JORMAR 5(1&2) 2008, 15 - 22 ISSN: 1597-3204

Mucuna beans seed as fertility control agent on Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Matured male and female Nile tilapia (*Oreochromis niloticus*) were fed 5.9 and 11.8 g/kg/day of ground mucuna beans seed (*Mucuna urens*) as low and high dose treatment respectively to find out if the treatment could induce sterility. During the 30 days treatment period no spawning occurred in any of the treatments but there was spawning in the control experiment. There was no spawning after the treatments were discontinued; no spawning occurred even when previously treated fish were later stocked (mated) with untreated fish. Both low and high doses induced irreversible sterility in male and female Nile tilapia. Histological sections of the gonads showed that mucuna bean seed acted directly on the gonads to induce sterility by eroding and causing malfunctioning of the germ cells. The treatments combined clumping of cells, destruction of cell membrane, staining, and masking together with eroding of cells to achieve sterility. Eroding effect of the Mucuna treated feed was more pronounced in the high dose treatment where the numbers of cells were greatly reduced. Male and female gonads were similarly affected. The study reveals that mucuna bean seed could be used to induce permanent sterility in Nile tilapia though high doses could be toxic.

INTRODUCTION

The fact that Nile tilapia (*Oreochromis niloticus*) had been introduced into all continents [1] is a testimony to the importance of this food fish in aquaculture. The reason for the widespread culture of this fish is that Nile tilapia is hardy and exhibits most of the desirable qualities of a suitable culture species enumerated by Huet [2]. Fitzsimmons [3] predicted that tilapia is poised to become the biggest aquaculture crop. Tilapia culture is however, hampered by problems of prolific breeding, overpopulation and stunting.

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Nile tilapia can become matured at a size as small as 20 g [4]. Uncontrolled reproduction of this species in ponds leads to the harvest of stunted fish with low commercial value [5].

Mair and Little [4] enumerated various methods for the control of prolific breeding in tilapia but each method has its short-comings. Treatment of tilapia with medicinal plants offers an alternative method. Different plant materials used in promoting sterility include Neem plant (*Azadinachta indica*) which Austin and Short [6] found to reduce pregnancy frequency in mice. African cucumber fruit (*Monordica charantia*) was reported to

disorganize seminiferous tubules in male albino rats, leading to a decrease in sperm production [7]. Papaya seeds (Carica papaya) had been used successfully to induce permanent sterility or reduce litter size in laboratory animals [8,9,10,11]. Mucuna bean seed (*Mucuna urens*) administered orally to mature male guinea pigs caused complete degeneration of sperm in testicular tubules and rendered the animals sterile [12].

Mucuna bean also commonly called horse eye bean is a climbing legume, which belongs to the plant family Papilionaceae. It is found and cultivated in West African rain forest zone stretching from Sierra Leone to Equatorial Guinea [13]. In southern Nigeria mucuna bean seed is an important soup ingredient, mainly used for thickening soup; it contains 20 – 27 % crude protein, 46 % carbohydrate and 18 % lipid [14], while the leaves and stem produce excellent dye. Mucuna bean seed is claimed to cause sterility in men who consume it. This may be true because mucuna bean seed contains a number of alkaloids and alkyl amines notable among which is 5-hydroxytryptamine, important in fertility control [11].

The aim of this study was to determine if mucuna bean seed could induce sterility in Nile tilapia, to verify the mode of action and to find out if the effect is reversible.

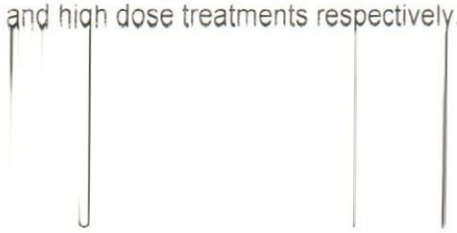
MATERIALS AND METHODS

Matured Nile tilapia used in this study were obtained from the University of Calabar Institute of Oceanography pond and acclimated in aquaria inside the Institute's hatchery where the study was conducted. Acclimation to aquaria conditions lasted for two weeks. During the period, the fish were fed a diet composed of 40.4 % wheat bran, 20.2 % groundnut cake, 20.2 % palm kernel cake, 6.2 % bone meal, 6.0 % blood meal, 6.0 % palm oil and 1 % vitamin premix. This diet is the same as that offered on regular basis to fish in the Institute's hatchery and ponds; it was also used as the control feed. To each kilogram of this control feed, 73.4 g of ground mucuna bean seed was added to prepare the low dose treatment while 146.8 g of mucuna bean seed was added to the same quantity of the control feed components to get the high dose treatment. After mucuna bean seed additions, nutrient contents of the three feeds were balanced using Pearson square method. Each feed obtained contained 28 % protein, 10 % fat and 18 % carbohydrate. Water (1L) was added to 1 kg of the dry ingredients of each feed and mixed properly. The dough prepared 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 Nile tilapia each weighing 40.0(±0.2) g, were stocked in each of nine aquaria. Dimensions of each aquarium used in this experiment were 95 cm x 50 cm x 30 cm and was filled to half its capacity with well-aerated water. The study was conducted in freshwater of temperature 27 ± 1 °C, pH 7, alkalinity of 30 ppm and 6.4 – 6.7 mg/l dissolved oxygen under continuous aeration.

Feeding of the fish commenced a day after stocking and lasted for 30 days. The fish were fed at 8 % of their initial body weight daily for the 30 days. This quantity of feed was divided into two equal parts and used in feeding the fish twice a day, first at 1000 h and then at 1500 h. Three aquaria formed the control experiment

while three aquaria constituted the low dose treatment and the remaining three aquaria constituted the high dose treatment. At that feeding rate the mucuna bean seed dosage was 5.9 g and 11.8 g per kg of fish per day for the low and high dose treatments respectively.



PVC pipes (8 cm dia) 30 cm long (two in number) were placed in each aquarium for the fish to hide in. Each aquarium was cleaned and observed daily as the experiment proceeded. At the end of the 30 days treatment period the fish were weighed and the weights were compared using analysis of variance. A male fish and a female fish from each aquarium of the treatments and the control were sacrificed and the gonads were removed for sectioning and histological examination while the livers, spleen and kidneys were examined for physical damage by the treatment. The remaining fish in each treatment and the control were all fed at the same rate as before with control feed for another 30 days to see if reproduction will occur in any treatment. After 30 days, male from the treated fish were stocked with untreated ripe female; while female from the different treatments were stocked with untreated ripe males. Stocking rate was two males and four female tilapia per aquarium. These and the control were set up in triplicate and fed with control feed. This setup was observed for another 30 days at the end of which the experiment was finally terminated.

The gonads so removed were fixed for 24 h in formal-saline solution made of equal volumes of 10 % formalin and 0.9 % sodium chloride solution. Standard method was followed in pretreatment of the gonads for microtome sections [15]. The microtome sections of the gonads were made at 8mμ thickness and each section was attached to a glass slide using egg albumen. Staining was done with haematoxylin and eosin following the method of Tseng and Chan [16]. The sections were dehydrated by subjecting them to ascending grades of ethanol (50

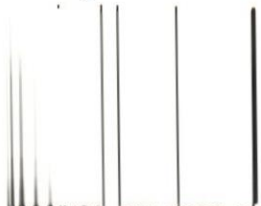
%, 70 % and 90 %) and again to three charges of absolute ethanol before they were cleared in Canada balsam. Each slide of tissue so prepared was mounted on a microscope and microphotographed at 40x magnification. One of the triplicate microphotographs prepared for each sex in each treatment and the control are presented in the result.

RESULTS

There was no spawning in any of the treated aquaria during the treatment and after the treatment had been discontinued, even when the treated fish were mated with untreated fish. In the control experiment, spawning was observed three weeks into the experiment and thereafter spawning continued at regular intervals of three weeks but in each case spawning was limited to one female per aquarium.

The histological section of Nile tilapia testis from the control experiment in Figure 1 illustrates normal and even sperm cell distribution. Figure 2 is a picture of Nile tilapia testis from fish which received low dose treatment of mucuna bean seed, it shows sperms clumping together and erosion of sperms cells in some area. The high dose treatment caused greater clumping of sperm cells and masking by dark stain in Nile tilapia testis section shown in Figure 3. Erosion of cells is more intense in the high dose treatment.

Ovary section of Nile tilapia from the control aquarium in Figure 4 gives a picture of normal egg cells evenly distributed. Microphotograph of histological section of ovary from fish that



received low dose of mucuna bean seed, presented in Figure 5, shows clumping of egg cells, the cells are masked and unevenly distributed. Eroding of cells, masking by stain and uneven distribution of eggs cells is more pronounced in Figure 6 which is a picture of ovary from Nile tilapia that received high dose treatment. Destruction of cell membrane and necrotic cells were also observed in this treatment.

Livers of fish that received high dose mucuna bean seed treatment had a pale colour compared to deep red colour of livers of fish in the low dose treatment and the control experiment. There were no differences in the colouration of kidneys and spleens. Table I shows the analysis of variance of the data obtained.

A significant difference ($P<0.05$) in weights (growth) of fish was found among treatment ($F = 14.29$, 2 and 87df). Duncan's Multiple Range test showed that weight of fish in high dose treatment caused the difference. Mean weights of fish from different treatments at the end of the experiment were as follows: control 43.1 ± 0.80 g; low dose 43.3 ± 0.79 g; high dose 42.27 ± 0.7 g

Table I: Analysis of variance for fish weights (g) in the control, low dose and high dose mucuna bean treatments. (one-way analysis of variance)

CONTROL	LOW DOSE	HIGH DOSE
44	43	42
42	43	43
43	42	42
43	44	43
43	43	42
42	43	42
43	44	42
43	43	43
42	42	41
44	43	43
43	44	42
44	44	43
43	44	42
42	43	41
44	43	42
43	44	42
42	42	41
43	44	43
43	43	42
45	44	44
44	45	43
43	43	42
44	44	43
42	42	41
44	44	43
43	43	43
43	44	42
42	42	41
44	44	43
43	43	42

Source of Variance

	Between Groups	Within Groups	Total
SS	18.02	54.87	72.89
df	2	87	89
VAR	9.01	0.63	
F	14.29		
F-crit	3.10		

Figure 1: Histological session of Nile tilapia testis (x40) from the control experiment showing normal sperm cell distribution

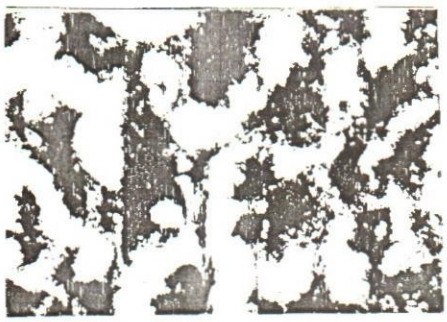


Figure 2: Histological session of Nile tilapia testis (x40) from the low dose mucuna bean treatment showing sperm cell erosion at some points and clumping of sperm cells (without membranes) at other points

Figure 3: Histological session of Nile tilapia testis (x40) from the high dose mucuna bean treatment showing sperm cells, greater clumping and masking of sperm cell without membranes

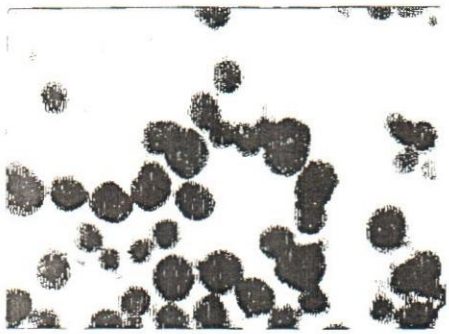


Figure 4: Histological session of Nile tilapia ovary (x40) from the control experiment showing normal egg cell distribution

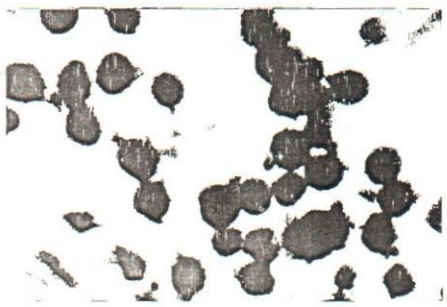


Figure 5: Histological session of Nile tilapia ovary (x40) from the low dose mucuna bean treatment showing clumping, uneven distribution, masking of egg cell at some points and erosion of egg cells at other points

Figure 6: Histological session of Nile tilapia ovary (x40) from the high dose treatment showing greater erosion and masking of egg cells

DISCUSSION

The lack of spawning by fish that received high and low dose treatments even after the treatments had been discontinued for 60 days demonstrated that sterility induced by mucuna bean seed is irreversible even at a dose as low as 5.9 g/kg/day. This is because even at that low dose the active ingredient in mucuna bean seed has the power to erode the cells, cause them to clump together and mask them. In the case of sperm cells the clumping action rendered them immobile and suppressed their fertilizing ability. Cell membranes were destroyed and this made it easier for the cells to get stained. The action of high dose clearly exposed the eroding ability of the active ingredient in mucuna bean seed as bigger egg cells were also eroded. Thus the antifertility agent in mucuna bean seed act directly to induce sterility by causing the cells to malfunction and erosion of cells both in male and female tilapia gonads. This could be compared to the complete degeneration of sperm in testicular tubules of the guinea pig reported by Udoh and Ekpenyong [12].

Spawning of fish in the control experiment, which was limited to a single female fish in each aquarium, may be attributed to limiting carrying capacity of the aquarium. The carrying capacity must have been filled immediately one female fish spawned and that could have suppressed the urge for further spawning by other female tilapia as discussed by Mair and Little [4].

As indicated by Duncan's Multiple Range test, weights (growth) of fish in the high dose treatment were lower than those in the low dose treatment and the control. This was probably due to the toxic effect of the high dose of mucuna bean which manifested in colour change of the liver. The high dose of mucuna beans must have interfered with the metabolism of the fish resulting in slow growth.

Decolouration of the livers of fish from the high dose treatment could have been induced from the presence of some chemical compounds, which, at low dose, can induce sterility in Nile tilapia but at high dose can be damaging to a vital organ, the liver. It is worthy of note that Udoh and Ekpenyong [12] found no toxic side effect on guinea pigs which were treated with mucuna bean seed; this is probably because the dosages they used were very low as it was measured in milligrams. One reason for using such high doses in the present study was to ensure adequate intake of the drug after some had been washed off the feed by water. Added to this is the fact that there was no such previous work on fish to provide guidelines on the adequate quantity of the treatment which could be used in a water environment.

ACKNOWLEDGEMENT

The authors are very 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 offered useful suggestions. We also thank Mr. Albert Ekanem for the help rendered in tissue processing.

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