HISTOLOGY OF GONADS IN *OREOCHROMIS NILOTICUS* (TREWAVAS) FED PAWPAW (*CARICA PAPAYA*) SEED MEAL DIETS

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Abstract

Pawpaw (*Carica papaya*) seed meal (PSM) 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 *Oreochromis niloticus* for 60 days to evaluate the effects on histology of the gonads. *O. niloticus* fed 0g PSM/kg diet showed normal testicular tissues and no lesions were observed. Fish fed 0.5g PSM/kg diet showed slight increase in interstitial cells. Fish fed 1.0g PSM/kg diet showed swollen sperm cells nuclei, increased interstitial cells and focal necrosis. Fish fed 1.5g PSM/kg diet exhibited atrophy of seminiferous tubules and in fish fed 2.0g PSM/kg diet, there were severe pathological changes as disintegration of sperm cells and necrosis of the testicular cells. Ovaries in fish fed with control diet (0g PSM/kg diet) showed normal ovary histology. No pathological lesions were observed. Typical bilateral lobes of the ovaries were evident and with normal olive green colour of ovaries. Fish fed 1.0g PSM/kg diet revealed ovarian colour change, hydropic degeneration, ruptured follicle, granulomatous inflammation in the insterstitium and necrosis. Ovaries in fish fed 2.0g PSM/kg diet showed severe atretic follicle. Histology of gonads in *O. niloticus* fed high dietary PSM revealed that pawpaw seeds may be effective as sterility-inducing agents.

INTRODUCTION

Pawpaw (*Carica papaya*) seeds had been used as fertility control agents in some animal models and even on human beings (Lohiya *et al.,* 2004; Udoh and Kehinde, 1999), respectively. They contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside (Emeruwa, 1982), the last of which caused sterility in male rats (Das, 1980). Histological observations made by Udoh and Kehinde (1999) revealed that at dose of 1 g/kg body weight after crude ripe pawpaw seeds were administered orally for eight weeks on male albino (Wistar) rat, degeneration of the germinal epithelium and germ cells, and the presence of vacuoles in the tubules were observed; while at a low or dose of 0.5 g/kg body weight little effect was observed.

Extracts from different parts of the pawpaw tree have been reported to decrease the testicular weight of Wistar rats when administered orally for eight weeks.

It was however reported that suppression of spermatogenesis was observed in these rats following the administration of pawpaw seed extract (Uche-Nwachi *et al*., 2001). In addition, water extract of papaya seeds, was administered orally to Sprague Dawley rats *ad-libitum* for eight weeks and results showed that three weeks after commencement of this administration, the lumina of the semiferous tubules were more prominent and empty in the experimental animals with no evidence of spermatid or spermatozoa. It also showed that the lateral walls of adjacent sertolil cells lost contact with each other.

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 methods in farmed tilapias have been reviewed (Guerrero, 1982; Mair and Little, 1991; Fagbenro, 2002) and include monosex culture, sex reversal, cage/tank culture, use of predators, high density stocking, sterilization, intermittent/selective harvesting, use of slow maturing tilapia species. However, all these population control methods have their limitations; e.g. use of reproductive inhibitors (chemosterilants and irradiation) has disadvantages of 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 technology to control undesirable tilapia recruitment in ponds using natural reproduction inhibitors found in plants.

The objective of this study was to investigate the effects of varying dietary levels of dry pawpaw seed meal (PSM) on the histology of testes and ovaries in *O. niloticus* fed for 60 days.

MATERIALS AND METHODS

Ripe fruits of pawpaw, *Carica papaya* Linn. (Caricaceae; Voucher No. RUBL 16590) of honey dew variety, obtained from farm settlements in southwest Nigeria, were cut open to remove the seeds which 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 (< 250 µm). A basal 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 prepared by adding 0.5, 1.0, 1.5 or 2.0 g of pawpaw seed meal to 1 kg of the basal diet, respectively. Nutrient imbalance caused by the addition of PSM was corrected by adding 2 g 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 mixed in a Hobart A-200T mixing/pelleting machine. Hot water was added at intervals to gelatinize starch. All diets were pelletized using a die of 8 mm diameter; 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

O. niloticus 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 *O. niloticus* (mean weight, 40 g) were separately stocked in each of 15 concrete tanks (2 m x 2 m x 1.25 m) 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 and weighed. Fish 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 *O. niloticus* fed varying dietary levels of pawpaw seed meal

Histological sections of testes in *O. niloticus* fed with the control diet (0g PSM/kg diet) showed normal testicular tissue and normal sperm cell distribution (Figure 1). No lesions were observed. In fish fed 0.5g PSM/kg diet, there was slight increase in interstitial cells of the testes (Figure 2). In fish fed 1.0g PSM/kg diet, there were swollen sperm cells nuclei, increased interstitial cells and focal necrosis was observed within the testicular tissue (Figure 3). In fish fed 1.5g PSM/kg diet, there was atrophy of seminiferous tubules (Figure 4) and fish fed 2.0g PSM/kg diet showed disintegration of sperm cells and necrosis of the testicular cells (Figure 5).

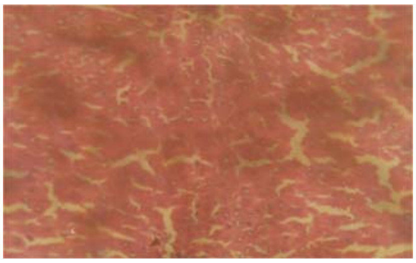

Figure 1. Section of testes in *O. niloticus* fed control diet showing normal testicular tissue and normal sperm cell distribution. Mag. X 40

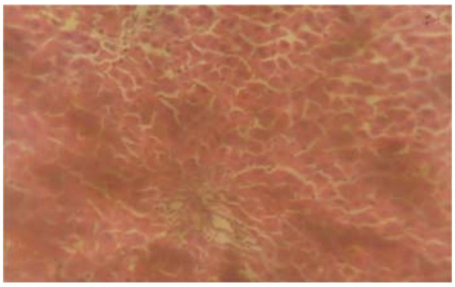

Figure 2. Section of testes in *O. niloticus* fed 0.5g PSM/kg diet showing increase in interstitial cells. Mag. X 40.

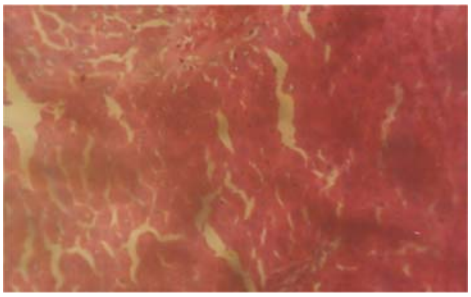

Figure 3. Section of testes in *O. niloticus* fed 1.0g PSM/kg diet showing swollen sperm cells nuclei, increased interstitial cells and focal necrosis. Mag. X 40.

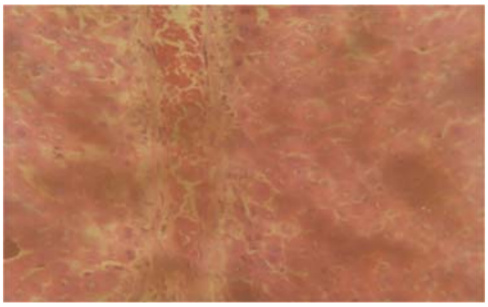

Figure 4. Section of testes in *O. niloticus* fed 1.5g PSM/kg diet showing atrophied seminiferous tubules. Mag. X 40.

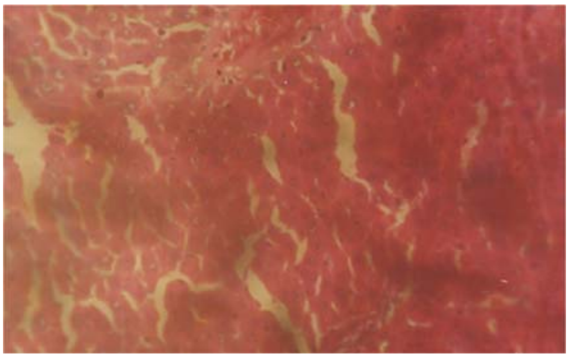

Figure 5. Section of testes in *O. niloticus* fed 2.0g PSM/kg diet showing severe disintegration of sperm cells and necrosis. Mag. X 40.

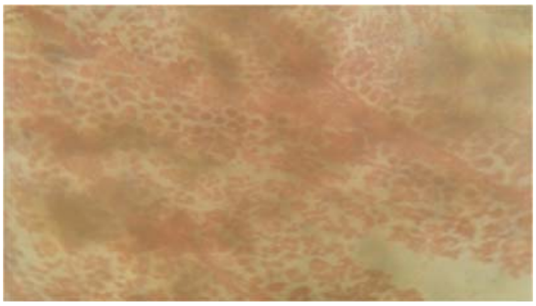

Figure 6. Section of ovary in *O. niloticus* fed diet 1 (control) showing normal histology and less visible atretic follicles. Mag. X 40.

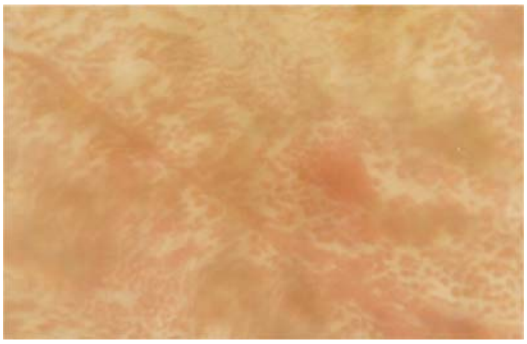

Figure 7. Section of ovary of *O. niloticus* fed 1.0g PSM/kg diet showing increased atretic follicles. Mag. X 40.

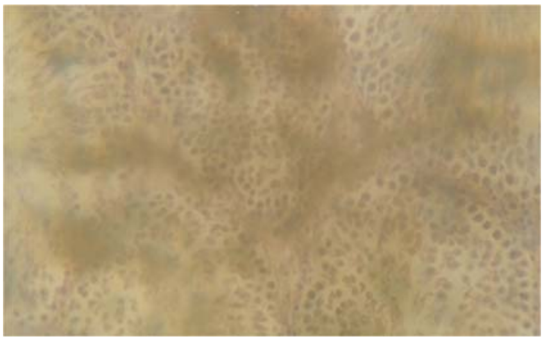

Figure 8. Section of ovary of *O. niloticus* fed 2.0g PSM/kg diet showing severe atretic follicle. Mag. X

In a related study, Ekanem and Okoronkwo (2003) observed similar histological changes in male *O. niloticus* fed much higher dietary PSM levels (5 or 10g PSM/kg diet) for 60 days, and in addition, a discolouration of the liver in *O. niloticus*, suggesting that pawpaw seeds contain ingredients that can be effective as sterility-inducing agents but can be damaging at a high dietary PSM levels. Das (1980) suspected that oleanolic glycoside is the active ingredient in pawpaw seeds responsible for sterility in the case of male rats.

Histology of ovaries in *O. niloticus* fed varying dietary levels of pawpaw seed meal

Histological sections of the ovary in *O. niloticus* fed with the control diet (containing no PSM) showed normal ovary histology. No lesions were observed, atretic follicles were less visible (Figure 6), and normal olive green colour of ovaries was maintained. Sections of the ovary in *O. niloticus* fed 1.0 or 2.0g PSM/kg diet revealed evidence of severe atretic follicle (Figures 7 and 8). Similarly, Cumaranatunga and Thabrew (1989) described the effects of legume (*Vigna catiang*) substituted diets on ovarian growth in *O. niloticus* in which histological sections indicated that fish fed with the control diet had a better ovarian growth than those fed with test diets containing *V. catiang*.

The dosage used in the present study (0.5-2.0 g PSM/kg diet) is much lower than those administered by Ekanem and Okoronkwo (2003). In this study however, the damage done to the testes and ovaries was minimal at lower dietary PSM level, while at higher dietary PSM level, it caused disintegration of many more cells, rendering the testes and ovaries devoid of spermatids and oocytes, respectively. This makes pawpaw seeds recommendable for use in the control of breeding in tilapias. Histological observations of gonads in *O. niloticus* fed diets containing PSM revealed that pawpaw seeds may be effective as sterility-inducing agents as they are destructive to gonads at high PSM levels. This study gives useful information towards the determination of the contraceptive efficacies of dietary PSM in combating problems of tilapia overpopulation in ponds.

REFERENCES

1. Cumaranatunga, P. R .T. and H. Thabrew. 1989. Effects of a legume (*Vigna catiang*) substituted diets on the ovarian development of *Oreochromis niloticus* (L.). pp. 333-344, In: Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. Japan.
2. Das, R. P. 1980. Effect of papaya seeds on the genital organs and fertility of male rats. Indian Journal of Experimental Biology 18: 408-409.
3. Ekanem, S. B. and T. E. Okoronkwo. 2003. Pawpaw seed as fertility control agent on male Nile tilapia. NAGA ICLARM Quarterly 26 (2): 8-10.
4. Emeruwa, A. C. 1982. Antibacterial substance from *Carica papaya* fruit extract. Journal of Natural Products 45: 123-127.
5. Fagbenro, O. A. 2002. Tilapia: fish for thought. 32nd Inaugural Lecture, Federal University of Technology, Akure, Nigeria. 77pp.
6. Guerrero, R. D. 1982. Control of tilapia reproduction. Pp. 309-316, in R.S.V. Pullin and R.H. Lowe- McConnell (eds.) The biology and culture of tilapias. ICLARM Conference Proceedings 7, Manila, Philippines.
7. Lohiya, N. K., B. Manivannan, P. K. Mishra, N. Pathak, S. Sriram, S. S. Bhande and S. Panneerdoss. 2004. Ultra-structural changes in the testis and epididymis of rats following treatment with the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya*. Phytotherapeutic Research 18 (4): 285-289.
8. Mair, G. C. and D. C. Little. 1991. Population control in farmed tilapia. NAGA, ICLARM Quarterly 14: 8-13.
9. Morrison, C. M., K. Fitzsimmons and J. R. Wright Jr. 2007. Atlas of tilapia histology. World Aquaculture Society, US. 96pp.
10. Udoh, P. and A. Kehinde. 1999. Studies on antifertility effects of pawpaw seeds (*Carica papaya*) on the gonads of male albino rats. Phytotherapeutic Research 13 (3): 226-228.
11. Uche-Nwachi, E. O., D. C. Ezeokoli, A. O. Adogwa and V. N. Offiah. 2001. Effect of water extract of *Carica papaya* seed on the germinal epithelium of the seminiferous tubules of Sprague Dawley rats. Kaibogaku Zasshi 76(6): 517-521.