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Research Article
Effect of *Martynia annua* (Devil's Claw) Leaf Meal on the Reproductive and Growth Performance of *Oreochromis niloticus* (*Nile tilapia*)

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Abstract

Background and objectives: Precocious maturity of *Oreochromis niloticus* results in overpopulation of fish enclosure and eventually poor growth and production rate hence the need to control its reproduction for better harvest. The specific objectives of this study were to: assess the nutrient utilization of *Oreochromis niloticus* fed *Martynia annua* leaf meal, determine the effect of feeding *Martynia annua* leaf meal diets on the reproductive performance (milt count, testis weight, ovary weight and fecundity) of both male and female *Oreochromis niloticus,* analyze the anti-fertility effects on the histology of testis and ovary of *Oreochromis niloticus* and evaluate the effect of increasing dietary levels of *Martynia annua* leaf meal on the histology of the liver and gills. **Materials and Methods:** The effect of feeding *Martynia annua* leaf meal for 72 days on the reproductive performance of *Oreochromis niloticus* (average weight; 10.87±0.50 g) was studied. Five treatments [0, 20, 40, 60 and 80 g kg^{-1} *Martynia annua* leaf meal (MALM)] with three replicates were used. Plastic tanks (75×45×25 cm) with 30 L capacity were filled with well water of 20 liters and 15 *Oreochromis niloticus* were introduced into each tank. The fish were fed at the rate of 4% of body weight two times a day at 9:00 and 17:00 h. **Results:** A reduction in milt count and fecundity was observed as the MALM increased in the diets of *Oreochromis niloticus*. In all the treatments, there were significant differences ($p \leq 0.05$) in pH and dissolved oxygen of the water used. The histology of the testis of *Oreochromis niloticus* fed 0 g and 20 g kg of MALM showed primary and secondary spermatogonia in the lumen of the seminiferous tubule while the fish fed 40 g, 60 g and 80 g kg^{-1} of MALM showed necrosis, abnormal gonadal development and increase in interstitial cell. The histology of the ovary in fish fed 0 g kg^{-1} MALM showed yoke droplets while the ones fed 20, 40, 60 and 80 g kg^{-1} MALM showed developing oocyte, necrotic oocyte, increase in interstitial cell and alteration in ovary development respectively. The liver showed normal hepatocellular architecture in fish fed 0 g of MALM kg^{-1} and increase in interstitial cell in fish fed 20 g kg^{-1} MALM, increase in portal vein in fish fed 60 g kg^{-1} MALM and severe degeneration in the hepatocytes architecture in the fish fed 80 g kg^{-1} MALM. **Conclusion:** The histological observation and milt analysis revealed that *Martynia annua* leaf meal is very effective fertility inhibitor in *Oreochromis niloticus* reproduction. Moreover, the inclusion of *Martynia annua* in the diet did not inhibit the growth performance of *Oreochromis niloticus*.

Key words: Reproduction rate, growth performance, *Matynia annua*, *Oreochromis niloticus*, aqua farming

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

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

INTRODUCTION

In sub-Saharan Africa, Nigeria is the largest aquaculture producers[1]. Nigeria produces over 1 million metric tons of fish, leaving a deficit of over 800,000 metric tons, which is imported annually[1]. *Oreochromis niloticus* is the most popular tilapia fish cultured in Nigeria and taxonomists have shown special interest in it for research purposes because of taste, flavor, reproduction within a short period of time (which must be controlled) and acceptability.

Cichlids is a highly productive and globally traded food fish. It matures in 2-3 months at a very small size of 8-10 cm (body length)[2] and reproduces in large quantities within a short period; this affects the growth and production rate due to competition for food, space, dissolved oxygen and other resources present in water body. *Oreochromis niloticus* (*Nile tilapia*) is an important food source and a major economic resource, it can survive in a wide range of environmental conditions such as low dissolved oxygen levels, high temperatures, high salinity and high ammonia concentrations and these characteristics make it very suitable for aqua farming.

Oreochromis niloticus reproduces through mass spawning of a brood within a nest made by the male and their young ones are cared for through mouth brooding (oral incubation of the eggs and larvae exclusively by the female). After spawning, the mother carries the young fry or eggs in the mouth for 12 days and sometimes pushes back the young into her mouth to avoid danger[3]. *Oreochromis niloticus* is a low-priced fish due to its overpopulation and small size and leads to low income and poor standard of living for fish farmers.

Several methods to control the population of tilapia have been described and these include; monosex culture where single sex fish are obtained through manual separation of sexes[4], periodic harvesting of fry and fingerlings, culture in cage[5], hormone augmentation and genetic manipulation, biological control, high density culture, eradication using organic toxicant and sterilization.

Different methods to control tilapia population has been the subject of extensive research but these methods are always very expensive and not readily available. Some aquaculturists had controlled tilapia population using different plants[6]: *Momordica charantia* leaf[7], *Gossypium herbaceum* root bark[8] and *Tragia benthanii*[9] and more research on plants with capacity to inhibit fertility in fish cannot be over emphasized.

The Devil's claw plant (*Martynia annua*) has been shown to possess sterilizing compounds which can control *Oreochromis niloticus* reproduction rate. It is an herb in the family of Martyniaceae and commonly known as devil's claw (English) and "iko odide" in Yoruba language[10]. This plant contains alkaloid, tannins, saponins, glycosides, flavonoids, anthocyanin, amino acid, steroids and phenols[10].

This study evaluated the effect of adding an inexpensive and readily available *Martynia annua* leaf meal in the diet of *Oreochromis niloticus* to reduce their precocious maturity.

MATERIALS AND METHODS

Experimental plant: The Devil's claw plant (*Martynia annua*) was collected from Ado-Ekiti in Ado Local Government Area of Ekiti State and identified at the Department of Plant Science, Ekiti State University. The leaves were detached from the stems and air dried at room temperature, milled into fine powder and packaged in air tight container.

Experimental fish: Apparently healthy 150 Juvenile Nile tilapia (*Oreochromis niloticus*) were purchased from Adegoke Fish Farm Orin, Ekiti State and randomly distributed into 15 labeled plastic tanks. The fish were acclimatized for 72 h before the commencement of the experiment.

Experimental design: The experiment was carried out in a rectangular shaped (75cm×45cm×25m) plastic tanks with 30 liters capacity and it was filled with 20 liters well water.

This study used a completely randomized design (CRD) consisting of 5 treatments and 3 replications. The treatments were T_1 (0 g kg^{-1} of *Matynia annua* leaf meal), T_2 (20 g kg^{-1} of *Matynia annua* leaf meal), T_3 (40 g kg^{-1} of *Matynia annua* leaf meal), T_4 (60 g kg^{-1} of *Matynia annua* leaf meal) and T_5 (80 g kg^{-1} of *Matynia annua* leaf meal).

Feed formulation: The ingredients used were menhaden fish meal (72%), soya bean meal (48%), corn meal (8.8%), cod liver oil, groundnut oil, vitamin-mineral premix as shown in Table 1. The ingredients were milled and mixed together using hammer mill and the mixture was gelatinized in a cool and dry place. Different rates of *Martynia annua* leaf meal was included in the diet as required for each treatment and the ingredients were mixed together thoroughly with hot water and pelletized using manually made pelleting machine with a die diameter size of 4.00 mm. The pellets were sun dried, packed into well labeled air tight containers and stored in cool

Table 1: Composition of diets using M*artynia annua* leaf (g kg^{-1})

Ingredients	T_1 (g kg^{-1} diet)	T_2 (g kg^{-1} diet)	T_3 (g kg^{-1} diet)	T_4 (g kg^{-1} diet)	T_5 (g kg^{-1} diet)
Menhaden (fish meal) 72%	280	280	280	280	280
Soya bean meal 48%	370	370	370	370	370
Corn meal yellow 8.8%	250	250	250	250	250
Cod liver oil	30	30	30	30	30
Groundnut oil	20	20	20	20	20
Vitamin-mineral mix	30	30	30	30	30
Starch	20	20	20	20	20
Martynia annua leaf	_	20	40	60	80
Total	1000	1000	1000	1000	1000

and dry place. The fish were fed for 72 days at the rate of 4% body weight twice per day at 09:00-09:30 and 17:00-17:30 h respectively.

Water analysis: The dissolved oxygen, temperature and pH of the water used were monitored every 15 days. A digital pH meter (model number: Mettle Toledo 320) was used to measure the water pH, mercury in glass thermometer calibrated in °C was used to measure the water temperature and digital dissolved oxygen meter was used to determine the level of the dissolved oxygen which was in mg L^{-1} (model number: mettle Toledo 320) using the method described by Swann[11].

Biological evaluation: The experimental fish were weighed every 15 days. At the end of the experiment, the female and male fish were dissected; ovary and testes were removed and weighed; fecundity, size of egg, milt count were determined using the method of Mims[12].

Weight gain and feed conversion ratio was calculated using the following equations:

$$\text{Weight gain} = \text{Final weight } (w_2)\text{-initial weight } (w_1)$$

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

Histological and statistical analysis: The female and male reproductive organs, gills and liver were dissected and the histology was carried out using Histology Laboratory Manual (2011-2012)[13]. The photo micrographs of the organs were taken and the organs were analysed.

Data were analyzed using one-way analysis of variance and differences among the means were tested using Duncan's multiple range tests (DMRT) with 5% level of significance.

RESULTS

Table 2 shows the water quality at day 1 and 15. The DO_2 reduced at day 15. The pH value for treatment 2 and 3 remained the same on day 1 while the pH value for treatments 1, 4 and 5 were significantly different. The temperature at day 1 and 15 did not differ.

The histologies of the ovary sections in *Oreochromis niloticus* fed *Matynia annua* leaf meal (MALM) diets are shown in Fig. 1. T_1 showed the lumen was filled with yoke droplets (YD) while T_2 revealed an ovary filled with developing oocytes (DO). T_3 showed developing oocytes and some necrotic oocytes (NO). The section in T_4 showed deteriorating oocytes and increase in interstitial cell (IIC) while T_5 revealed no oocyte in the lumen and alteration in ovary development (AOD).

The histology of the testes *in Oreochromis niloticus* fed *Matynia annua* leaf meal is shown in Fig. 2. In T_1, secondary (SES) and primary (PS) spermatogonia are found in the lumen of the seminiferous tubule, the section of testes in T_2 showed secondary spermatogonia in the lumen of the seminiferous tubule. T_3 showed increase in interstitial cell (IIC) and necrosis (NE) while T_4 showed abnormal gonadal development (AGD) and necrosis. T_5 revealed eroded connective tissue (ECT), hydropic degeneration (HD) and necrosis.

Figure 3 shows the section of liver in *Oreochromis niloticus* fed 0 g of *Matynia annua* leaf meal diet, T_1 showed normal hepatocellular architecture. The vein was lined by a thin endothelium (EN) and erythrocytes (ER) was seen in the liver. Section of Liver in T_2 showed increase in interstitial cell. T_3 showed degeneration in the hepatocytes (DIH) and increase in interstitial cell while T_4 showed portal vein (POV) surrounded by exocrine pancreas and degeneration in hepatocytes. T_5 also showed severe degeneration in the hepatocytes (SDIH) architecture.

The histology of the gills in *Oreochromis niloticus* fed different levels of M*atynia annua* leaf meal is shown in Fig. 3. T_1 showed normal architecture of gill lamellae (GL) and gill ray (GR) while T_2 showed dilation of gill ray (DGR). T_3 showed necrosis (NE) and erosion of the efferent filament artery (EFA) and T_4 showed disintegration (DIS) on the left side of the section and necrosis while T_5 showed necrosis and erosion of the efferent filament artery.

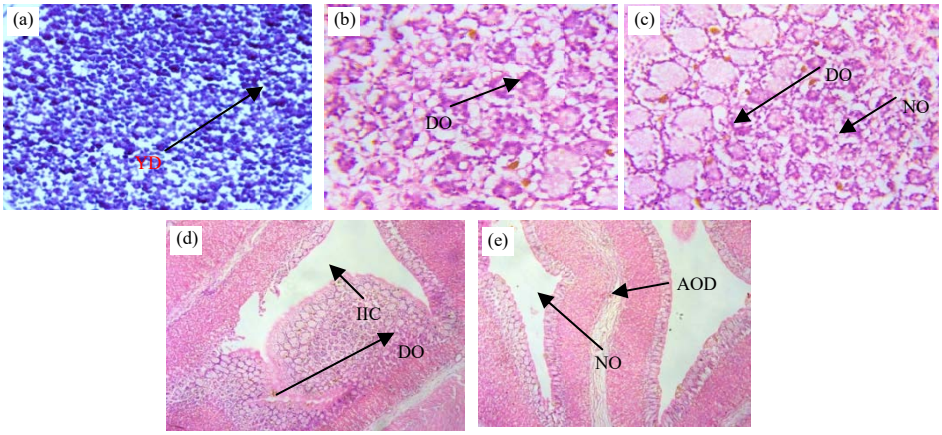

Fig. 1(a-e): Section of testes (Mag. ×100) (a) T_1, (b) T_2, (c) T_3, (d) T_4 and (e) T_5

Table 2: Water quality analysis at day 1 and day 15

	Day 1			Day 15		
Treatments	DO_2	pH	Temperature	DO_2	pH	Temperature
1	5.11±0.02^a	6.52±0.01^b	23.50±0.3^a	4.07±0.01^b	5.77±0.18^a	23.50±0.3^a
2	4.72±0.36^a	6.31±0.02^a	21.35±0.35^b	4.00±0.06^b	6.43±0.02^c	22.17±0.45^b
3	4.48±0.02^a	6.10±0.02^a	21.18±0.46^b	3.32±0.02^{ab}	6.11±0.03^a	21.83±0.60^b
4	4.29±0.00^a	6.15±0.25^b	23.67±0.33^a	3.21±0.04^a	5.70±0.15^a	24.00±0.29^a
5	4.93±0.03^a	5.40±0.01^a	23.00±0.29^a	3.00±0.01^a	5.37±0.01^a	23.00±0.17^b

Table 3: Growth and nutrient utilization of *Oreochromis niloticus* fed with *Martynia annua* leaf meal at week 10

Parameters	T_1 (0 g)	T_2 (20 g)	T_3 (40 g)	T_4 (60 g)	T_5 (80 g)
Initial weight (g)	10.87±0.27^a	11.27±0.43^a	10.90±0.12^a	10.73±0.15^a	12.14±0.87^a
Final weight (g)	19.42±0.32^d	21.02±0.71^d	19.99±0.80^d	18.91±0.29^c	19.30±0.06^d
Weight gain (g)	8.55±0.22^b	9.75±0.33^c	9.09±0.56^c	8.18±0.25^b	7.16±0.69^a
Feed intake	31.32±23^a	32.46±43^a	31.39±50^a	30.90±22^a	34.96±32^a
Feed conversion ratio	3.66±44^a	3.33±17^a	3.45±56^a	3.77±27^a	4.88±16^b

Table 3 shows that weight gain was the highest in treatment 2 and not significantly different from treatment 3. Treatment 5 had the lowest weight gain and differed from all the treatments used. Feed intake was not significantly different in all the treatment while feed conversion ratio differed in all the treatments except in treatment 5.

Table 4 shows that the ovary weights reduced as the concentration of *Martynia annua* leaf meal increased and differed in all the treatments. The testes weights did not differ in all the treatments while fecundity reduced as the concentration increased in the diets of the fish. The milt counts reduced as the level of *Martynia annua* leaf meal increased. The sizes of the egg were significantly different in treatments 1, 2 and 3 but the same in treatments 3 and 4; 2 and 5.

DISCUSSION

Histological investigation of ovary of *Oreochromis niloticus* fed diet supplemented with *Martynia annua* leaf meal (MALM) showed that lumen was filled with yoke droplets in treatment 1 (Fig. 1), this result agrees with those of Akin-Obasola *et al.*[9] who fed *Tragia Benthani* leaf meal to *Oreochromis niloticus* and the histological analyses of the gonads was performed. Treatment 2, 3 and 4 revealed that ovary was filled with developing oocytes and some necrotic oocytes respectively, this result agrees with a previous study conducted by Jegede[14] where reproduction of *Oreochromis niloticus* was controlled using Hibiscus (*Rosa-sinensis*) leaf meal at concentration of 1.0 g. Treatment 5 revealed no oocyte in the lumen and alteration in ovary development. The results also agree with previous study by Akin-Obasola and Jegede[15] who fed *Gossypium herbaccum* root bark meal to *Oreochromis niloticus* to control reproduction rate.

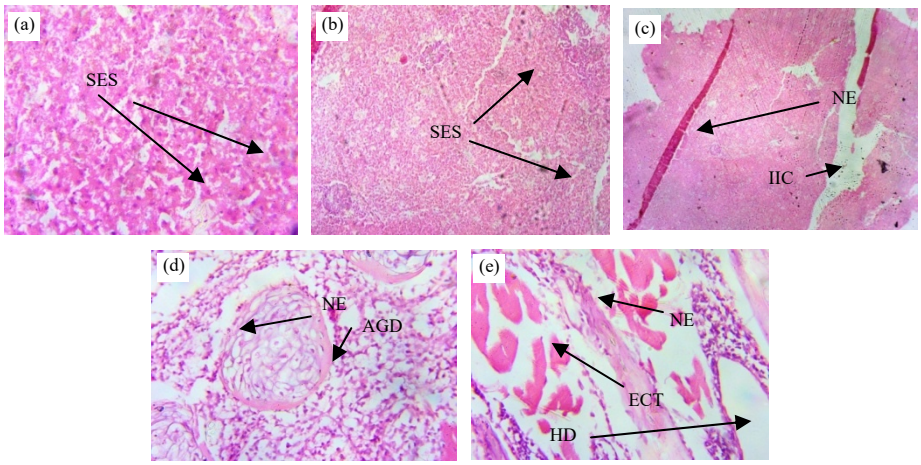

Fig. 2(a-e): Section of testis (Mag. ×100), (a) T_1, (b) T_2, (c) T_3, (d) T_4 and (e) T_5

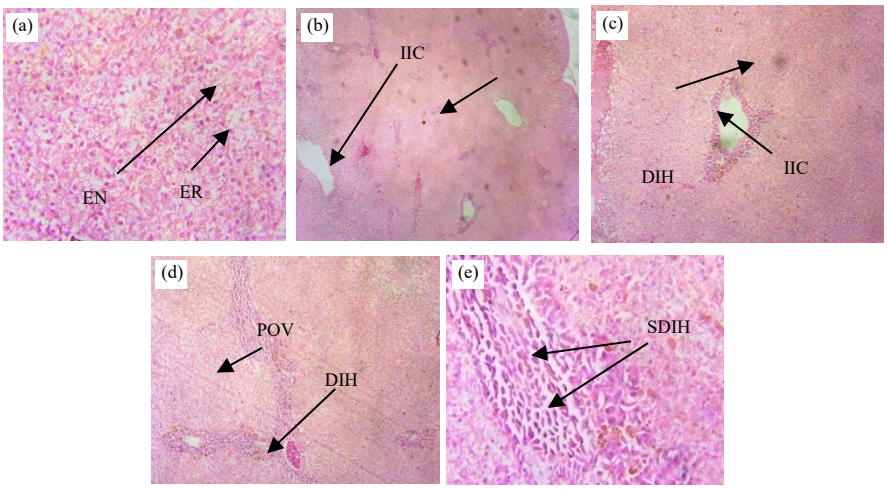

Fig. 3(a-e): Section of liver, (a) T_1, (b) T_2, (c) T_3, (d) T_4 and (e) T_5

The section of testes in treatment 1 revealed secondary and primary spermatogonia in the lumen of the seminiferous tubule, similar results were reported by Akin-Obasola and Oyekanmi[16] who clearly observed primary and secondary spermatocytes in the lumen of the seminiferous tubule. The section of testes in treatment 2 showed secondary spermatogonia in the lumen of the seminiferous tubule (Fig. 2). Similar results were obtained by Kushwaha[17] when Aloe vera (liliaceae) was fed to Nile tilapia to control fertility. The section of testes in treatment 3 showed increase in interstitial cell and necrosis, the result of this research is in accordance with the results of Akin-Obasola and Jegede[15] who fed diet supplemented with *Gossypium hebaceum* (cotton) root bark as fertility inhibitor to male and female *Oreochromis niloticus* (Nile tilapia) to control reproduction Section of testes in treatment 4, showed abnormal gonadal development and necrosis, this result agrees with those of Mali *et al.*[18] who fed diet supplemented with 50% ethanol extract of *Martynia annua* and observed significant decreases in the weight of testes, epididymides, seminal-vesicle and ventral prostate, moreover, reduction in the testicular sperm count, epididymal sperm count and motility number of male fertile. The ratio between delivered and inseminated female was significantly reduced due to serum concentration of luteinizing hormone and testosterone activity.

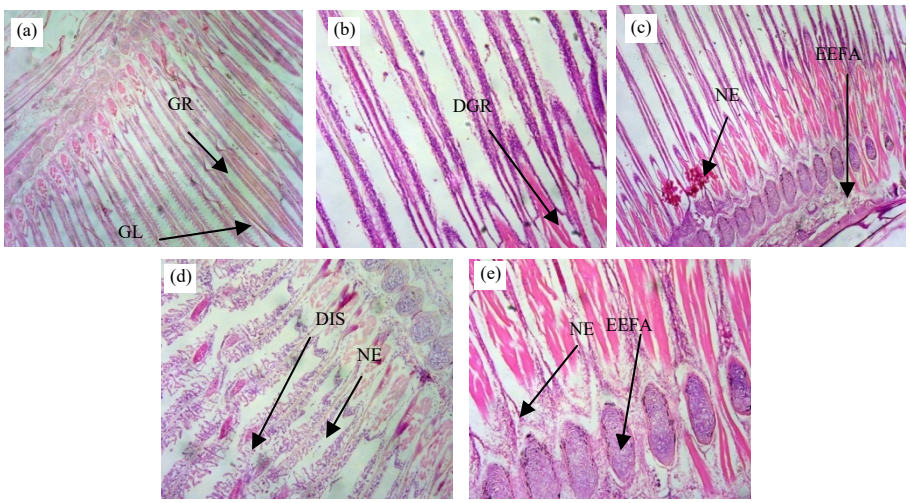

Fig. 4: Section of gills , (a) T_1, (b) T_2, (c) T_3, (d) T_4 and (e) T_5

Table 4: Reproductive performance of *Oreochromis niloticus* fed *Matynia annua* leaf meal

Parameters	T_1 (0 g)	T_2 (20 g)	T_3 (40 g)	T_4 (60 g)	T_5 (80 g)
Ovary weight (g)	1.63±0.00^e	1.20±0.00^d	1.18±0.00^c	1.09±0.00^b	1.00±0.00^a
Testes weight (g)	0.55±0.00^a	0.53±0.00^a	0.52±0.00^a	0.52±0.00^a	0.52±0.00^a
Fecundity	176.00±0.00^e	100.00±0.00^d	97.00±0.00^c	94.00±0.00^b	90.00±0.00^a
Milt count (S min^{-1})	2.91±0.00^c	2.01±0.00^c	1.72±0.00^b	1.45±0.00^b	0.96±0.00^a
Size of egg (g)	0.91±0.00^b	0.93±0.00^c	0.86±0.00^a	0.85±0.00^a	0.81±0.00^c

T5 revealed eroded connective tissue, hydropic degeneration and necrosis (Fig. 2). Similar observation was expressed by Akin-Obasola and Jegede[19] who controlled the reproduction of male *Oreochromis niloticus* (Nile tilapia) using *Gossypium herbaceum* (Cotton) root bark meals as fertility inhibitor, necrosis, very few matured germinal cells in fish fed 60g leaf meal was reported.

As shown in Fig. 3, the section of liver in *Oreochromis niloticus* fed 0g of *Matynia annua* leaf meal showed normal hepatocellular architecture. This result is consistent with a previous study conducted by Akin-Obasola and Jegede[19] who recorded normal hepatocellular architecture of *Oreochromis niloticus* fed 0 g of *Gossypium herbaceum* root bark (GHRB kg^{-1}) diet. The vein was lined by a thin endothelium and erythrocytes were seen in the liver. Section of Liver in treatment 2 showed increase in interstitial cell. Treatment 3 showed degeneration in the hepatocytes and increase in interstitial cell while treatment 4 showed portal vein surrounded by exocrine pancreas and degeneration in hepatocytes (Fig. 3). Similar results were reported by Akin-Obasola and Oyekanmi[16] who recorded histological changes in *Oreochromis niloticus* fed *Momordica charantia* leaf meal at the higher concentrations.

Histology of the gills in *Oreochromis niloticus* fed different levels of M*artynia annua* leaf meal revealed normal architecture of gill lamellae and gill ray, dilation of gill ray, erosion of the efferent filament artery, disintegration and necrosis in treatment 1, 2, 3, 4 and 5 respectively (Fig. 4). These results are corroborated by Akin-Obasola and Oyekanmi[16].

The growth performance of *Oreochromis niloticus* fed *Martynia annua* leaf meal changed with time. The best growth performance was noticed in the control treatment. Feed conversion ratio was higher than the recommended level (2.0-2.5)[20].

Table 2 shows the physico-chemical parameter of the water used for the experiment. The pH level and dissolved oxygen were significantly different at day 1 and 15. This May be due to the presence of ammonia from fish waste, fish urine and faeces. There were no significant differences in temperature at different weeks. The water temperature during the experiment was maintained according to the FAO[21] recommendation of 11°C and 42°C as the lower and upper sub-lethal value for tilapia production. However, it disagrees with those of Wairimu *et al.*[22] who recommended the values of pH (7.3), Temperature (27°C) and dissolved oxygen (7.6-7.9 mg L^{-1}) for maximum growth of tilapia.

CONCLUSION

Plants are used as an anti-fertility agent for animal in many parts of the world today. Medicinal plants such as *Martynia annua* leaf exist abundantly in many thick forest or vegetative environment of sub-sahara Africa, making their accessibility easier, faster and cost effective. However, medicinal plants often possess several traits which include contraceptive, inhibitory, involuntary, abortifacient and other traits that are capable of altering the histological arrangement and reproductive performances of animal's sexuality. Hence, the result of the experiment has shown that *Martynia annua* leaf meal has anti-fertility effects that can help control tilapia reproduction and its use is advocated in developing world where hormones are expensive and not readily available.

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