THE HISTOLOGICAL EVALUATION OF THE EFECTS OF *Moringa oleifera* LEAF MEAL INCLUSIONS ON THE LIVER AND OVARIES OF FEMALE *Oreochromis niloticus*

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ABSTRACT
The present study was carried out to examine the histological effects of Moringa oleifera leaves as a reproduction inhibitor in controlling the unusual reproduction tendency of female O.niloticus. Thirty- five percent crude protein formulated tilapia feeds of various level of M.oleifera leaf inclusions were fed to 120 females of O.niloticus of average weight of 28.56 ±0.46g for 90 days at 4% feeding level of total biomass. The fishes were randomly assigned in triplicates into 12 transparent plastic tanks at a stocking density of 10 fishes per tank. The water quality parameters were monitored weekly throughout the study period. The histological evaluation of the liver and ovaries of O.niloticus fed with 0% Moringa leaf meal (MLM) showed normal liver and ovarian tissue characteristics. In general, it was observed that MLM had alterative effects on the ovaries characteristics. The degree of alterations was directly proportional to the level of MLM inclusion. The histological evaluations showed mild to severe stages of ovarian lamellae deformities, degenerations of oocytes cytoplasm, depletion of yolk particles, oocyte atresia, buckling of oocytes and necrosis. Liquefaction of the nucleus was pronouncedly observed in 10 % and 15% MLM. However, it was observed that MLM only caused vacuolation of the hepatopancrease cells. The alteration of the ovaries' integrity can be attributed to the abundant phytochemicals inherent in M.oleifera leaves. Therefore, the results obtained from the photomicrographs points clearly that M.oleifera leaves meal can be used to curtail the prolific reproduction of female O.niloticus without affecting its health.
Keywords: Moringa leaves; Liver; Ovaries; Alterations; Phytochemicals; histology.

INTRODUCTION

The phenomenal expansion of tilapia from its native land Africa, to other continents is quite remarkable (Costa – pierce and Rakocy, 2000 and Altun etal. 2006).Tilapia has been acknowledge as a unique aquaculture species by aquaculturist and is being cultivated in commercial level in both developing and developed countries (El-Sayed, 2006 and Fitzsimmons, 2010).The general perception on Nile tilapia and other fast growing tilapias have improved and they have come to be accepted as aquatic chicken (Fitzsimmons, 2000 and Hussain etal. 2000). More than 475 million pounds of tilapia is consumed in the United States which qualifies tilapia as a perfect industry fish (FAO, 2012). Global tilapia production is expected to reach 7.3 million tons by 2030.(FAO, 2009, FAO, 2014 and Shelton, 2002, Shelton & Popma, 2006) .Fish continue to be one of the most traded commodities worldwide and it is especially important for developing countries, sometimes worth half the total value of their traded commodities.(FAO, 2015).

Fish constitutes more than 41% of the total animal protein intake by the average Nigerian, hence there is great demand for fish in the country (NANTS, 2014).Nigeria spent over N100 billion on the importation of frozen fish in 2010. The estimated annual fish demand in Nigeria was about 2.66 million as against the annual domestic production of about 0.78 million, giving a demand-supply gap of about 1.8 million metric tons (NANTS, 2014) This massive importation of frozen fish in the country has ranked Nigeria the largest importer of frozen fish in Africa (NANTS, 2014 and FMARD ,2010).If this amount of money is judiciously invested in the aquaculture industries yearly, the feedback would be tremendously overwhelming and consequently boost the countries chances of being a major world aquaculture and aquatic product exporter and also secure food security for its citizenry.

The country has a vast amount of natural resources, a great diversity of high-value indigenous fish species, thousands of hectares of irrigated land, man-made ponds and numerous wetlands and ditches. If these resources can be properly exploited and made productive through aquaculture; the benefit would be beyond the country's expectations (Abdel, 2011).

Recently, several research interests have shifted to medicinal plants and their potent and efficacious phytochemicals compositions. Some of these medical plants have proven to be suitable alternative to chemicals used in controlling reproduction in tilapia (Ganzera etal. 2001, Green and Kelly, 2009, Citarasu, 2010, Ghosal and Chakraborty, 2014 and Gabriel etal. 2015).Some medicinal plants possess antifertility and abortificient properties when administered orally to farm animals (Obaroh and Chionye–Nzeth, 2011). Plants such as *Moringa oleifera*, *Carica papaya*, *Aloe vera*, *Azadirachta indica*, *Hibiscus rosasinensis*, *Tribulus terrestris* extracts have been reported to have a direct impact on gonad morphology (Gabriel etal. 2015).

The purpose of this study is to examine the histological effects of the various levels of *Moringa oleifera leaf* meal inclusions on the ovaries and liver of female Nile tilapia as an endocrine disruptor and reproduction inhibitor in controlling the undesirable and unusual reproduction tendencies of female Nile tilapia (*Oreochromis niloticus*).The study therefore, hypothesis that:

1. Feeding a fish with a diet that contain *M.oleifera* leaf will alter its gonadal structure and functions.
2. Increasing the level of *M.oleifera* leaf in a fish diet will have proportional adverse effects on its gonadal activities.
3. Feeding a fish with a diet of *M.oleifera* leaf will have little or no pathological effect on its liver integrity.

MATERIALS AND METHODS

The experimental facilities consist of 20 transparent plastics tanks of 80 litres carrying capacity equipped with air pumps, air stones and a water reticulated system. The tanks were washed every morning before first feeding and the faeces removed.

Basic water quality parameters such as the pH, temperature, and DO were monitored weekly. The average values of temperature, pH and dissolve oxygen concentration throughout the study period were $T = 27 \pm 0.73\ ^{o}C$, $pH = 7.02 \pm 0.26$, $DO = 6.8 \pm 36\ mgl^{-1}$.Before,the experimental diets(MLM) were administered, the fishes were starved for two days and fed at feeding level 4% of the body biomass for 90 days.

A total of 120 fishes were randomly assigned in triplicate into 12 plastic tanks at a stocking density of 10 fishes per tank. Before the experiment, two female fishes were selected at random from each treatment group, sacrificed and the conditions of their ovaries and liver observed histologically in the laboratory.

Moringa oleifera leaves were harvested fresh from a private Moringa farm, shade dried and milled into fine particle and used for feed formulation. Other components of the diets were purchased from the local market in Rumuokoro, Port Harcourt, Nigeria. Prior to the feed formulation, the proximate analysis and the qualitative phytochemical analysis of the dried *Moringa oleifera* leaves was carried out and presented in table 1 and 2.

Four isonitrogenous and isoenergetic diets were formulated to provide 35% crude protein. Moringa leaves were included in the diets at levels: 0%, 5%, 10% and 15% of total dietary protein (Table 3). The four diets were pelletized and air-dried. The dried pelletized diets were grinded, sieved into small pellet sizes, packed into labeled polythene bags and stored. The proximate composition (g/100) of the experimental diets fed to the *O.niloticus* is presented in table 4.

Matured 150 female Nile tilapia (*Oreochromis niloticus*) were obtained from the tilapia farm of Root Crop Research Institute, Umuahia and transported to the wet laboratory of Michael Okpara University of Agriculture Umudike, Umuahia and allowed to acclimatize for two weeks in plastic tanks during which the fishes were feed commercial diet

RESULTS

The histological analysis of the ovaries of *O.niloticus* fed with 0% MLM showed normal ovarian tissue architecture with no appreciable or histopathological degeneration and lesion. The fish ovarian lamellae contained oocytes at various stages of oogenesis, capable of producing viable gametes. The photomicrograph is presented in figure 1.

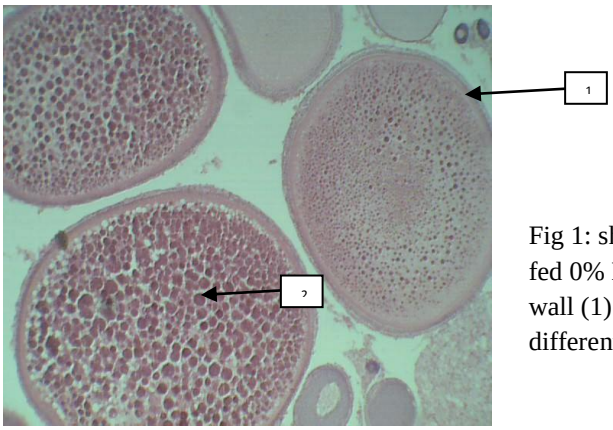

Fig 1: showing photomicrograph of ovaries in *O.niloticus* fed 0% MLM inclusion. The ovaries are surrounded by thin wall (1) containing normal structured oocytes (2) at different stages of maturation. X10.

The histopathological evaluation of female fishes fed with 5% MLM, showed reduced number of oocytes, large areas of severe deformation of ovarian lamellae, severe oocyte cytoplasm degeneration, depletion of yolk particles, ruptured follicles of the oocytes ,oocyte atresia, liquefaction of nucleus and necrosis as shown in figure 2.

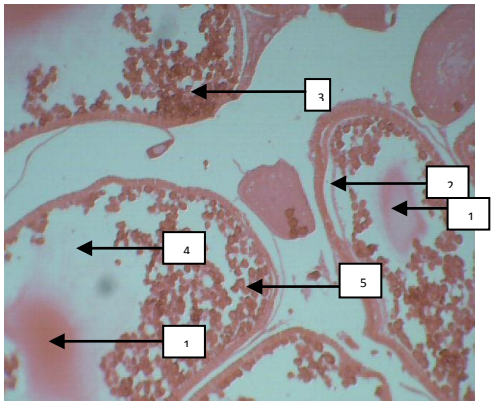

Fig 2: showing photomicrograph of ovaries in O.niloticus fed 5 % MLM inclusion. The ovaries show liquefied nuclei (1), severely damaged ovarian lamellae (2), reduced number of oocytes (3) near empty cytoplasm (4), deformed oocyte follicles (5), etc. X10.

The ovaries of the fishes fed 10% MLM showed scanty oocytes, large areas of severe deformation of ovarian lamellae, severe oocyte cytoplasm degeneration, depletion of yolk particles, and ruptured follicles of the oocytes. Furthermore, the nuclear membrane were observed to be ruptured and its content severely damaged and showing extended liquefaction of nuclei and increased necrosis as shown in figure 3.

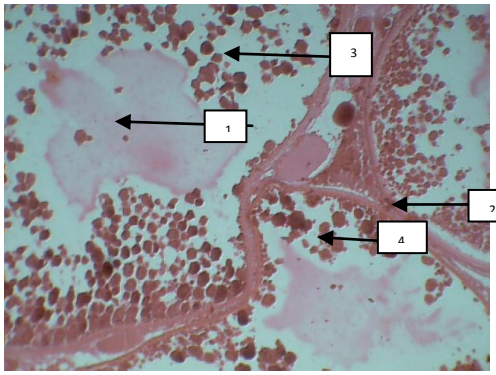

Fig 3: showing photomicrograph of ovary in O.niloticus fed 10% MLM inclusion. The ovaries show liquefied nuclei (1), severely damaged ovarian lamellae (2), reduced number of oocytes (3), deformed oocyte follicles (4), oocyte atresia, necrosis etc. X10.

The ovaries fed 15% MLM were observed to have very severe ovarian characteristics such as ruptured ovarian lamellae, absence of nuclear membrane and extended liquefaction of nucleus, very scanty and degenerated cytoplasm with empty oocytes due to extensive rupturing of ovarian follicles. The observed ovaries showed extensive necrosis as shown in figure 4.

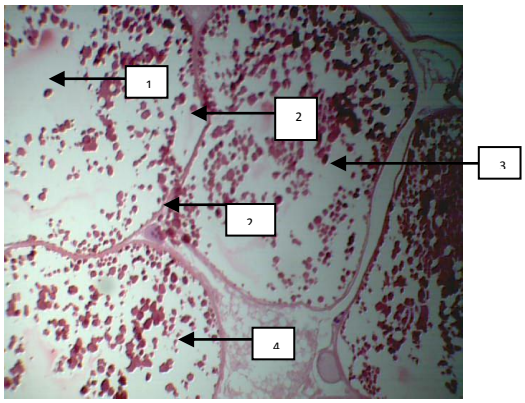

Fig 4: showing photomicrograph of ovary in O.niloticus fed 15% MLM inclusion. The ovaries show liquefied nuclei (1), severely damaged ovarian lamellae (2), reduced number of oocytes (3), deformed oocyte follicles (4), oocyte atresia, necrosis etc. X10.

The photomicrograph in Fig 5 and 6 below show normal liver hepatocytes and hepatopancreas of female O.niloticus fed 0% and 5% MLM. There is no observable damage or lesion in the liver structures.

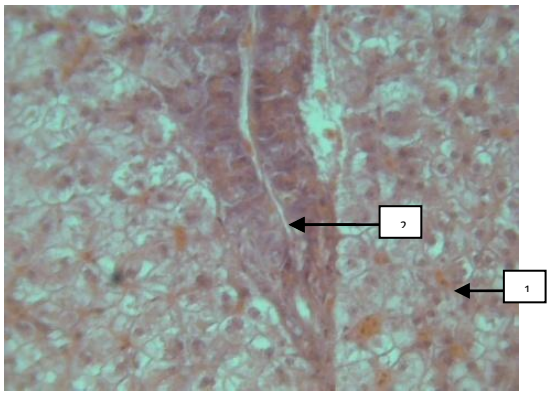

Fig5. Photomicrograph showing normal liver hepatocytes(1) and hepatopancreas(2) of female O.niloticus fed 0% MLM. X40

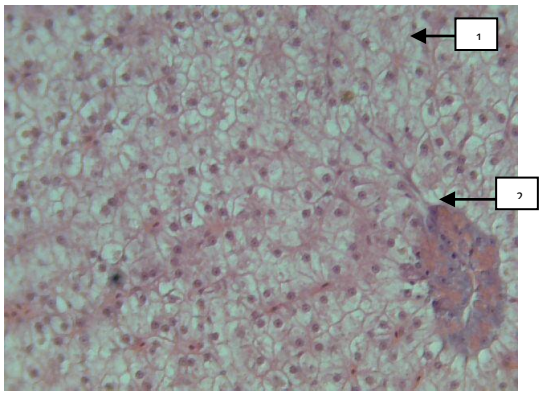

Fig 6. Photomicrograph showing normal liver hepatocytes (1) and hepatopancreas (2) of female O.niloticus fed 5% MLM inclusion. X40.

The liver of the fish fed with 10% MLM shows normal liver hepatocytes but vacuolated hepatopancreas. The thick wall (membrane) of the hepatopancreas is still intact as shown in Fig7 below.

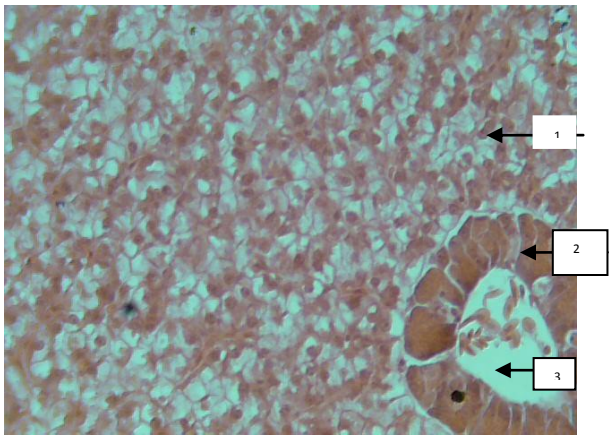

Fig 7.Photomicrograph showing normal liver hepatocytes (1) with vacuolated hepatopancreas content (3) but normal hepatopancreas membrane of female O.niloticus fed 10% MLM. X40.

The liver of the fish fed with 15% MLM show normal liver hepatocytes with vacuolated hepatopancreas. The thick wall (membrane) of the hepatopancreas is seen to be ruptured or eroded,as shown in Fig7 below.

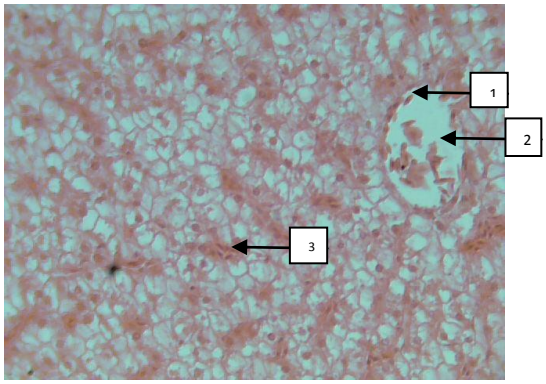

Fig8. Photomicrograph showing normal liver hepatocytes (3) with increased vacuolation (2) and ruptured hepatopancreas tissue membrane (1) of female *O.niloticus* fed 15% MLM. X40.

DISCUSSION

The histological analysis of the liver and ovaries of *O.niloticus* fed with 0% MLM showed normal liver hepatocytes and ovarian tissue architecture with no histopathological degeneration or observable lesion. Their ovarian lamellae contained oocytes at various stages of oogenesis and capable of producing viable gametes. The photomicrograph is presented in figure 1.

The histopathological evaluation of female fishes fed with 5% MLM inclusion, showed large areas of severe deformation of ovarian lamellae, severe oocyte cytoplasm degeneration, depletion of yolk particles ruptured follicles of the oocytes ,oocyte atresia, liquefaction of nucleus and necrosis as shown in figure 2.

The ovaries of the fishes fed 10% MLM showed large areas of severe deformation of ovarian lamellae, severe oocyte cytoplasm degeneration, depletion of yolk particles, and ruptured follicles of the oocytes. Furthermore, the nuclear membrane were observed to be ruptured and its content severely damaged and showing extended liquefaction of nucleus and increased necrosis as shown in figure 3. The ovaries fed 15% MLM were observed to have very severe ovarian characteristics such as ruptured ovarian lamellae, absence of nuclear membrane and extended liquefaction of nucleus, very scanty and degenerated cytoplasm with empty oocytes due to extensive rupturing of ovarian follicles. The observed ovaries showed extensive necrosis as shown in figure 4.

The histological evaluation of the livers of female *O.niloticus* fed 0% and 5% MLM showed normal hepatocytes and prominent hepatopancreas. The photomicrographs are presented in figure 5 and 6.The livers fed 10% MLM were observed to have normal hepatocytes with vacuolated hepatopancreas as shown in fig 7. The female *O.niloticus* fed 15% MLM showed normal hepatocytes with increased vacuolation and ruptured hepatopancreas tissue membrane as represented in fig 8.

The results obtained from the histological evaluation of ovaries of *O.niloticus* in this study were similar to those observed by Ekanem and Okoronkwo, 2003, Jegede and Fagbenro, 2008, Ayotunde etal., 2011, Ampfoyeboah, 2013, and Akin-Obasola and Jegede 2014. Das (1980) reported that Oleanolic glycoside which can be extracted from M.oleifera seed and leaves is responsible for inducing sterility in rat. In a review by Kumar et al. (2012), they reported that oleanolic acid-3-glucoside and β-sitosterol are some of the phytochemicals that have shown 100% antifertility activities, which are present in the Moringa plants. These phytochemicals in Moringa make them potential endocrine disrupters.

Ampfoyeboah, 2013, Ayotunde etal. 2011, and Jegede and Fagbenro 2008, reported severe alterations of the liver cells of fishes administered plant phytochemicals extracts. However, the histological observations showed normal hepatocytes for all the fishes as described by Morrison etal. (2006) except for vacuolation of the hepatopancreas observed in female *O.niloticus* fed 10% and 15% MLM. The severity of alteration of the liver may be due to absence of hepatoprotection factor in most plant seeds but inherent in M.*oleifera* leaves. Fakurazi etal.(2008) have reported the hepatoprotective effect of *M. oleifera* leaves as a result of its potency to bring about the restoration of the liver enzymes in rat induce with acetaminophen. They were of the opinion that the plant extracts had some roles in preserving the architectural characteristics of hepatocellular membrane, consequently inhibiting enzymes leakage into the blood circulation system. They suggested that the hepatoprotective effects of M.*oleifera* was due to its potential to catalyze phase II detoxification mechanism through the activation of gonadotropic stimulating hormone fusion with the poisonous metabolic waste products produced from CYP450 mechanism (Fakurazi etal., 2008). This hepatoprotective ability of *M. oleifera* leaves would have accounted for the near normal liver integrity of all the fishes fed with the different inclusion levels of MLM observed in this study. Therefore, *M.oleifera* leaf meal which is socio-economically and ecologically friendly to use, can be used to curtail the undesirous reproduction prowess of female O.*niloticus* without affecting their health.

CONCLUSION

The photomicrographs of the ovaries in this study show clearly that *M.oleifera* leaf alters the structure and functions of the ovaries of female *O.niloticus.* Increasing the level of inclusion *M.oleifera* leaf in the diet increases its adverse effects on the ovaries integrity, thereby conferring on the ovaries the status of sterility. However, the hepatocytes were not adversely affected by the inclusion *M.oleifera* leaf in the diet. Therefore, the results obtained from this study point clearly that *M.oleifera* leaves can be used to curtail and manipulate the undesirous prolific reproduction of female *O.niloticus* without adversely affecting its health.

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