

Effect of *Mucuna pruriens* Ovo-Testis Histology of Giant African Land Snail (*Archachatina marginata*)

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Target Audience: Snail farmers, Animal Scientist

Abstract

*A study was conducted to evaluate effect of *Mucuna pruriens* on ovo testis histology of giant African land snail (*Archachatina marginata*). Thirty (30) snails with weight range of 100- 150g were used for the study. Ten (10) snails were assigned to each of the three dietary treatments which include: Concentrate (CON), mixture of concentrate and *Mucuna* seed powder (CON + MSP) and *Mucuna* seed powder (MSP) only. At the end of eight (8) weeks, three (3) snails each per treatment were selected and dissected. Ovo testis was harvested from each treatment and processed for histology. Parameters monitored were: ova and spermatozoa formation in ovo testis. Results showed that snails fed with mixture of *Mucuna* seed powder and concentrate had well formed ova but scanty spermatozoa, while those fed with *Mucuna* seed powder only had severe diffuse vacuolar degeneration and necrosis of the spermatozoa. Similarly, those snails fed with concentrate alone followed the same trend with those fed with *mucuna* only. It was concluded that MSP contain substances that could affect spermatozoa formation but support and ova production in right quantity in giant African land snail (*Archachatina marginata*). It was recommended that MSP be processed to further evaluate its potential for spermatogenic activities as professed by different authors.*

Keywords: *Mucuna*, Ovo-testis, Snail, *Archachatina marginata*

Description of Problem

Snails are highly appreciated by Africans and Nigerians in particular for its role as a source of protein and for its traditional usage (1). Currently, there is growing interest in modern way of rearing this animal to break seasonal barriers in other to boost productivity. The biology of this animal has clearly shown centre of reproductive activity to be ovo-testis. Any manipulation that

will assist reproduction must start with the ability to influence ovo-testis activity (2). Recently, different feed types have been tried since this animal had been denied of their natural preference in the wild. It also becomes imperative to also evaluate the effect of feed given under intensive system at cellular level on target organs of reproduction, especially those feed reported to promote reproduction in

other species. The use of *Mucuna pruriens* to influence fertility in human has been reported by (3). Its positive role on testicular measurement, sperm motility, concentration and reduction in sperm abnormality was also reported by (4). If the use of this plant may also have positive influence on ova production, then its incorporation into the feed of this animal will guarantee reproduction during breeding and off season. This study therefore aimed to evaluate the effect of *Mucuna pruriens* on ovo-testis histology of giant African land snail (*A. marginata*).

Materials and Method

Location of Study

The research was carried out at the Snail physiology Research Unit of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture Abeokuta, Ogun State. The location lies within the rainforest belt of West Nigeria, latitude 7°N, longitude 3° 2' E and altitude 76° m.a.s.l. The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 34.7°C and mean relative humidity of 82% (5).

Materials

Thirty (30) *Archachatina marginata* weighing between 100g to 150g were used for this experiment. Fifteen (15) plastic cages with each having a dimension of 30 cm by 40 cm by 24 cm, with small plastic feeding and drinking troughs in each cage were used. Other materials include: Sensitive weighing scale, tape rule, mucuna seed powder, mixture of concentrate and mucuna seed powder in ratio 1:1 v/v, w/w, micropipette and dissecting kit.

Snails and their Management

The plastic cages were cleaned prior to the commencement of the experiment. Snails were fed *ad-libitum* with three types of feed (concentrate, mucuna seed powder and mixture of concentrate and mucuna seed powder). Drinking water was also provided daily *ad-libitum* in drinking troughs. Feed and water troughs were washed daily while the cages were also cleaned daily. Snails were acclimatized for four weeks before the commencement of the experiment. The experiment lasted for eight (8) weeks

Experimental Procedure

The snails were randomly assigned in to three (3) different treatments with 10 replicates for each treatment as follows:

Treatment 1: Concentrate (CON)

Treatment 2: Mucuna seed powder (MSP)

Treatment 3: Concentrate and mucuna seed powder (CON + MSP)

Table 1 below shows the composition of concentrate used in this study:

Table 1: Composition of the concentrate (g/100g)

Ingredients	Quantity (g)
Maize	50
Wheat offal	27.5
Groundnut cake	12
Soy bean meal	4
Bone meal	3
Oyster shell	3
Premix	0.25
Salt	0.25
Total	100

Each 2.5kg of premix contains: Vit. A 10,000,000i.u., Vit D3 2,000,000i.u., Vit. E 20,000i.u., Vit. K 2,250mg, Thiamine B1 1750, Riboflavin B2 5000mg, Pyridoxine B6 2750mg, Niacin 27500mg, Pantothenic acid 7500mg, Vit. B12 15mg, Folic acid 7500mg, Biotin H2 50mg, Cholin Chloride 400gr, Cobalt 200mgr, Copper 5g, Iodine 1.2g, Iron 20gr, Manganese 80g, Selenium 200mg, Zinc 50g, Antioxidant 125g. Recommended inclusion is 2.5kg per tonne of feed.

At the end of eight weeks (8), representative snails per treatment (5) were selected and dissected. Ovo-testis was harvested from the snails and processed for histology. Thereafter, spermatozoa and ova formation were assessed.

Histology

Ovo-testis removed after dissections were fixed in 10 % formalin, dehydrated in series of alcohol (70%, 90% & 100 %), cleared in xylene, embedded in paraffin wax after which the tissue were sectioned (5 mm) and stained with H&E (Hematoxylin and eosin).

Morphological Assessment of Spermatozoa and Ova

Representative slides from each treatment were observed under microscope to assess vacuolar degeneration, arrest of ova development and necrosis of ova. For spermatogenic activity in the acini of ovo-testis, degeneration of germinal epithelium cells and necrosis of germ cells were also assessed.

Statistical Analysis

Data generated from this study were subjected to Least squares analysis of variance using SYSTAT Statistical

Computer Package (6) in a Completely Randomized Design (CRD). Significant treatment means were separated using Duncan multiple range test (7). The statistical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where

Y_{ij} = Dependent variable

μ = Population mean

T_i = Effect of feed types (i=1 - 3)

e_{ij} = Random residual error.

Results and Discussion

Table 2 and 3 shows histopathological changes and severity of lesion in both ova and spermatozoa of giant African land snail (*Archachatina marginata*) fed with different dietary treatments. Table 1 showed that vacuolar degeneration and necrosis of ova was absent in snails fed mixture of MSP and concentrate. This is an indication that MSP contain substances that support oogenic functions if combined with other feed ingredients. It was also observed that levels of severity were more prominent in mucuna seed powder fed snails and those fed concentrate only. The overall effect of MSP on spermatogenesis was high considering degeneration of germinal epithelium cells and necrosis of germ cells in the acini of ovo-testis.

Table 2: Comparative histopathological changes in ova and severity of lesion in the ovo - testis of giant African land snail *Archachatina marginata* fed different dietary treatments.

LESIONS (OVA)	FEED TYPES		
	CON	MSP+CON	MSP
Vacuolar degeneration	+++	-	+++
Arrest of development	++	+	+++
Necrosis of ova	+++	-	+++

KEY

CON= Concentrate, M.S.P= Mucuna seed powder

+: mild ++: more +++: severe ++++: More severe

According to (8), *Mucuna* seed powder has been found to *enhance libido and sexual performance, increase energy levels, improve cholesterol profile and regenerate organs*. Similarly, Reports from (4), (9) who asserted that oral administration of *Mucuna pruriens* seed powder improves spermatogenesis, testicular measurement, motility, concentration and plasma testosterone are completely different from the observation of this study. The acini of the ovo-testis responsible for both ova and

spermatozoa productions were severely affected via vacuolar degeneration and necrosis in the ovo-testis. Similar effects were also seen in those snails fed with concentrate only as well. The reason for this observation may be as a result of anti-nutritional compound which have been reported to reduce nitrogen retention, growth, mineral and protein availability (10) which may affect the normal spermatogenic activities and normal oval formation when concentration is high especially in an unprocessed mucuna seed powder.

Table 3: Comparative histopathological changes in spermatozoa and severity of lesion in the ovo -testis of giant African land snail *Archachatinamarginata* fed with different dietary treatments.

LESIONS (SPERMATOZOA)	DIETARY TREATMENTS		
	CON	MSP+CON	MSP
Absence of spermatogenesis	++	++	++++
Degeneration of the germinal epithelium cells	+++	++	+++
Necrosis of germ cells	++	+	++

KEY

CON= Concentrate, M.S.P= Mucuna seed powder

+: mild, ++ : more, +++: severe

Plate 1-6 showed the effect of dietary treatment on histology of ovo-testis considering ova and spermatozoa formation. Snails fed with concentrate had severe degeneration and necrosis of ova and also show loss of spermatogonia cells (Figure 1 and 2) while those fed with mixture of mucuna seed powder and concentrate had few spermatozoa and spermatids i.e few spermatogenesis, but had a well formed ova (Figure 3 and 4) while those fed with mucuna seed powder only had diffuse severe degeneration and necrosis of the germ cells of the seminiferous tubules and presence of multinucleated germ cells of the seminiferous tubules in the spermatozoa. Observation made in the

acini producing sperm cells may be as a result of toxic effect of unprocessed mucuna used in this study. This observation further confirm the gonadotoxic effects of some anti-nutritional factors present in this seed examples of which are trypsin and chymotrypsin-inhibiting activities that decrease protein digestibility and nitrogen retention and thus reduced growth (11). It also contains phytate that may reduce mineral and protein availability. The morphological hallmark of apoptosis which have been detected in spermatogonia and primary spermatocytes might primarily result from mitotic arrest due to toxic effect of this plant. However, absence of vacuolar

degeneration and necrosis of ova in those snails fed mixture of MSP and concentrate is a further pointer that MSP contain substances that have potential to

influence positively oogenic activity in this animal and if well processed to eliminate anti-nutritional factor, it is hopeful that it may influence reproduction.

++++ : More severe

Plate 1: Severe degeneration and necrosis of the ova

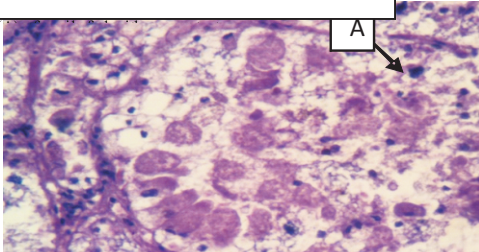


Plate 2: lost Spermatogonia, spermatozoa (A) cell in the acini

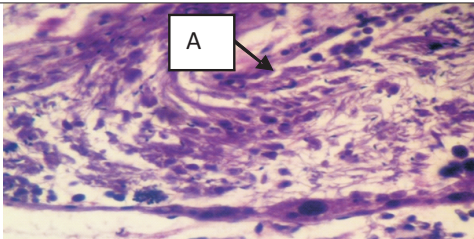


Plate 3: Well-formed ova (A) in ovo-testis of snail fed with mixture of mucuna seed powder and concentrate

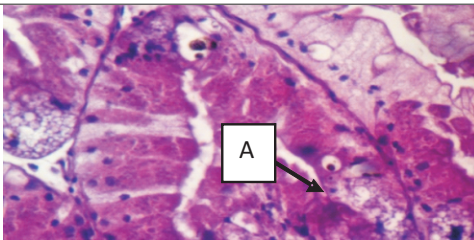


Plate 4: Few spermatozoa (A) and spermatogonia cells (B) in the ovo-testis of snails fed with mixture of mucuna seed and powder and concentrate.

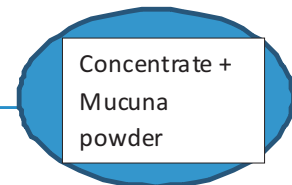
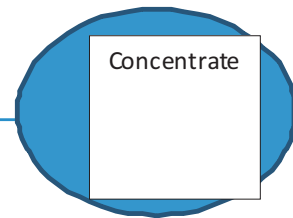
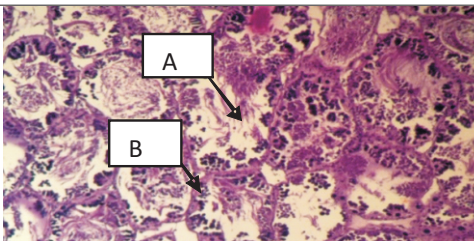


Plate 5: Severe diffuse vacuolar degeneration and necrosis of the ova in the ovo-testis of snails fed with mucuna seed powder alone

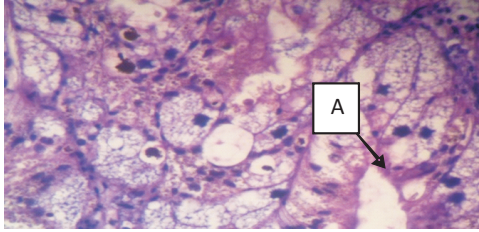
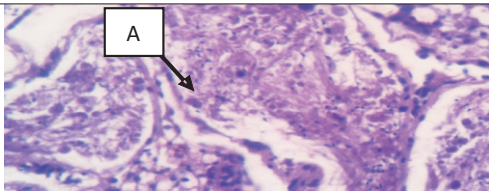


Plate 6: Degeneration and necrosis of germ cells in the acini of ovo-testis of snails fed mucuna seed powder alone



Mucuna powder alone

Conclusion and Application

The morphological alterations observed in spermatozoa formation as a result of *Mucuna pruriens* seed powder usage provides reasonable evidence that

1. Raw mucuna seed powder contain substances that could alter normal spermatogenic activities in ovo-testis which is a key organ in molluscan reproduction.
2. However, MSP plays supportive role in ova production.
3. Hence, processed seed powder of this plant at varying levels should be tried in further studies to justify various positive roles professed by different authors on spermatozoa formation.

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