Control of Reproduction in *Oreochromis niloticus* (Linnaeus 1758) Using *Hibiscus Rosa-sinensis* (Linn.) Leaf Meal as Reproduction Inhibitor

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Abstract

Hibiscus rosa-sinensis leaf (HLM) was added to a basal diet (350g crude protein and 18.5MJ gross energy/kg diet) at 0, 1.0, 2.0, 3.0 or 4.0 g/kg diets and fed to mixed-sex *Oreochromis niloticus* for 60 days to evaluate the effects on growth, feed conversion ratio, reproduction traits, and histology of gonads. There were no variations ($p > 0.05$) in growth parameters and feed conversion ratio. Indices of reproduction traits decreased with increasing dietary HLM levels. Fish fed with the basal diet had higher and better indices of reproduction traits ($P < 0.05$) than the fish fed with HLM diets. Fish fed 0g HLM/kg diet showed normal testicular and ovarian tissues, and no lesions were observed. Fish fed 1.0g HLM/kg diet showed slight increase in interstitial cells in testes. Fish fed 2.0g HLM/kg diet showed swollen spermatids nuclei, increased interstitial cells and focal necrosis in testes; and hydropic degeneration, ruptured follicles, granulomatous inflammation in the insterstitium and necrosis in ovaries. Fish fed 3.0g HLM/kg diet exhibited atrophy of seminiferous tubules in testes. Fish fed 4.0g HLM/kg diet, there was disintegration of spermatids and necrosis in testes and severe atretic follicles in ovaries. Reproduction traits and histological observations of gonads in *O. niloticus* fed high dietary HLM levels revealed that *Hibiscus rosa-sinensis* leaves may be effective as a reproduction inhibitor in *O. niloticus*.

Keywords: *Hibiscus rosa-sinensis*, Reproduction inhibitor, Gonad histology, *Oreochromis niloticus*

1. Introduction

Red Hibiscus (*Hibiscus rosa-sinensis*) is a widely grown evergreen ornamental herbs, shrubs and trees of the tropics and sub-tropics which are grown as landscape plants, attractive roadside plants, border plants or as a container plants in greenhouses (Hou *et al* 2005). It belongs to the family Malvaceae and are reported to posses various medicinal properties viz: regulation of menstral cycle, curing hypoglycemia, potentiate hair growth, antihypertensive, anti-tumour, antioxidant and even treatment of veneral diseases{Hirunpanich *et al* (2006), Chang *et al* (2006), Herrara (2004), Palaniswamy (2003) and Telefor *et al* (1998)}.

Hibiscus rosa-sinensis had also been used as fertility control agent in some animal models {Zhou (1998), Jiang (1998), Tan (1983), Farnsworth (1982) and Tiwari (1982)}. The flowers have been reported to posses anti-implantation and anti-spermatogenic activities (Murthy *et al* 1997).Vasudeva and Sharma (2007) reported a post-coital activity of ether extract of *Hibiscus rosa-sinensis* roots administered orally to colony-breed female albino rats(Wister strain) and adult albino mice.

Tilapias constitute one of the most productive and internationally traded food fish in the world (Modadugu and Belen 2004). They are a major protein source in many of the developing countries. The commodity is not only the second most important farmed fish globally (next to carp) but also described as the most important aquaculture species of the 21st century (Shelton 2002).

(FAO 2006) reported that farmed nile tilapia (*Orochromis niloticus*) production reached 1,703,125mt, which is about 84% of total farmed tilapia production in 2006.

However, Tilapias are yet to reach their full aquaculture potential because of the problem 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 (Guerrero, 1982; Mair and Little, 1991); 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, 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 in some plants.

Over its natural range, *Oreochromis niloticus* (Linnaeus 1758) occurs in Africa and the coastal rivers of Israel; Nile from below Albert Nile to the delta; Jebel Marra; Lake Chad basin and the rivers Niger, Benue, Volta, Gambia and Senegal. It is widely introduced for aquaculture, with many existing strains. *O. niloticus* is a maternal mouth brooder and becomes sexually matured in 4-5 months at small size (10 cm; 20-50 g) in ponds; each female lays about 1,500-2,000 eggs/spawning and 3 spawnings/year (Balarin and Hatton, 1979). The objective of this study was to investigate the effects of varying dietary supplementation levels of dry *Hisbiscus rosa sinensis* leaf meal (HLM) on some reproduction traits (gonad development stages, gonadosomatic index (GSI), fecundity, egg size (length, diameter, volume), egg weight (wet and dry basis), histology of gonads) in *O. niloticus* fed for 60 days.

2. Materials and methods

Hisbiscus rosa sinensis leaves (tropical variety) were obtained from *Hisbiscus rosa sinensis* trees during the raining season in southwest Nigeria, where they are planted as ornamental plant. 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 basal diet (D1, 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 1.0, 2.0, 3.0, or 4.0g of HLM to 1 kg of basal diet, respectively. Nutrient imbalance caused by the addition of HLM was corrected by adding 4.0g of cellulose (non-nutritive ingredient) to the basal diet (D1) and 3.0, 2.0, 1.0, and 0g of cellulose to test diets D2, D3, D4 and D5, respectively. The feedstuffs were thoroughly mixed in a Hobart A-200T mixer. 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.

O. niloticus 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 *O. niloticus* (mean wt., 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 *O. niloticus* samples were randomly taken from each treatment, dissected, and the testes and ovaries removed and weighed for the gonadosomatic index (GSI) calculations (gonad wt./total body wt. x 10^2). Gonad development stages in male and female *O. niloticus* 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 oven-dried 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 gonads were 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.

Statistical comparisons of the results were made using the one-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).

3. Results and Discussion

Growth performance and feed conversion by *O. niloticus* fed varying dietary *Hibiscus rosa sinensis* Leaf meal (HLM) *levels.*

Dietary supplementation of HLM 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 (FCR) by fish fed with the experimental diets are presented in Table 2. The best overall growth response was obtained in fish fed with the basal diet, while weight gain,

% weight gain and average daily growth (ADG) were poorer ($P<0.05$) in fish fed with the HLM diets. A similar trend was observed with the specific growth rate (SGR); as the values decreased with increasing dietary HLM levels while the FCR values showed an inverse relationship.

Reproduction traits and histology of testes in *O. niloticus* fed varying dietary *Hibiscus rosa -sinensis* Leaf meal (HLM) *levels*

Table 3 shows that GSI values decreased ($P<0.05$) as the dietary HLM levels increased; which was similarly reported by Jegede and Fagbenro (2008) and is attributable to the poor development of testes tissues. However this result is in discrepancy with that reported by Tan (1983) which reported that *Hibiscus rosa sinensis* extracts had no effects on weights of the male reproductive organ in a study on rats. Histological sections of testes in *O. niloticus* fed 0g HLM/kg diet (basal diet) showed normal tissue architecture and spermatids distribution (Table 3). Fish fed 1.0g HLM/kg diet showed alterations in the testis architecture and cystic seminiferous tubules. In fish fed 2.0g HLM/kg diet, there was atrophy, while fish fed 3.0g HLM/kg diet showed cystic seminiferous tubules and atrophy. In fish fed 4.0g HLM/kg diet, there was severe tissue atrophy, spermatids disintegration and necrosis. This result corroborate that reported by Farnsworth (1982) that intake of alcoholic extracts of *Hibiscus rosa -sinensis* flowers led to decreased spermatogenic elements of testis and epididymal sperm count. Also in related studies, Jegede *et al*.(2008a) obtained similar histological effects in male redbelly tilapia (*Tilapia zillii*, Gervais 1848) fed varying dietary supplementation levels (0.5-2.0 g/kg diet) of neem (*Azadirachta indica*) leaf meal (NLM) tested as reproduction inhibitors. However, Ekanem and Okoronkwo (2003) obtained much severe histological effects in male Nile tilapia fed higher dietary Pawpaw seed meal (PSM) supplementation levels (4.9 and 9.8g PSM/kg diet).

Reproduction traits and histology of ovaries in *O. niloticus* fed varying dietary *Hibiscus rosa -sinensis* Leaf meal (HLM) *levels*

Relative distribution of gonad development stages was very homogenous among replicates in each dietary HLM 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 2.0g or 4.0g HLM/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 unclear, 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 HLM levels.

As with the male *O. niloticus*, GSI values as well as other reproduction traits decreased ($P<0.05$) as the dietary HLM levels increased (Table 4); which was similarly reported by Jegede and Fagbenro (2008) and is also attributable to the poor development of ovarian tissues as suggested by Cumaranatunga and Thabrew (1989). In *O. niloticus* fed with the basal diet (0g HLM/kg diet), typical bilateral lobes of the ovaries were evident; and the normal olive green colour was maintained. Sections of ovaries in *O. niloticus* fed with the basal diet showed normal ovary histology. No pathological lesions were observed, atretic follicles were less visible (Table 4). In fish fed 2.0 or 4.0g HLM/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* was fed with varying dietary supplementation levels (1.0-2.0 g/kg diet) of (NLM) neem leaf meal used as reproduction inhibitors.

In this study, the damage done to tissues of the testes and ovaries was minimal at lower dietary HLM levels (1.0 or 2.0 g/kg diet), and at higher dietary HLM levels (3.0 or 4.0 g/kg diet), it caused disintegration of many more cells, rendering the testes and ovaries devoid of spermatids and oocytes, respectively. This makes air dried *Hibiscus rosa sinensis* leaf recommendable for use in the control of breeding in tilapias. Histological observations of testes and ovaries in *O. niloticus* fed the basal diet supplemented with HLM revealed that *Hibiscus rosa sinensis* leaf 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 HLM in combating

problems of tilapia overpopulation in ponds. Other than infertility, literature did not indicate any adverse reactions from the consumption of *Hibiscus rosa sinensis* leaf.

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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.

Table 2. Growth performance and feed conversion by Oreochromis niloticus fed Hisbiscus rosa-sinensis leaf meal (HLM) diets

	Dietary HLM level (g/kg diet)				
	0	1.0	2.0	3.0	4.0
Final weight (g)	66.53a	66.03a	62.48ab	58.37b	58.18b
Initial weight (g)	40.23	40.23	40.23	40.23	40.23
Weight gain (g)	26.30a	25.80a	22.25b	18.14c	17.95c
% weight gain[1]	65.37a	64.13a	55.31b	45.09c	44.62c
ADG[2]	0.44a	0.43a	0.37b	0.30c	0.29c
SGR[3]	0.84a	0.82a	0.73b	0.62b	0.61b
FCR[4]	1.82a	1.90b	2.05b	2.12bc	2.17c

[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)

Table 3. Reproduction traits and histological description of male Oreochromis niloticus fed HLM diets

Treatments (g HLM/kg diet)	GSI (%)	Histological description
0	1.75a	normal testicular tissue architecture and normal spermatids distribution
1.0	1.09b	increase in interstitial cells
2.0	0.82c	swollen spermatids nuclei, increased interstitial cells and focal necrosis
3.0	0.73c	atrophied seminiferous tubules
4.0	0.41d	severe disintegration of sperm cells and necrosis

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

Table 4. Reproduction traits and histological description of female Oreochromis niloticus fed HLM diets

Treatments (g HLM/kg diet)	GSI (%)	Fecundity	Egg traits					Histological description
			Diameter (mm)	Length (mm)	Volume (mm^3)	Wet weight (mg)	Dry weight (mg)	
0	1.95a	465	2.92a	3.05a	7.14a	7.7a	2.8	normal histology and less visible atretic follicles
2.0	1.36b	340	2.70b	2.81b	6.53b	6.0b	2.7	increased atretic follicles and hydropic degeneration
4.0	1.11b	280	2.56b	2.12c	6.31b	5.4b	2.5	increased atretic follicles, ruptured follicles and necrosis

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

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