# HISTOLOGY OF GONADS IN *TILAPIA ZILLII* (GERVAIS) FED NEEM (*AZADIRACHTA INDICA*) LEAF MEAL DIETS

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***Abstract***

Neem (*Azadirachta indica*) leaf meal (NLM) was added to a basal diet (350g crude protein and 18.5MJ gross energy/kg diet) at 0, 0.5, 1.0, 1.5 or 2.0 g/kg diets and fed to mixed-sex *Tilapia zillii* for 60 days to evaluate the effects on histology of the testes and ovaries. Male *T. zillii* fed 0g NLM/kg diet showed normal testicular tissues; and no pathological lesions occurred. Fish fed 0.5g NLM/kg diet showed alteration in testicular architecture and cystic seminiferous tubules. Fish fed 1.0g NLM/kg diet showed severe testicular atrophy. Fish fed 1.5g NLM/kg diet exhibited cystic seminiferous tubule and atrophy of tissue. Fish fed 2.0g NLM/kg diet showed severe tissue atrophy, sperm cells disintegration and necrosis. Female *T. zillii* fed with control diet (0g NLM/kg diet) showed normal ovary histology and no pathological lesions were observed. Typical bilateral lobes of the ovaries were evident and with normal olive green colour of ovaries. Fish fed 2.0g NLM/kg diet revealed ovarian colour change. There was evidence of hydropic degeneration, ruptured follicle, granulomatous inflammation in the insterstitium and necrosis. Histological observations of testes and ovaries in *T. zillii* fed high dietary NLM levels revealed that neem leaves may be effective as sterility-inducing agents as they were destructive to testes and ovaries tissues.

## INTRODUCTION

Medicinal plants have successfully been used to induce sterility in laboratory animals (Gary and Garg, 1971; Bodharker *et al.*, 1974; Das, 1980). Neem tree (*Azadirachta indica* A. Juss) is a large evergreen tree with edible fruits and aromatic leaves. A mature tree can produce 350 kg of leaves a year. Neem has been used worldwide in traditional medicine for various therapeutic purposes, anti-bacterial, anti-fungal, anti-viral and anti-fertility properties (Jegede and Fagbenro, 2007) and phytochemical analyses showed that the neem tree has more than 100 unique bio-active compounds, among which is sodium nimbinate which has potential applications as spermicide in animal care and for even regulating human fertility (NRC 1992).

Neem oil as a vaginal contraceptive inhibits the spread of micro-organisms including *Candida albican*, *C. tropicalis*, *Niesseria gonorrhoea*, herpes, simplex-2 and HIV-1 as well as resistant strains of *E. coli* and *Staphylococcus aureus*, in part by boosting immune-system activity in vagina (Shakli *et al.*,1990). Sinna and Riai (1985) reported that Rhesus monkey and human spermatozoa became totally immobile within 30 seconds of contact with undiluted Neem oil. *In vivo* studies in rats (20), rabbits (8), Rhesus monkeys (14) and human volunteers (10) proved that Neem oil applied initially before sexual intercourse prevents pregnancies in all species. Neem oil also has anti implantation/ abortifacient effect in rats and rabbits if applied initially on day 2-7 of expected pregnancy.

Tilapias are yet to reach their full aquaculture potential because of the problems of precocious maturity and uncontrolled reproduction, which often results in the overpopulation of production ponds with young (stunted) fish. Population control in farmed tilapias has been reviewed by Guerrero (1982), Mair and Little (1991) and Fagbenro (2002). Such control methods include monosex culture, sex reversal by androgenic hormones, cage culture, tank culture, the use of predators, high density stocking, sterilization, intermittent/selective harvesting, and the use of slow maturing tilapia species, among others. However, all these population control methods have their limitations; e.g. the use of reproductive inhibitors, such as irradiation, chemosterilants has disadvantages which are: expensive technology, hatchery facilities and skilled labour are required and hormones are expensive and difficult to obtain. There is need therefore to examine less expensive and appropriate technology to control unwanted/undesirable tilapia recruitment in ponds using natural reproductive inhibitory agents occurring in some plants.

The objective of this study was to investigate the effects of varying dietary levels of dry neem leaf meal (NLM) on the histology of testes and ovaries in *Tilapia zillii* fed for 60 days.

## MATERIALS AND METHODS

Leaves were collected from neem trees in southwest Nigeria, where they occur naturally/planted as decorative plant, wind break or as a shade tree. They were shade-dried and milled into fine particle size (< 250 µm); and kept in a dry, clean, air-tight transparent plastic container. Feedstuffs were purchased from a local feedstuff market and were separately milled to small particle size (< 250 µm). A control diet (D 1, 350g crude protein and 18.5MJ gross energy/kg diet) was prepared as formulated in Table 1. Four test diets (D2, D3, D4, D5) were formulated by adding 0.5, 1.0, 1.5 or 2.0g of NLM to 1 kg of control diet, respectively. Nutrient imbalance caused by the addition of NLM was corrected by adding 2.0g of cellulose (non-nutritive ingredient) to the basal diet (D1) and 1.5, 1.0, 0.5, and 0g of cellulose to test diets D2, D3, D4 and D5, respectively. The feedstuffs were thoroughly mixed in a Hobart A-200T mixing/pelleting machine. Hot water was added at intervals to gelatinize starch. All five diets were pelletized using a die of 8 mm diameter. The diets were air-dried at ambient temperature for 72 hours; broken, sieved into small pellet sizes, packed in air-tight containers, labelled and stored.

Table 1. Ingredient composition of basal diet

| | g/kg Diet |
|---|---|
| Menhaden fish Meal | 280 |
| Soybean meal | 370 |
| Corn meal | 250 |
| Cod liver Oil | 30 |
| Corn Oil | 20 |
| Vitamin-mineral mix | 30 |
| Corn starch | 20 |

*T. zillii* fingerlings were acclimated for 14 days in concrete tanks during which they were fed with a commercial diet. After acclimation, 10 male and 10 female *T. zillii* (mean weight, 40g) were stocked in each of 15 concrete tanks (2 x 2 x 1.25m) supplied with 400 litres of fresh water (water temperature, 27 $^{o}$C; pH, 7.3; alkalinity, 50 ppm; dissolved oxygen, 7.6-7.9 mg/L). The treatments were replicated thrice and fish were fed at 4% body weight/day in two instalments at 0900-0930 h and 1700-1730 h for 60 days; after which they were removed, sorted by sex and weighed. Fish samples were randomly taken from each treatment, dissected, and the testes and ovaries removed for sectioning and histological examination. The testes and ovaries were fixed for 24 hours in formalin-saline solution made of equal volumes of 10% formalin and 0.9% NaCl solution. Histological sections of 8µ thickness were prepared following standard procedures. Photomicrographs were taken with Leitz (Ortholux) microscope and camera and compared with those of Morrison *et al.* (2007).

## RESULTS AND DISCUSSION

### Histology of testes in *T. zillii* fed varying dietary levels of neem leaf meal

Sections of testes in *T. zillii* fed 0g NLM/kg diet (control diet) showed normal testicular tissue architecture and sperm cells distribution (Figure 1). Fish fed 0.5g NLM/kg diet showed alterations in the testicular architecture and cystic seminiferous tubules (Figure 2). In fish fed 1.0g NLM/kg diet, sections of the testes showed severe testicular atrophy (Figure 3), while fish fed 1.5g NLM/kg diet, showed cystic tubules and atrophy in the testicle (Figure 4). In fish fed 2.0g NLM/kg diet, sections of the testes showed severe testicular tissue atrophy, sperm cells disintegration and necrosis (Figure 5).

### Histology of ovaries in *T. zillii* fed varying dietary levels of neem leaf meal

Histological sections of the ovary in *T. zillii* fed with the control diet (containing no NLM) showed normal ovary histology. No pathological lesions were observed, atretic follicles were less visible (Figure 1), and normal olive green colour of ovaries was maintained. In fish fed 1.0 or 2.0g NLM/kg diet, there were changes in colour of ovaries, increased atretic follicles, ruptured follicles and necrosis (Figures 7 and 8).

In this study, the damage done to tissues of the testes and ovaries was minimal at lower dietary NLM level (1.0 g/kg diet), and at higher dietary NLM level (2.0 g/kg diet), it caused disintegration of many more cells, rendering the testes and ovaries devoid of spermatids and oocytes, respectively. This makes dry neem leaves recommendable for use in the control of breeding in tilapias. Histological observations of testes and ovaries in *T. zillii* fed diets containing NLM revealed that neem leaves may be effective as sterility-inducing agents as they were destructive to testes and ovary tissues; and is useful in the determination of the contraceptive efficacies of dietary NLM in combating problems of tilapia overpopulation in ponds. Other than infertility, literature did not indicate any adverse reactions from the consumption of neem leaves.

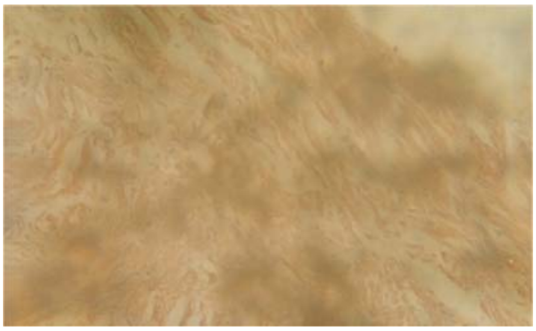

Figure 1. Section of testes in *T. zillii* fed 0g NLM/kg diet showing normal tissue architecture and normal sperm cell distribution. Mag. X 40.

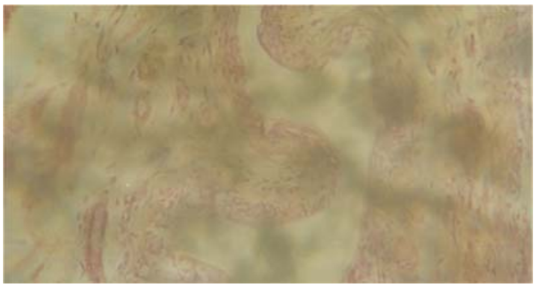

Figure 2. Section of testes in T. zilliii fed 0.5g NLM/kg diet showing alteration in the normal testicular architecture and cystic seminiferous tubules. Mag. X 40.

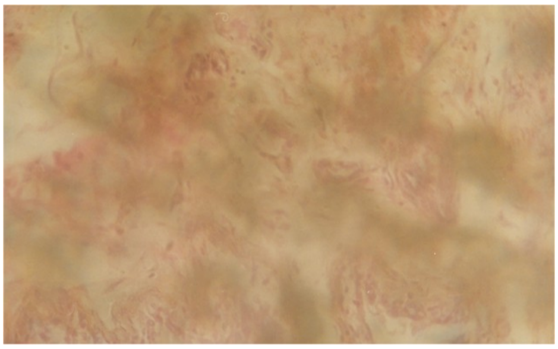

Figure 3. Section of testes in *T. zillii* fed 1.0g NLM/kg diet showing atrophy of tissue. Mag. X 40.

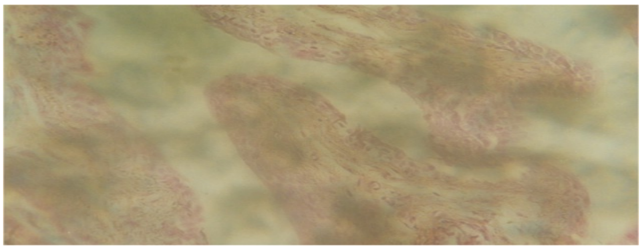

Figure 4. Section of testes in T. zillii fed 1.5g NLM/kg diet showing cystic tubule and atrophy in the testicle. Mag. X 40.

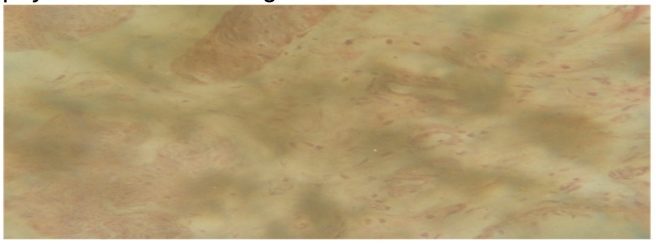

Figure 5. Section of testes in *T. zillii* fed 2.0g NLM/kg diet showing severe testicular tissue atrophy, sperm cells disintegration and necrosis. Mag. X 40.

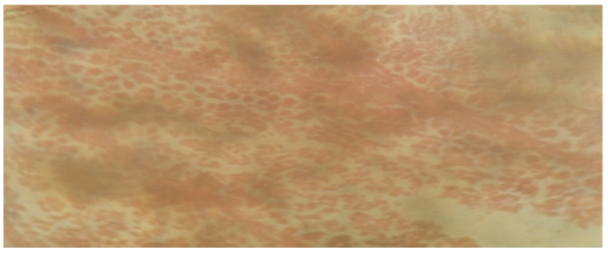

Figure 6. Section of ovary in *T. zillii* fed diet 1 (control) showing normal histology and less visible atretic follicles. Mag. X 40.

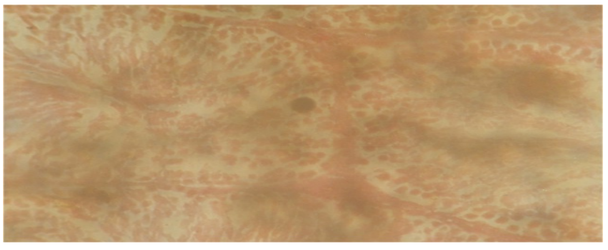

Figure 7. Section of ovary in *T. zillii* fed 1.0g NLM/kg diet showing increased atretic follicle and hydropic degeneration. Mag. X 40.

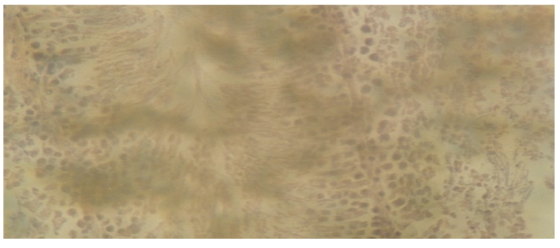

Figure 8. Section of ovary in *T. zillii* fed 2.0g NLM/kg diet showing increased atretic follicles, ruptured follicles and necrosis. Mag. X 40.

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