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EFFECT OF FEED TYPE ON GROWTH, SPERMATOZOA PRODUCTION AND GONADO-SOMATIC INDEX IN GIANT AFRICAN LAND SNAIL (*Archachatina marginata)*

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ABSTRACT

A study was conducted on the effect of feed type on growth, spermatozoa production and gonado-somatic index in giant African land snail *(Archachatina marginata)*. Thirty snails (30) of weight range of 150 - 180g were used for this study. Ten snails (10) were assigned to each of the three feed types which included: Poultry layers mash (PLM), dried Pawpaw leaves (PL) and mixture of both Pawpaw leaves and Poultry layers mash (PLM+PL). Weight gain was monitored weekly. At the end of seven weeks, snails were dissected; visceral weight and ovo-testis were measured. Five (5) snails each per treatment were used for both histology and spermatozoa concentration estimation. Ovo-testis was processed for histology while little hermaphrodite duct was used for spermatozoa concentration. Results showed that feed type had significant ($P<0.001$) effect on sperm concentration. Snails fed on PL had higher sperm concentration, followed by those fed on PL+PLM while the least was found in PLM group. For live weight gain, those fed with PLM had the highest weight followed by those fed with PL+PLM while PL had least weight gain. Results for gonado-somatic index showed that PL had the highest means while those fed on PLM and PL+PLM were not significantly different from each other. It was also found that snails fed with PL had high spermatogenic activity in the acini of snail ovo-testis while those fed with PLM+PL had medium activity and those fed with PLM had the least activity. The findings from this study suggest that pawpaw leaves have agents which promote spermatogenesis and can therefore be used as diet for breeding snails to enhance spermatogenesis.

Keywords: Morphology, Haemocyte, *Archachatina marginata, Achatina achatina*, Haemolymph, African land Snail, Feed type

INTRODUCTION

The need for intensive rearing of snails for both private and commercial purposes has necessitated the quest for good quality feed which can enhance reproduction of this animal in captive. This challenge requires better understanding of the biology and especially that of growth and reproduction (Leahy, 1984). Growth and reproduction are directly influenced by diet (Raut *et al*., 1992; Furtado *et al*., 2002). Energy content and nutritional value in the diet influence growth rate and reproductive success in molluscs (Thomas *et al*., 1983; Mcshane *et al*., 1994;

Foster *et al*., 1999). In the wild, the animal is open to varieties of feeds which can benefit or harm them, but they select those preferable to them, although effect of temperature among other factors may also influence their reproductive activity (Lusis, 1966; Imhof, 1973; Sokolove and McCrone, 1978; Price, 1979; Bohlken and Joosse, 1982; Sokolove *et al*., 1984; Dogterom *et al*., 1984; Joosse, 1984). In captive rearing, the animals have no choice but to consume which ever feed given to them. At times, these feeds may promote their growth, but have negative effect on their reproductive apparatus/organs. Ajayi *et al* (1978) observed that some food given to this animal may lead to a retrogressive growth, while some may cause some pathological conditions. As such, replication may be very difficult. The reason for such reproductive delay may not be understood if conducive environments are provided for the animal. It therefore becomes very important that feed given to intensively raised animal especially snails in which not much work has been done on the standardization of their feed be given proper evaluation before any feed is given to them. This study was therefore designed to determine the effect of feed type on spermatozoa production in giant African land snail (*Archachatina marginata*).

MATERIALS AND METHODS

Experimental Area

The research was carried out at the Snail Physiology Research Unit of the College of Animal Science and Livestock Production and Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, Ogun State. The location lies within the rainforest belt of Western Nigeria, latitude 7 ^{o}N, longitude 3 ^{o} 2' E and Altitude 76 meter above sea level (m.a.s.l). The climate is humid with a mean annual rainfall of 1,037 mm, mean temperature of 34.7 ^{o}C and mean relative humidity of 82 % (Google earth 2010).

Materials

A total of thirty (30) *Archachatina marginata* snails weighing between 150g to 180g were used for this experiment. Fifteen (15) plastic cages, each of dimension 30 cm by 40 cm by 24 cm, with small plastic feeding and drinking troughs in each cage, Sensitive weighing scale, tape rule, layers mash, dried pawpaw leaves, mixture of dried pawpaw leave and poultry layers mash in ratio 50:50 w/w, micropipette, dissecting kit and haemocytometer were used for this study.

Snails and their management

The plastic cages were cleaned prior to the commencement of the experiment, two weeks was set aside as a period of acclimatization. The snails were fed *ad libitum* with the three types of feed provided. 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. The experiment lasted for seven (7) weeks.

Experimental Procedure

The snails were randomly assigned into three (3) different treatments with 10 replicates for each treatment, making a total of thirty snails (30).

Treatment 1: Dried pawpaw leaves (PL)
Treatment 2: Poultry layers mash (PLM)
Treatment 3: Pawpaw leaves and Poultry layers mash (PL + PLM)

At the end of the 7th week, the snails were weighed and dissected. Ovo-testis and little hermaphrodite duct were removed. Little hermaphrodite ducts removed were used for spermatozoa PLM centration estimation while ovo-testis were preserved for histol-

ogy.

Gonado-somatic index

After dissection, visceral organ and ovo-testis were weighed separately. Ovo-testis weight was expressed as proportion of visceral organ weight and multiplied by 100 according to Barber and Blake (1983). The formula is shown below:

$$\textbf{GSI} = \frac{\text{Ovo-testis weight}}{\text{Visceral mass}} \times 100$$

Determination of Spermatozoa concentration

Six snails were selected per treatment for sperm concentration determination making a total of 18 snails. After dissection, little hermaphrodite duct were removed and homogenised in 1ml of normal saline. A dilution of 1:19 was made with the aid of formalin-bicarbonate solution after which loading onto improved haemocytometer was carried out. Sperm cells found in four squares were counted. Thereafter, numbers of cells counted were multiplied by a conversion factor (50,000) to obtain the sperm concentration.

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).

Statistical Analysis

The data generated from this study were subjected to Least squares analysis of variance using SYSTAT statistical computer package (SYSTAT, 1992) in a Completely Randomized Design (CRD). Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez, 1985). The statistical model used was:

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

Where

Y_{ij}	=	Dependent variable
μ	=	Population mean
T_i	=	Effect of feed types (i=1 - 3)
$å_{Ij}$	=	Random residual error.

RESULTS AND DISCUSSION

The results of least-square mean showing the effect of feed type on spermatozoa concentration is shown in Table 1. Feed types had significant effect on spermatozoa concentration. Pawpaw leaves (PL) had the highest number of sperm concentration (6.625 **±** 0.726), while both mixture of dried pawpaw leaves and Poultry layers mash (PL + PLM) and Poultry layers mash were not significantly different from each other (3.500 **±** 0.726; 2.238**±** 0.726). This observation is an indication that ovo-testis activity is influenced positively by pawpaw leaves (Dried and milled), which negate the report of Oyekunle and Omope (2010) who observed reduced spermatozoa count after administration of aqueous extract of *Carica papaya* leaf to Wistar rat. The reason for this disparity in observation may be as a result of differences in the processing methods used in the leaf preparation. Also there may be slight differences in metabolic pathway of this animal as compared to the rat which is a mammal.

Table 1: Least-square means showing effect of feed type on spermatozoa PLM centration in giant land snail (*Archachatina marginata*)

Feed type	Sperm centration (x10^6)	SEM (±)
Pawpaw leaves (PL)	6.625a	0.726
Poultry layers mash (PLM)	2.238b	0.726
Pawpaw leaves and Poultry layers mash (PL + PLM)	3.500b	0.726

[ab] Means in the same column with different superscripts differ significantly

That substance which alters spermatogenesis in rat may be altered during processing via chemical changes and may support spermatozoa production in snails after elimination via processing method. The reports of some authors have shown therapeutic potential of this plant (Lohiya *et al*., 1992; Ghost *et al*., 1998). Figures 1-3 show effect of different feed types on the histology of the ovo-testis. Snails fed with pawpaw leaves showed very good spermatozoa formation compared to mixture of pawpaw leaves and poultry layers mash. This observation further negates the report of Oyekunle and Omope (2010) who reported marked degeneration of seminiferous tubule epithelium after administration of aqueous extract of pawpaw leaf. Processing methods as earlier mentioned could also be the reason for this observation.

Table 2 shows the least-squares means effect of feed types on weight gain of giant African land snail (*Archachatina marginata*). Out of the three feed types used, poultry layers mash (PLM) had the highest means (6.303 ± 0.72), followed by the mixture of pawpaw leaves and poultry layers mash (PL + PLM) (3.680 ± 0.72) while pawpaw leaves (PL) (1.497±0.72) recorded least. The result on feed types which showed weight gain to be more in poultry layers mash and least in pawpaw leave is an indication that poultry layers mash is richer in protein, energy and minerals which are required for growth in the body of animal compared to both the mixture and pawpaw leave alone. Study carried out by Osinowo *et al*. (2007) on growth rate of *Archachatina marginata* fed both poultry layers mash and mixture of pawpaw leave showed that appreciable growth was recorded in both feed types.

Reports by several authors have also showed that energy content, nutritional value, texture and structure as well as digestibility and absorption of items in the diet influence growth rates and reproduction in molluscs (Thomas *et al*., 1983; Mcshane *et al*., 1994; Foster *et al*., 1999). The report of Bessa and Araujo (1996) who asserted that artificial diet favours growth and reproduction contradicts the observation made in this study. However, the report of Ribas (1986) who observed that artificial diets are much better than plant diets to increase growth in culture snails is in line with the result of this study. Report of Gomot *et al.* (1989) who indicated that diet based on green vegetables do not ensure a good growth rate of terrestrial snails

also corroborates the findings of this study. This observation may be as a result of low energy and protein present in the vegetable based diet.

Fig 1: PL

Fig 2: PLM+PL

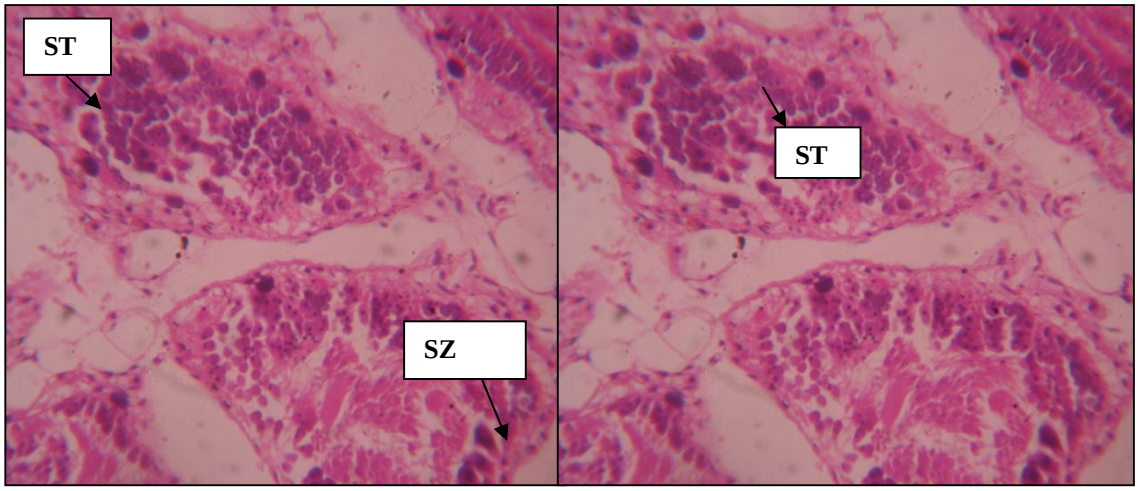

Fig 3: PLM

ST: Spermatogonia cell in the acini of giant African land snail (*Archachatina marginata*) ovo-testis

SZ: Spermatozoa cell in the acini of giant African land snail (*Archachatina marginata*) ovo-testis

EM: Empty acini of giant African land snail (*Archachatina marginata)*

Table 2: Least-square means showing effect of feed types on weight gain of *Archachatina marginata*

Feed type	Weight gain	SEM (±)
Pawpaw leaves (PL)	1.497c	0.72
Poultry layers mash (PLM)	6.303a	0.72
Pawpaw leaves and Poultry layers mash (PL + PLM)	3.680b	0.72

abc Means in the same column with different superscripts differ significantly

The results of Least-square means showing effect of feed type on gonadosomatic index (GSI) in giant African land snail (*Archachatina marginata*) is presented in Table 3. Feed types had significant effect (P<0.01) on gonadosomatic index. The results revealed that pawpaw leaves recorded the highest means for GSI, while mixture of poultry layers mash and pawpaw leave together with poultry layers mash alone were not significantly different from each other.

Table 3: Least-square means showing effect of feed type on gonadosomatic index (GSI) in giant *Archachatina marginata*

Feed types	GSI	SEM (±)
Pawpaw leaves (PL)	0.086a	0.004
Poultry layers mash (PLM)	0.072b	0.004
Pawpaw leave and Poultry layers mash (PL + PLM)	0.066b	0.004

abc Means in the same column with different superscripts differ significantly

A decrease in testicular weight and GSI is a better way to assess the damage to testes in relation to its spermatogenic activity and hormone production (Simanainen *et al*., 2008). Also, in the absence of any known pathology, testis weight in mammal is highly related to daily sperm production (Zindy *et al*., 2001). The role played by pawpaw leaves in boosting the GSI of those snails fed with pawpaw leaves is a clear indication that it contains substance/substances which promote the development and activity of ovotestis thus enhancing the GSI compared to those fed with mixture of pawpaw leave and poultry layers mash and also poultry layers mash alone. Yusha'u *et al*. (2009) reported that extract gotten from pawpaw leaves was confirmed to contain steroids. Values recorded for spermatozoa concentration in this study is a further proof of enhancing

ability of pawpaw leaves in this study. Although, several medicinal roles have been attributed to the extract of pawpaw leaves (Ranasinghe *et al*., 2012). Role played in the boosting of spermatozoa concentration in this study may an additive function of its content.

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