DIETARY NEEM (*AZADIRACHTA INDICA*) LEAF MEAL AS REPRODUCTION INHIBITOR IN REDBELLY TILAPIA, *TILAPIA ZILLII*

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Abstract

There is need to control unwanted/undesirable tilapia recruitment in ponds using natural reproductive inhibitory agents in plants because they are less expensive and constitutes appropriate technology in developing countries. 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 growth and feed utilization, reproduction traits, and histology of gonads. There were no significant variations ($p > 0.05$) in the growth parameters and food conversion ratio. Indices of reproduction traits decreased with increasing dietary NLM levels. Fish fed with the control diet had significantly higher and better indices of reproduction traits ($P<0.05$) than the fish fed with NLM diets. Fish fed 0g NLM/kg diet showed normal testicular and ovarian 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, hydropic degeneration, ruptured follicle, granulomatous inflammation in the insterstitium and necrosis in the ovaties. Reproduction traits and histological observations of gonads in *T. zillii* fed high dietary NLM levels revealed that neem leaves may be effective as reproduction inhibitory agents in *T. zillii*.

INTRODUCTION

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. Hence there is need to examine less expensive and appropriate technology to control tilapia recruitment in ponds using natural reproductive inhibitory agents occurring in some plants.

Over its natural range, *Tilapia zillii* (Gervais 1848) occurs from west Africa through the Chad basin to the Nile, Lake Albert and Lake Turkana into Israel and the Jordan Valley (Trewavas, 1982). *T. zillii* is widely used in aquaculture in west and central Africa, and was introduced into the Gulf of Mexico ecosystem, as well as to many other areas of the United States, primarily for aquatic weed control, to control noxious aquatic insects, and for culture as a food fish (Courtenay *et al.*, 1984). *T. zillii* is a bi-parental substrate spawner and becomes sexually matured in 3-5 months at small size (10 cm, 20-50 g) in ponds, each female lays about 7,000 eggs/spawning and 6 spawnings/year (Balarin and Hatton, 1979).

The objective of this study was to investigate the effects of varying dietary supplementation levels of dry neem leaf meal (NLM) on some reproduction traits (gonad development stages, gonadosomatic index (GSI), fecundity, egg size (length, diameter, volume), egg weight (wet and dry basis), histology of testes and ovaries) in *T. 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.

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[1]	30
Corn starch	20

[1]Fish pre-mix. Colborne Dawes Nutrition Ltd., United Kingdom.: vitamin A, 1600 IU, vitamin D, 2400 IU, vitamin E, 160 mg, vitamin K, 16 mg, thiamin, 36 mg, riboflavin, 48 mg, pyridoxine, 24 mg, niacin 288 mg, panthotenic acid, 96 mg, folic acid, 8 mg, biotin, 1.3 mg, cyanocobalamin, 48 mg, ascorbic acid, 720 mg, choline chloride, 320 mg, calcium 5.2 g, cobalt, 3.2 mg, iodine, 4.8 mg, copper, 8 mg, iron, 32 mg, manganese, 76 mg, zinc, 160 mg, Endox (antioxidant) 200 mg.

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.

T. zillii fingerlings, obtained from a single spawn, 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, 40.23g) were stocked in each of 15 concrete tanks (2m x 2m 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 diet 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. Sex determination was done through visual examination of the gonad. Fish mortality was monitored daily. Growth and feed utilization indices were then estimated.

Six male and six female *T. zillii* samples were randomly taken from each treatment, dissected, and the testes and ovaries removed and weighed for the gonadosomatic index (GSI) calculations (gonad weight/total body weight x 10^2). Gonad development stages in male and female *T. zillii* were classified according to Kronert *et al.*(1989) and Oldorf *et al.*(1989), respectively. Fecundity was estimated from gonads of six fishes from each treatment in the final maturation stage from a sample representing at least 50% of ovary weight then reported to the total weight of the ovary. Egg weight (dry and wet basis) was determined using 50-count egg samples: a sample of 50 eggs was weighed and then dried in an oven at 80 ^{o}C for 24 hours. Thirty (30) eggs were measured using a microscope eye-piece graticule for length (L) and width (H). Egg volume was calculated by the formula: $V = n/6LH^2$ (Rana, 1985). The testes and ovaries were for sectioned, 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).

Statistical comparisons of the results were made using the on-way Analysis of Variance (ANOVA) test. Duncan's New Multiple Range Test was used to evaluate the differences between means for treatments at the 0.05 significance level (Zar, 1996).

RESULTS AND DISCUSSION

Growth performance and feed conversion by *T. zillii* fed varying dietary NLM levels

Dietary supplementation of NLM but did not reflect in the nutrient composition of the diets as both crude protein and gross energy contents were similar for all diets, and satisfied the nutrient requirements for tilapias (Jauncey, 2000). Water quality during the feeding trial was within the acceptable range for tilapia culture (Ross, 2000). No mortality was recorded in all diet treatments. Acceptance of the diets was good and fish became accustomed to the diets within the first week. Weight gain, growth response, feed conversion ratio by fish fed with the experimental diets are presented in Table 2. The best overall growth response was obtained in fish fed with the control

diet, and weight gain, % weight gain and average daily growth (ADG) were poorer (P<0.05) in fish fed with the NLM diets. A similar trend was observed with the specific growth rate (SGR), as the values decreased with increasing dietary NLM levels while the feed conversion ratio (FCR) values showed an inverse relationship. As suggested by Cumaranatunga and Thabrew (1989), lower final body weights and poor growth may be linked to reduced GSI values (Tables 3 and 4).

Table 2. Growth performance and feed conversion by *T. zillii* fed neem leaf meal diets.

	Dietary NLM level				
	0 g/kg	50 g/kg	100 g/kg	150 g/kg	200 g/kg
Final Weight (g)	58.60a	58.45a	58.20ab	57.71bc	56.89c
Initial weight (g)	40.23	40.23	40.23	40.23	40.23
Weight gain (g)	18.37a	18.22a	17.97b	17.48b	16.66c
% weight gain[1]	45.66a	45.29a	44.67b	43.45bc	41.41c
ADG[2]	0.306a	0.304a	0.300ab	0.291b	0.278c
SGR[3]	30.62a	30.37ab	29.95b	29.13c	27.77d
FCR[4]	2.17a	2.23b	2.23b	2.24bc	2.27c

[1] % weight gain (%. fish^{-1}) = [(final wt. – initial wt.)/initial wt.)] x 100

[2] average daily growth (g) = [(final wt. – initial wt.)/no of days

[3] specific growth rate (%. day-^{1}) = [(ln final wt. – ln initial wt.)/no of days] x 100

[4] feed conversion ratio = feed intake (g)/body weight gain (g)

a, b, c – Mean values in a row followed by dissimilar letters are significantly different (P<0.05)

Reproduction traits and histology of testes in *T. zillii* fed varying dietary NLM levels

Table 3 reveals that the GSI values decreased (P<0.05) as the dietary NLM levels increased, which was similarly reported by Jegede *et al.*(2008a) and is attributable to the poor development of testes tissues. Sections of testes in *T. zillii* fed 0g NLM/kg diet (control diet) showed normal tissue architecture and sperm cells distribution (Table 3). Fish fed 0.5g NLM/kg diet showed alterations in the testis architecture and cystic seminiferous tubules. In fish fed 1.0g NLM/kg diet, there was atrophy, while fish fed 1.5g NLM/kg diet, showed cystic seminiferous tubules and atrophy in the testicle. In fish fed 2.0g NLM/kg diet, there was severe tissue atrophy, sperm cells disintegration and necrosis. In a related study, Jegede *et al.*(2008a) obtained similar histological effects with male *T. zillii* fed varying dietary levels of pawpaw (*Carica papaya*) seed meal, tested as a reproduction inhibitor.

Table 3. Reproduction traits and histological description of male *Tilapia zillii* fed NLM diets.

Treatments	GSI (%)	Histological description
0 g NLM/kg diet	1.05a	normal tissue architecture and normal sperm cell distribution
0.5 g NLM/kg diet	0.82b	alterations in testis architecture and cystic seminiferous tubules.
1.0 g NLM/kg diet	0.56c	atrophy of testicular tissue
1.5 g NLM/kg diet	0.44c	cystic seminiferous tubules and atrophy in the testicle.
2.0 g NLM/kg diet	0.26d	severe tissue atrophy, sperm cells disintegration and necrosis

a, b, c, d – Mean values in a column followed by dissimilar letters are significantly different ($P<0.05$).

Reproduction traits and histology of ovaries in *T. zillii* fed varying dietary NLM levels

Relative distribution of gonad development stages was very homogenous among replicates in each dietary NLM treatment. As no differences were found in replicate tanks of a same treatment, data from replicate tanks were pooled. However, inter-treatment comparisons revealed significant differences in fecundity among treatments. High percentages of stage 4 was observed in fish fed 1.0g or 2.0g NLM/kg diet, in which several oocytes that were going to be laid were atretic, suggesting that physiological conditions were not optimal for oocyte development and eventual spawning. Dry weights of eggs were similar ($P>0.05$). The reasons for this are not clear, but may reflect differences in the relative moisture content of eggs. Even though egg diameter was not significantly different among treatments (Table 4), GSI and other reproductive traits decreased with increasing dietary NLM levels.

As with the male *T. zillii*, the GSI values as well as other reproduction traits decreased ($P<0.05$) as the dietary NLM levels increased (Table 4), which was similarly reported by Jegede *et al.*(2008b) and is also attributable to the poor development of ovarias tissues (Cumaranatunga and Thabrew, 1989). 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 (Table 4), and normal olive green colour of ovaries was maintained. Typical bilateral lobes of the ovaries were evident and with normal olive green colour of ovaries. 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. Similar histological effects were reported by Jegede *et al.*(2008b) when female *T. zillii* were fed with varying dietary levels of pawpaw seed meal, investigated as a reproduction inhibitor.

In this study, the damage done to tissues of the testes and ovaries was minimal at lower dietary NLM levels (0.5 or 1.0 g/kg diet), and at higher dietary NLM levels (1.5 or 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.

Table 4. Reproduction traits and histological description of female *Tilapia zillii* fed NLM diets.

Treatments (NLM/kg diet)	GSI (%)	Fecundity	Egg traits					Histological description
			Diameter (mm)	Length (mm)	Volume (mm^3)	Wet weight (mg)	Dry weight (mg)	
0g	1.90a	579a	1.87a	2.45a	5.77a	5.3a	2.1	normal histology and less visible atretic follicles
1.0g	1.27b	468b	1.75b	2.23b	5.38b	4.9b	2.1	increased atretic follicle and hydropic degeneration
2.0g	0.91b	401b	1.68b	2.03c	5.01b	4.6b	2.0	increased atretic follicles, ruptured follicles and necrosis

a, b - Mean values in a column followed by dissimilar letters are significantly different ($P<0.05$)

APPENDICES

Table 5. Gonad development stages in male and female tilapia

Stage		Males (Oldorf *et al.* 1989)
1	Immature	Thread-like, colourless
2	Inactive	Translucent, wider than above
3	Inactive-active	Flesh coloured, still thin
4	Active	White/yellowish, thickened, no milt apparent when cut
5	Active-ripe	Cream coloured, thick and enlarged
6	Ripe	Distended fully over length of visceral cavity, milt evident when cut
7	Ripe-running	White/silvery, milt runs freely under pressure
Stage		Females (Kronert *et al.* 1989)
1	Immature/Inactive	No eggs visible
2	Inactive-active	<20 eggs visible, size <0.2mm
3	Active	>20 eggs visible, size <0.2mm
4	Active-ripe	Eggs yellow, size 0.2-1.1mm
5	Ripe/Ripe-running	Eggs yellow, size >1.1mm
6	Spent	Absorption of yolk material, eggs white

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