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Effects of *Aloe vera* (Liliaceae) on Gonad development in Nile tilapia, *Oreochromis niloticus* (L.) during intensive aquaculture

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ABSTRACT

There is need to control undesirable tilapia recruitment in ponds using natural reproductive inhibitory agents in plants because they are less expensive and constitutes appropriate technology in developing countries. *Aloe vera* latex (AL) was added to a basal diet (350 g crude protein and 18.5 MJ gross energy/kg diet) at 0, 0.5, 1.0, 1.5 or 2.0 ml/kg diets and fed to mixed-sex *Oreochromis niloticus* for 60 days to evaluate the effects on growth and feed utilization, reproduction traits, and histology of gonads. There were no significant difference ($P > 0.05$) in the growth parameters and food conversion ratio. Indices of reproduction traits decreased with increasing dietary *A. vera* latex (AL) levels. Fish fed with the control diet and 0.5 ml AL/kg diet had significantly higher and better indices of reproduction traits ($P < 0.05$) than the other fish fed with 1.0, 1.5 and 2.0 ml AL/kg diets respectively. Fish fed 0.0 ml AL/kg diet (control) showed normal testicular and ovarian tissues architecture, typical bilateral lobes of the ovaries were evident, normal olive green colour was maintained; and no pathological lesions occurred. Fish fed 0.5 ml AL/kg diet showed no visible alteration in the ovaries, testicular architecture and cystic seminiferous tubules. Fish fed 1.0 ml AL/kg diet showed testicular atrophy. Fish fed 1.5 ml AL/kg diet exhibited cystic seminiferous tubule and atrophy of tissues. Fish fed 2.0 ml AL/kg diet showed severe tissue atrophy, sperm cells disintegration and necrosis. Also, in fish fed 2.0 ml AL/kg diet, oviectomy revealed a change in the colour of ovaries, histology showed ruptured follicle, granulomatous inflammation in the interstitium and necrosis of the ovaries. Reproduction traits and histological observations of gonads in *Oreochromis niloticus* fed high dietary levels of AL revealed that *A. vera* latex may be effective as reproduction inhibitory agent.

Keywords: Nile Tilapia, *Aloe vera*, Gonad Development, aquaculture.

1. Introduction

Aloe vera is a succulent, almost sessile perennial herb. Its leaves 30-50 cm long and 10 cm broad at the base; colour is pea green (when young they are spotted with white) and has bright yellow tubular flowers, 25-35 cm in length arranged in a slender loose spike. It contains a colourless mucilaginous gel called *A. vera* gel [2]. *A. vera* is grown commercially, especially in the Himalayan Kingdom of Nepal, for the latex which is used medicinally [3]. It is frequently used in herbal medicine and can be grown as an ornamental plant. Preliminary evidences have shown that *A. vera* extracts (bitter yellow *A. vera* latex) are useful in the treatment of fungi and bacterial infections in humans and as laxative [4]. Compounds extracted from *A. vera* have been used as an immunostimulant that aids in fighting cancers in cats and dogs [5]. Active compounds in *A. vera* include polysaccharides, mannans, anthraquinones, lectins, salicylic acid, urea, nitrogen, cinnamic acid, phenol and sulphur has also been reported [6]. It also contains amino acids, lipids, sterols tannin and enzyme [5, 6].

Though many scientific studies on the use of *A. vera* have been undertaken [7], some of them are said to be conflicting [8] and this include the fact that the bitter yellow latex from *A. vera* leaf (which active ingredients are aloe-emodin, aloin and barbaloin) can cause abdominal cramps, impair fertility or cause miscarriage in humans and animals during overdose or misuse [8]. Active ingredients from *A. vera* latex are used extensively in commercial laxative preparation, though its use has been banned [7].

Tilapias constitute one of the most productive and internationally traded food fish in the world [9]. They are a major protein source in many of the developing countries [7].

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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 [10]. FAO reported [11] that farmed Nile tilapia (*O. 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 [12], which often results in the overpopulation of production ponds with young (stunted) fish. Population control in farmed tilapias has been reviewed [13]; 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 [12]. 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 [12].

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 [14]. The objective of this study was to investigate the effects of varying dietary inclusion levels *A. vera* latex on some reproduction traits (gonad development stages, fecundity, egg size (length and diameter) and histology of gonads in Nile tilapia (*O. niloticus*) fed for 60 days.

2. Materials and Methods

A. vera leaves were cut with a sharp, clean knife and the bitter yellow latex were collected and stored in a dry, clean, air-tight transparent plastic container, labelled and refrigerated at -20 °C. Feedstuffs were purchased from a local feedstuff market and were separately milled to small particle size (< 250 µm). A basal diet (D1, 350 g crude protein and 18.5 MJ 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 2.0 ml of *A. vera* latex (AL) to 1 kg of basal diet, respectively. The feedstuffs were thoroughly mixed in a Hobart A-200T mixer. Hot water was added at intervals to gelatinize starch. The five diets were pelletized using a die of 8 mm diameter and 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 |
|---------------------|------------------|
| Fish meal | 280 |
| Soybean meal | 370 |
| Corn meal | 250 |
| Cod liver oil | 30 |
| Corn oil | 20 |
| Vitamin-mineral mix | 30 |
| Corn starch | 20 |

Nile tilapia (*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, 5 male and 5 female *O. niloticus* (mean wt., 30.38 ±0.16 g) were stocked in each of 15 glass tanks (75 cm x 40

cm x 40 cm) supplied with 60 litres of fresh water (water temperature, 27 °C; pH, 7.3; alkalinity, 50 ppm; dissolved oxygen, 7.6-7.9 mg/L). Continuous aeration was provided using a blower and air stones (Tecas air pump AP-3,000; 2 ways). The treatments were replicated thrice. Fish were fed at 4% body weight/day of the basal diet 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.

Six male and six female *O. niloticus* samples were randomly taken from each treatment, dissected, and the testes and ovaries removed and weighed. Gonad development stages in male and female *O. niloticus* were classified [5, 16], respectively. Fecundity was estimated from gonads of six fish 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. Thirty (30) eggs were measured using a microscope eye-piece graticule for length (L) and width (H) (Rana, 1985) [17]. Short and long axis of two egg samples from each spawn (treatment) were measured using light microscope containing a calibrated eye piece graticule [16].

Mean egg diameter was calculated from each spawn (treatment) as follows:

$$\text{Mean egg diameter (mm)} = \frac{\text{length of long axis} + \text{length of short axis}}{2}$$

2

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 8 µ 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 [18].

3. Results and Discussion

3.1 Reproduction traits and histology of testes in *O. Niloticus* fed varying inclusion levels of *Aloe vera* Latex (AL) diets.

In fish fed with the control diet, milt motility from its initial level of 84.84% for *O. niloticus* dropped to 17.88%, for those fed with 2.0 ml AL/kg diet for 60 days. In a similar study [19, 20, 21] it has been reported that the chloroform extract, the benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate sub-fractions and the isolated compounds of *Carica papaya* showed significant effects on sperm parameters by oral administration in rats and rabbits. Also, milt count dropped from its initial level of 163,600 to 138,000 in *O. niloticus* fed with 2.0 mlAL/kg diet for 60 days. This was corroborated by Sadre *et al.* (1983) [22] who studied male antifertility activity of neem on mice, rats, rabbits, and guinea pigs by daily oral administration of a cold-water extract of fresh green neem leaves and reported infertility effect in treated male rats, as there was a 66.7% reduction in fertility after six weeks, 80% after nine weeks, and 100% after 11 weeks [21]. There were no inhibition of spermatogenesis, no decrease in body weight and no manifestation of toxicity, but there was a marked decrease in the motility of spermatozoa.

The infertility in rats was reported not to be associated with loss of libido and that the animals maintained normal mating behaviour [21]. Milt morphology was also evaluated using the evaluation criteria suggested by World Health Organization (WHO) [23] and examined under Olympus microscope model CX 40. The milt showed normal milt morphology; the oval head and distinctive and well defined tail. At low treatment dosage i.e. 0.5 ml or 1.0 ml AL/kg basal diet, no appreciable visible changes was noticed in the milt morphology, while at high treatment dosage 1.5 ml or 2.0 ml AL/kg basal diet, deleterious changes in the plasma membrane of the head was observed, consequently suggesting that the milt were infertile [24].

Histological sections of testes in *O. niloticus* fed 0 ml AL/kg diet (basal diet) showed normal tissue architecture and spermatids distribution (Table 2). Fish fed 0.5 ml AL/kg diet showed no visible alterations in the testicular architecture and cystic seminiferous tubules. In fish fed 1.0 ml AL/kg diet, there was atrophy, while fish fed 1.5 ml AL/kg diet showed cystic seminiferous tubules and atrophy. In fish fed 2.0 ml AL/kg diet, there was severe tissue atrophy, spermatids disintegration and necrosis.

Table 2: Histological description of male *O. niloticus* fed *Aloe vera* latex (AL) diets.

| Treatments (ml AL/kg diet) | Histological description |
|----------------------------|---|
| 0 | normal testicular tissue architecture and normal spermatids distribution |
| 0.5 | no visible alterations in the testis architecture and cystic seminiferous tubules |
| 1.0 | Atrophy |
| 1.5 | cystic seminiferous tubules and atrophy |
| 2.0 | severe tissue atrophy, spermatids disintegration and necrosis |

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

This result corroborate that reported [25] that oral intubation of ethanol extracted *Momordica charantia* at 1.3 mg/kg treated guinea pigs shows degeneration of tubules from connective tissue. Also in a related study obtained [26] similar histological effects (severe alteration in the testicular architecture and necrosis) in male redbelly tilapia (*Tilapia zillii*, fed varying dietary inclusion levels (0.5-2.0 g/kg diet) of neem (*Azadirachta indica*) leaf meal (NLM). Also, in a similar study on male albino rats [27] administered intramuscularly Papaya seed extract at a dose of 0.5 mg/kg/day for 7 days, a much severe decrease in the contractile response of epididymal tubules was obtained when compared with the control.

3.2 Reproduction traits and histology of ovaries in *O. niloticus* fed varying inclusion levels of *Aloe vera* Latex (AL) diets. The inclusion of *A. vera* latex (AL) at varying levels in the diets of *O. niloticus* fed for 60 days treatment period, revealed no deleterious changes in the shape of the eggs when observed under an electronic microscope. Normal oval shape of eggs was observed. This was corroborated by the normal egg shape of tilapia is oval [28]. Egg size and fecundity decreased (2.25 ± 7.07 mm- 2.13 ± 3.54 mm and 228 ± 4.24 - 150 ± 1.41 respectively) as the inclusion level of *A. vera* latex (AL) increases (Table 3). This result agrees with Coward and Bromage (2004) [29] who reported that eggs produced by mouth brooders (*O. niloticus*) normally exceed 2 mm in diameter and that fecundity is usually less than 350 in mouth brooders. In *O. niloticus* fed with the basal diet (0 ml AL/kg diet), typical bilateral lobes of the ovaries were evident; and the normal olive green colour was still maintained.

Table 3: Egg sizes (mm) and fecundity of *Oreochromis niloticus* fed ALM diets

| | ALM diet treatments (ml/kg) | | | | |
|------------------|-----------------------------|--------------------|--------------------|--------------------|--------------------|
| | 0 | 0.5 | 1.0 | 1.5 | 2.0 |
| Egg size | 2.33 ± 1.77 | 2.20 ± 0.00 | 2.20 ± 0.00 | 2.13 ± 3.54 | 2.18 ± 3.54 |
| Fecundity | 258.0 ± 2.12 | $228.0^* \pm 4.24$ | $218.0^* \pm 2.12$ | $203.0^* \pm 1.41$ | $198.0^* \pm 2.12$ |

*The mean difference is significant to control at the 0.05 level

Sections of ovaries in *O. niloticus* fed with the basal diet showed normal ovary histology. No pathological lesions was observed, atretic follicles were less visible (Table 4). Also in fish fed low AL/kg basal diets (0.5 and 1.0 ml AL/kg diet) no visible changes were noticed, normal ovarian colour was maintained; ovary histology was similar to that of the control

except for few pockets of lesions. In fish fed 1.5 and 2.0 ml AL/kg diet, ovariectomy reveals a change in colour of ovaries, while histology reveals ruptured follicles, inflammation of the granulomatous in the interstitium, evidences of abnormal gonadal development and necrosis.

Table 4: Histological description of female *Oreochromis niloticus* fed *Aloe vera* latex (AL) diets.

| Treatments (ml AL/kg diet) | Histological description |
|----------------------------------|---|
| 0 | normal histology and less visible atretic follicles |
| 1.0 | ovary histology was similar to that of the control except for few pockets of lesions; normal ovarian colour was maintained; |
| 2.0 | Change in colour of ovaries was noticed, ruptured follicles, inflammation of the granulomatous in the interstitium, evidences of abnormal gonadal development and necrosis. |

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

Similar histological effect was reported [30] where the damage done to tissues of the testes and ovaries were minimal at lower dietary *Hibiscus rosa sinensis* leaf meal (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.

Although *A. vera* gel had been reported to provide evidence of anti-genotoxic against mutagenicity induced by alkylating agent ethyl methane sulfonate [31], nothing of such had been reported on *A. vera* latex. This indicates that *A. vera* latex at high levels of concentration causes histological damage to the gonads of both male and female *O. niloticus*, thereby potentially impairing reproduction.

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