# Pawpaw seed as fertility control agent on male Nile tilapia

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## Abstract

To find out if pawpaw (*Carica papaya)* seeds can induce sterility in male Nile tilapia (*Oreochromis niloticus*) and to determine if sterility so induced is reversible or otherwise, mature male tilapia of mean weight 40 g were treated for 30 days with a low dose (4.9 g/kg/day) and a high dose (9.8 g/kg/day) of ground pawpaw seeds incorporated into their feed. Fish of similar sizes in the control experiment were fed with feed that did not contain pawpaw seed. The pawpaw seeds induced permanent sterility in the fish that received the high dose, while sterility in the low dose treatment was reversible. Fish in the control experiment spawned two weeks into the experiment and again in the fifth week. Fish in the low dose treatment spawned three weeks after the treatment had been discontinued. Histological sections of the testes showed that pawpaw seeds produced swollen nuclei in the low dose treatment and disintegrated cells in the high dose treatment. The study showed that pawpaw seeds, which are easy to obtain, can be incorporated into fish feeds and used by farmers to control prolific breeding of Nile tilapia.


## Introduction

The importance of Nile tilapia (*Oreochromis niloticus)* in aquaculture is evident from the fact that it has now spread to all continents of the world (Pullin 1994). The reason for this spread is that it is hardy and exhibits most of the desirable qualities of a culture species (Bardach et al. 1972) and tolerates a wide range of salinity. Tilapia culture is, however, fraught with the problems of prolific breeding, overpopulation and stunting. *O. niloticus* sexually matures at about 20 g weight (Mair and Little 1991). Uncontrolled reproduction of this species in ponds leads to the harvest of stunted fish with low nutritional and commercial value (Beardmore 1996).

For profitable culture, the prolific breeding and stunting problem associated with it have to be solved. Mair and Little (1991) enumerated various methods and techniques available for the control of prolific breeding in tilapia. However, each of them has its own shortcoming. As the search for a better solution to this problem continues, medicinal plants offer some possibilities. Medicinal plants have successfully been used to induce sterility in laboratory animals (Gary and Garg 1971; Bodharkar et al. 1974; Das 1980). Pawpaw (*Carica papaya*) seed, administered orally to male albino rats, effectively controlled their reproduction (Udoh and Kehinde 1999). It is therefore not out of place to expect a similar effect on male tilapia. This method of control could be easier to adopt by poor fish farmers since pawpaw seeds are available all year round in the tropics and sub-tropical regions. Pawpaw seeds contain active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor, and oleanolic glycoside, the last of which had been found to cause sterility in male rats (Das 1980).

The objectives of this study were to determine if pawpaw seeds could induce sterility in male *O. niloticus* when administered through feed, to determine its mode of action on the testes, and to determine if the effect is reversible.

## Materials and Methods

*O. niloticus* used in this study were obtained from the University of Calabar Institute of Oceanography pond and acclimated in aquaria in the Institute's hatchery where the study was conducted. Acclimation to the aquarium environment lasted for two weeks during which the fish were fed with a formulated feed.

Feed for the control experiment was compounded with 40.4 per cent wheat bran, 20.2 per cent groundnut cake, 20.2 per cent palm kernel cake, 6.2 per cent bone meal, 6.1 per cent blood meal, 1 per cent vitamin premix and 6.1 per cent palm oil. To 1 kg of this control feed component, 61 g of ground pawpaw seed were added to prepare the low dose treatment while double the quantity of pawpaw seed (122 g) was added to 1 kg of the control feed to get the high dose treatment. Nutrient imbalance created by the addition of pawpaw seed was corrected by adding 122 g of sawdust to each kilogram of the control feed and 61 g of sawdust to each kilogram of low dose pawpaw seed-treated feed. The dough formed by adding water was dried in the sun and stored. This was used to feed the fish for two weeks before a new batch of feed was prepared.

After acclimation, five male and five female *O. niloticus*, with a mean weight of 40 g, were stocked in each of six aquaria of 95 cm x 50 cm x 30 cm dimension. The study was conducted at water temperature of 27°C, pH 7, alkalinity of 30 ppm and 6.3 to 6.7 mg/L of dissolved oxygen. The number of aquaria available for the study permitted only duplication of the experiment.

Feeding of the fish commenced a day after stocking and lasted for 30 days. The fish were fed at 8 per cent of their body weight daily, in two instalments at 1000 and 1500 hours. Fish in the first two aquaria were fed with the control feed; those in the second set of two aquaria were fed with the low dose treatment feed while fish in the last set of aquaria were fed with the high dose treatment

feed. At that feeding rate, the pawpaw seed dosage for the low dose treatment was 4.9 g/kg of fish/day while the high dose treatment was double that quantity (i.e., 9.8 g/kg of fish/day).

Eight-centimeter diameter pipes were introduced into the aquaria for the fish to hide. Each aquarium was cleaned and observed daily as the experiment proceeded. At the end of the 30-day treatment period, the fish were weighed, a male fish from each treatment and the control were sacrificed and the testes were removed for sectioning and histological examination. The liver, spleen and kidney were examined for possible damage by the active ingredient in pawpaw seed. The remaining fish in all aquaria were fed with the control feed and observed for another 30 days. Then the fish were weighed again before the experiment was finally terminated.

The removed testes were fixed for 24 hours in formalin-saline solution made of equal volumes of 10 per cent formalin and 0.9 per cent sodium chloride solution. Histological sections of 8 μ thickness were prepared following standard procedures.

## Results

The liver of fish from the high dose pawpaw seed treatment (9.8 g/kg/day) had a pale color compared to the deep red color of liver from fish in the low dose treatment and the control experiment. There was no clear difference in the coloration of the kidney and spleen.

No spawning occurred in any of the treated aquaria during the 30-day treatment period whereas spawning was observed in the control experiment two weeks into the experiment. Thirty days after the treatment had been discontinued, there was still no spawning in aquaria that earlier received the high dose treatment of pawpaw seed. However, there was spawning in the aquaria that previously received low dose treatment. Spawning recurred in the fifth week into the experiment in the control aquaria. In the control experiment 51 to 59 fry were counted during the second spawning while 36 to 44 fry were counted in low dose treatment aquaria.

Histological sections of testes from the control fish showed normal cell distribution in the testes (Fig. 1), while swollen nuclei on the sperm cells were observed in fish from the low dose treatment (Fig. 2). A high dose of pawpaw seed had caused disintegration of the sperm cells and the formation of more swollen nuclei in the sperm cells (Fig. 3).

Fish that received pawpaw seed treatments showed a decrease in weight during the 30-day treatment when compared to the control, but analysis of the variance test did not find the difference to be significant ($P > 0.05$). Fish that received a high dose treatment gained some weight after the treatment had been discontinued; however, the weight difference was not significant ($P > 0.05$).

## Discussion

Discoloration of the liver of fish from the high dose treatment shows that the active ingredients of pawpaw seeds are strong chemicals that at a moderate dose can be effective as sterility-inducing agents but can be damaging at a high dose. The findings in this preliminary study could be used in the crude form, but further studies can confirm if oleanolic glycoside is the ingredient responsible for sterility in *O. niloticus* as was the case in rats (Das 1980). Isolating and studying the active ingredients separately can reveal whether or not the other active ingredients have similar effects and whether they act in unison or in combination with one another. From these studies, refinements could be made in the drug.

Spawning of the fish in the low dose treatment after the treatment had been discontinued amply demonstrated that pawpaw seed can be used reversibly to control breeding of *O. niloticus*. This result is similar to that obtained by Udoh and Kehinde (1991) who administered pawpaw seed at a high dose of 100 mg/kg/day and a low dose of 50 mg/kg/day to male rats. The dosage used in the present study is, however, much greater than that administered by Udoh and Kehinde (1991), the reason being that there was no such previous work on fish to provide the guiding information on the dosage. This study has now provided such data, which could be used in future studies. It is worth noting that the high dose used on the fish was to ensure adequate intake of the drug after some might have dissolved or have been washed off the feed by water.

The reversible effect of pawpaw seeds in the low dose treatment was due to the fact that damage done to the testes was minimal and could be repaired within a few weeks. This fact makes the drug recommendable for use at this low dose for control of breeding in *O. niloticus*. The high dose of the drug caused disintegration of many more sperm cells, rendering the testes devoid of sperm cells.

Lower numbers of fry produced in the low dose treatment compared to that of

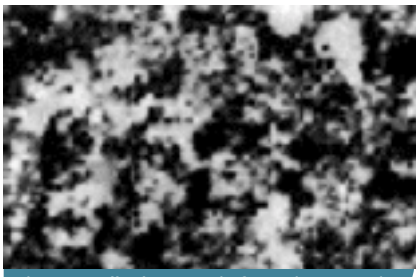

*Fig. 1.* O. niloticus *testis from the control experiments showing normal sperm cell distribution*

*Fig. 2.* O. niloticus *testis from low dose pawpaw seed treatment showing swollen sperm cells nuclei*

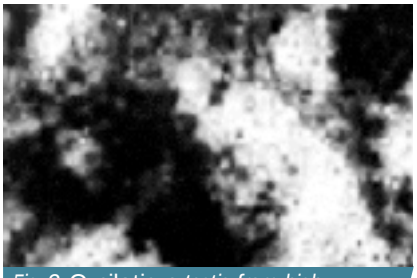

*Fig. 3.* O. niloticus *testis from high dose pawpaw seed treatment showing disintegration of sperm cells*

the control experiment shows that even the low dose had a deleterious effect on the testes as well as the ovary of this species. Such results warrant further studies to determine an effective dose that will not produce deleterious effects. Reduction in the number of fry produced by fish, which previously received low dose treatment is similar to the results obtained by Gary and Garg (1971) and that of Udoh and Kehinde (1991) for rats.

It appears that reproduction in the aquarium was limited to only one female per aquarium in both the control and the low treatment aquaria. This is probably due to limited carrying capacity of each aquarium. The carrying capacity must have been filled immediately when one female spawned and that could have suppressed the urge for further spawning by other female tilapia as discussed by Mair and Little (1991). This will be investigated further in another study in earthen ponds with adequate space.

## Acknowledgements

The authors are grateful to the staff of the Institute of Oceanography Fish Hatchery, University of Calabar, and particularly to Dr. M. Taege who provided the facilities and made useful suggestions. Mr. Albert Ekanem's assistance in tissue processing is greatly appreciated. Miss Patience Jackson is gratefully acknowledged for typing the manuscript.

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