted in another 90 µl MFR to make 100 µl (Dilution 2) and 0.1µl of the milt was loaded on Neubauer haemocytometer and counted under the microscope at X400 magnification (Sharma *et.al*. 2011).

Assessment of egg quality

Two lobes of egg were removed from two randomly selected matured female fish that their ovary contained ruptured follicles, oocytes in vitellogenetic and protoplasmic growth, as well as oogonia. The ssamples of egg representing 50% of ovary weight was counted and reported to the total weight of the ovary, mean egg size was determined using microscope eye-piece graticule to measure the Length and width of egg (Rana, 1985). The short and long axes of two egg samples from each sample were measured using light microscope containing a calibrated eye piece graticule. Mean egg diameter was calculated from each treatment as follows:

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

Ovary weights, relative fecundity, gonadosomatic index, number of fries, % fertilization and % survival was calculated using Rana, 1985 method.

At the end of the 80 days of experiment two male and female fishes were taken from each tank, killed by decapitation and the testes and the ovary were removed for sectioning and histological examination. The organs were fixed in formal-saline solution. Using microtone, histological sections of 5 µm thicknesses were prepared following the standard procedures (Histology Laboratory Manual, 2011-2012). Sections were fixed on clean slides and stained with haematoxylin and eosin. Photomicrographs were taken with Leitz (Ortholux II) microscope and camera, standard model BHTU-111.

$$\textbf{Growth rate} = \frac{\ln W_1 \text{ x} \ln W_0}{T}$$

W_1 = Fish weight at the end of study
W_0 = Initial weight at the start of study
T = Time interval in days

Feed conversion ratio =Dry weight of feed fed (g) / Fish weight gain (g)

Statistical analysis

Using SPSS package, the data was subjected to Analysis of Variance (ANOVA) to compare means and where significant differences were found, a post-hoc test was conducted and the means were separated using Turkey's Honestly Significant Difference (HSD).

Growth rate and feed conversion ratio were determined using the following formulas.

Water analysis was done using APHA (1987) method.

Results

Sections of *Oreochromisniloticus*ovary

Plate 2 shows normal histology with lumen filled with yolk droplets (YD) that tends to coalesce (CYD) and become larger while in Plate 3, there was increase in interstitial cell (IIC) and mild degeneration of oocyte (MD). Plate 4 shows that there were few oocytes (FO) in the ovary. Plate 5 shows abnormal gonadal development (AGD) and ovary almost devoid of oocytes. Also Plate 6 shows few oocytes (FOC), abnormal gonadal development (AGD) and necrosis (NE)

Sections of *Oreochromisniloticus* ovary fed *Momordicacharantia* leaf meal

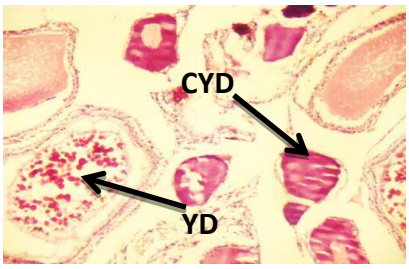

Plate 2: Ovary section in Diet A

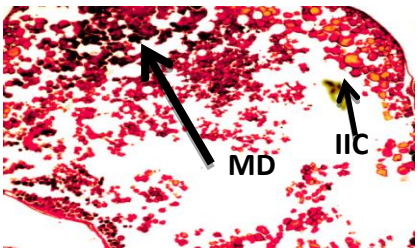

Plate 3: Ovary section in Diet B

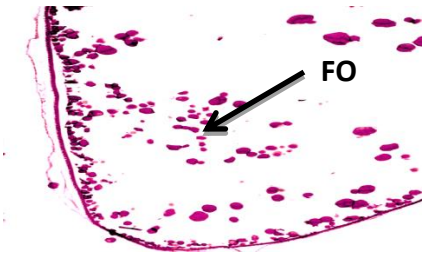

Plate 4: Ovary section in Diet C

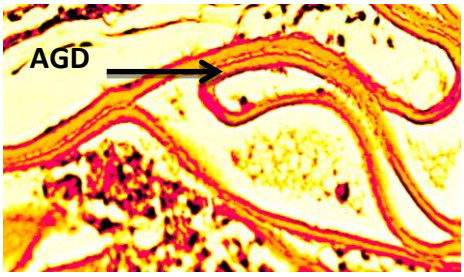

Plate 5: Ovary section in Diet D

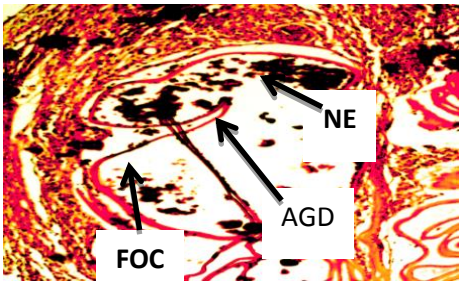

Plate 6: Ovary section in Diet E

Sections of *Oreochromis niloticus* testis

Plate 7 shows primary (PSP) and secondary spermatocytes (SSP) in the lumen of the seminiferous tubule. The connective tissue was also very prominent. Plate 8 shows eroded connective tissue (ECT) and hydropic degeneration (HD). Plate 9 is showing hydropic degeneration (HD) and disintegration in the seminiferous lobule (DSL) and interlobular tissue (DIT). Plate 10 shows hydropic degeneration (HD). Plate 11 revealed increase in interstitial cell (IIC), the connective tissue and seminiferous lobule were disintegrated (DSL).

Sections of *Oreochromis niloticus* testis fed *Momordica charantia* leaf meal

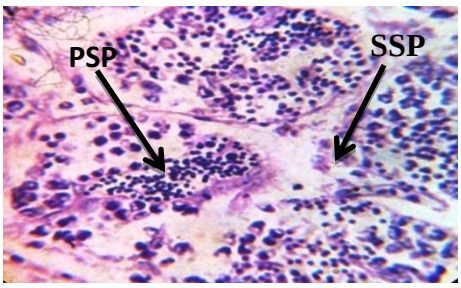

Plate 7: Testis section in Diet A

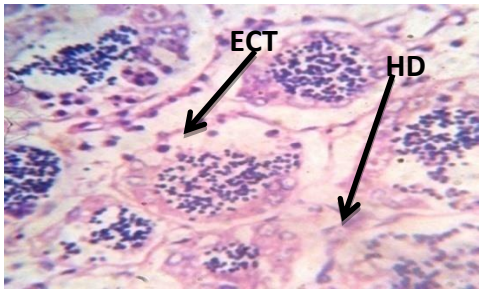

Plate 8: Testis section in Diet B

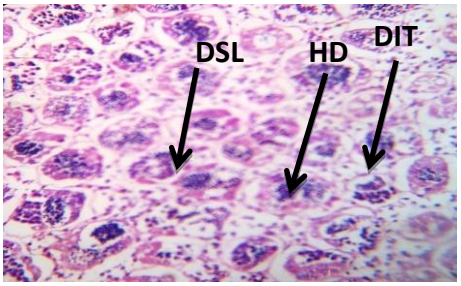

Plate 9: Testis section in Diet C

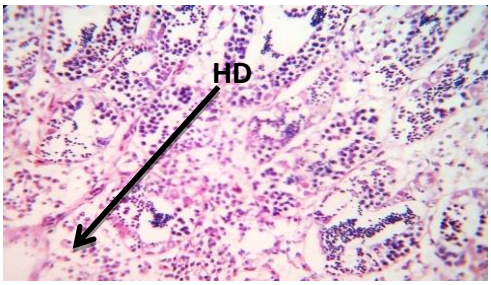

Plate 10: Testis section in Diet D

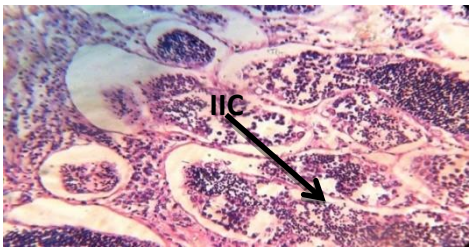

Plate 11: Testis section in Diet E

Reproductive analysis

The ovary weight reduced with the increase in MCLM concentration in the diets used (Table 3). The fecundity, relative fecundity and gonadosomatic index also reduced with increase of dietary MCLM. Ovary weights, fecundity, relative fecundity, gonadosomatic of the different treatment levels were significantly different statistically.

Table 3: Reproductive parameters of adult female *Oreochromisniloticus* fed *Momordicacharantia* leaf meal.

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Ovary weight (g)	3.05±0.08^{a}	2.33±0.17^{abc}	2.33±0.03^{b}	2.17±0.07^{bc}	1.87±0.03^{cd}
Egg wet weight (mg)	0.010±0.0003^{a}	0.013±0.0010^{ab}	0.013±0.00^{ab}	0.016±0.0006^{b}	0.017±0.0015^{b}
Diameter of egg (mm)	1.83±0.03^{a}	1.87±0.03^{a}	1.73±0.03^{ab}	1.70±0.06^{ab}	1.63±0.03^{b}
Fecundity	284.67±0.88^{a}	179.00±2.65^{b}	1.76.33±2.40^{bc}	136.00±1.53^{d}	112.00±10.54^{ab}
Relative fecundity	3.89±0.02^{a}	2.37±0.03^{b}	2.21±0.04^{b}	1.73±0.02^{c}	1.54±0.15^{bc}
Gonadosomatic index	4.17±0.10^{a}	3.08±0.23^{b}	2.92±0.05^{bc}	2.76±0.07^{c}	2.56±0.04^{d}

Means with the same letters are not significantly different with SPSS at 5% probability.

Table 4 shows that the final weight of the female fish fed the diets with MCLM inclusion differed significantly. Fish fed diet C had the highest weight which did not differ from diet D but both differs significantly from Diet B which in turn differed from Diet A and Diet E that gave similar weight .

Table 4: Growth parameters and nutrient utilization of adult female *Oreochromisniloticus*.

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Fish initial weight(g)	30.84±0.25^{a}	30.45±0.14^{a}	31.20±0.34^{a}	30.36±0.13^{a}	30.93±0.24^{a}
Fish final weight(g)	73.10±0.06^{a}	75.67±0.33^{c}	79.83±0.33^{b}	78.57±0.47^{b}	72.80±0.40^{a}
Feed Fed(g)	98.69±0.81^{a}	97.45±0.44^{a}	99.84±1.07^{a}	97.16±0.41^{a}	98.96±0.77^{a}
Weight Gain	42.26±0.27^{a}	45.21±0.46^{b}	48.63±0.63^{c}	48.20±0.59^{c}	41.87±0.29^{a}
Growth Rate	1.08±0.01^{a}	1.14±0.01^{b}	1.17±0.02^{c}	1.19±0.01^{c}	1.07±0.01^{a}
Feed Conversion Ratio	2.34±0.03^{a}	2.16±0.03^{b}	2.06±0.05^{b}	2.01±0.03^{b}	2.37±0.02^{a}
No of Fries	67.00±2.08	0	0	0	0
% Fertilization	23.67±0.88^{a}	0	0	0	0
Survival in 60 days	41.67±1.67	0	0	0	0
% Survival	62.33±2.73	0	0	0	0

Means with the same letters are not significantly different with SPSS at 5% probability.

Table 5 shows that milt volume, milt count and motility duration of sperm collected from fish samples that were raised on diets containing high concentration of MCLM reduced significantly (P=0.05). The testes weight was lowest in the fish samples fed Diet B (0.53±0.01) and highest in Diets C but the values were not significantly different.

Table 5: Analysis of reproductive parameters of adult male *Oreochromis niloticus* fed *Momordica charantia* leaf meal.

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Milt volume (ml)	0.60±0.00^{a}	0.53±0.12^{a}	0.53±0.07^{a}	0.37±0.09^{a}	0.23±0.03^{b}
Milt count x10^{9} (s/ml)	3.59±0.07^{a}	2.21±0.09^{b}	1.67±0.35^{c}	1.18±0.02^{d}	0.27±0.03^{e}
Testes weight (g)	0.54±0.02^{a}	0.53±0.01^{a}	0.56±0.01^{a}	0.54±0.01^{a}	0.55±0.01^{a}
Motility duration (min)	4.29±0.05^{a}	3.09±0.03^{b}	2.47±0.28^{abc}	1.18±0.09^{c}	0.15±0.02^{d}

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

As shown in Table 6, feed conversion ratio (FCR) in the adult male fish was highest in Diet D which differed significantly from Diet A, B, C and E with similar values. Weight gain was not significantly different between Diets A, C and E while Diet D gave the highest value of growth rate which differs from other treatments .

Table 6: Growth parameters and nutrient utilization of adult male *Oreochromisniloticus*

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Fish initial weight(g)	30.82±0.44^{a}	30.70±0.32^{a}	30.49±0.10^{a}	30.83±0.41^{a}	30.87±0.34^{a}
Fish final weight(g)	90.07±0.07^{a}	84.90±2.60^{b}	90.50±0.15^{a}	82.15±0.04^{b}	88.00±0.28^{a}
Feed Fed(g)	98.61±1.39^{a}	98.25±1.02^{a}	97.56±0.32^{a}	98.67±1.31^{a}	98.78±1.10^{a}
Weight Gain	59.25±0.37^{a}	54.20±2.58^{b}	60.01±0.08^{a}	51.31±0.38^{b}	57.13±0.13^{a}
Growth Rate	1.34±0.02^{a}	1.27±0.04^{a}	1.36±0^{a}	1.22±0.02^{b}	1.31±0.01^{a}
FCR	1.66±0.03^{a}	1.82±0.09^{a}	1.63±0^{a}	1.92±0.04^{b}	1.73±0.02^{a}

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

Discussion:

The histological section of the ovary of *O. niloticus* fed Diet A (Plate 2) showed normal appearance with the lumen filled with oocytes. As earlier reported by Morrison *et. al,* (2006). The inclusion of MCLM at different levels in the diet of *O. niloticus* showed increasing level of degeneration in the ovary. The lower concentration only increased the interstitial cell and mild degeneration of the oocytes but at higher level of inclusion, there was abnormal gonadal development and ovary devoid of oocytes. These results are in agreement with Jegede (2008) who administered different doses of *C. papaya* seed meals on *O. niloticus* and reported;

hydropic degeneration, ruptured follicle and necrosis. A similar histological effect was reported on the ovaries of *O. niloticus* fed *Hibiscus rosasinensis* leaf meal (Jegede, 2010) and *Aloe vera (Liliaceae)* latex (Jegede, 2011).

The sections of the testes in *Oreochromis niloticus* fed Diet A showed normal testicular tissue architecture and normal spermatids distribution but eroded connective tissue and hydropic degeneration in fish fed Diet B while Diets C, D and E caused hydropic degeneration, increase in interstitial cells, disintegration in connective tissue, seminiferous lobule and interlobular tissue respectively. These results agree with Jegede (2011) that *O. niloticus* fed *Aloe vera* gel meals had testes sections which showed spermatids disintegration, cystic seminiferous tubules and necrosis at higher concentrations.

In the female *O. niloticus,* the inclusion of MCLM in the diets at varying levels revealed normal oval shape of egg and no harmful change was noticed in the shape of eggs when observed under electronic microscope. This result agrees with Jegede (2011) who reported normal oval shape of egg in the control experiment when Aloe vera was fed to *O. niloticus*. The size and diameter of egg in the fish were not affected by MCLM inclusion in the diets but fecundity decreased. Sonia *et. al.* (2011) reported the anti-fertility effects of bitter melon (*Momordica charantia)* on female mice, and reversible anti-fertility on fecundity when the leaf (1.0ml) of the water extract was administered orally. In male *O. niloticus,* the milt volume, milt count and motility were higher in control diet and decreased as MCLM increased. Jegede (2011) reported similar decrease in milt motility and milt count when *O. niloticus* was fed 1.0, 1.5 and 2.0 ml/kg diets of *Aloe vera* in the diets for 60 days.

The best overall growth response were obtained in control experiment which was fed basal diet. Weight gain, feed efficiency ratio, specific growth rate and feed conversion ratio were optimal in the control and Diet C. The Feed conversion ratio was between 1.5-2.0; which also agrees with Ofori *et al.* (2009) recommendation of 1.4 - 2.5.

Conclusion

Several methods of reproduction control have been discovered and used but they are not readily available, expensive and require skilled labour to apply. The use of *Momordica charantia* leaf meal as reproduction inhibitor could be the panacea to the problem of early maturation and uncontrolled reproduction of tilapia most especially in developing countries which are characterized by poor and low income. The method is cheaper and accessible to poor farmers.

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