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EFFECTS OF AESTIVATION DURATION ON REPRODUCTIVE TRACT DIMENSION AND SPERMATOZOA PRODUCTION OF GIANT AFRICAN LAND SNAIL (*Archachatinamarginata*) DURING DRY SEASON

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ABSTRACT

Aestivation is a process of metabolic suspension which plays a key role in the survival of land snails during a period of drought. Human shave also used this process to transport the animal for commercial purpose. To examine the effect of this physiological process on reproductive apparatus, thirty (30) Giant African Land snail (*Archachatinamarginata*) with average weight of 130 -150g were used for this study. The snails were allotted to three different treatments with ten (10) replicate each after four weeks of acclimatization period which include: zero (0) week, three (3) weeks and six (6) weeks of aestivation. Parameters measured were: Internal organ weight, ovo-testis weight, reproductive tract length (cm) and weight (g). Also gonado-somatic index and spermatozoa concentration were also determined. Results showed that aestivation duration had significant influence on organ weight, ovo-testis weight, reproductive tract length and weight and spermatozoa concentration. However, gonado-somatic index (GSI) was not significantly influenced. It was observed that organ weight decreased as aestivation duration increased. For ovo-testis, snails with aestivation duration of six weeks had smaller weight compared to the control (0 week) while those at three weeks aestivation duration were not significantly different from the control group and those of six weeks aestivation duration. For reproductive tract weight, there was a significant($P<0.01$)decrease in weight of the tract for both snails under three and six weeks aestivation duration compared to the control group (0 week). Spermatozoa count also decreased as aestivation duration increased. It can therefore be concluded that aestivation duration had significant effect on organ weight, ovo-testis weight, reproductive tract weight and spermatozoa concentration. For quick target of reproduction and snail economic value, it is recommended that aestivation duration should not be allowed to reach three weeks and above for whatever reason.

Keywords: Aestivation duration, reproductive tract, spermatozoa count, Giant African Land snail, dry season

INTRODUCTION

An understanding of reproductive process is central to the management of a species (Barber and Blake, 1983). This is because information provided on the recruitment and population dynamics of the species is central to effective planning of production process. Understanding the reproductive cycle of a species is also a requirement for the successful cultivation of the species (Barber

and Blake 1983). In snail production, moisture availability is the key climatic variable needed for effective production while dry season constitute a serious bottle neck (Michael, 2011).

Dry season is characterized by high temperature which reduced feed consumption and general activity of snail. As such, metabolic activities are thus affected which in turn affect the growth rate of the animal. It is not also impossible that the immune status of the animal are also compromised (Hermes-Lima and Storey, 1998; Nowakowska*et al*. 2009; AL-Rawadeh, 2010). On the long run, this may also lead to mortality which may deplete the population of stock. Cumulative effects may lead to reduction in the economic value of snail during this period if effective steps are not taken. Prominent among the effect of this period is the hindrance to reproduction of the animal (Abdussamad*et al*., 2010; Omoyakhi and Osinowo, 2010).

During this period, egg production is affected (TerMaat*et al*., 1989). But information on spermatozoa production is scarce. However, lack of adequate care during this period may make the animals go into aestivation which results to metabolic suspension (Storey and Storey, 2012; Guppy, 2004; Withers and Cooper, 2010). The implication of this mechanism is that the available energy reserve will be used for the sustenance of life via physiological activities. But duration of aestivation mayalso have relationship with reproduction, especially when this period is closer to raining season as period of recovery may take appreciable period of time for the system to be set for reproductive activity. Energy budget for reproductive activity may also be affected during aestivation since metabolic activity is suspended. It is therefore necessary to evaluate effect of aestivation on reproductive apparatus of giant African land snail (*Archachatinamarginata*) during dry season in order to pre-empt how to bring to an end on time the impending danger of this physiological mechanism.

MATERIALS AND METHODS

Experimental site

The research work was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Abeokuta, Nigeria. Abeokuta lies within the Rain forest vegetation zone of Western Nigeria on latitude 7° 10'N, longitude 3° 2'E and altitude 76 m above sea level. The climate is humid with a mean annual rainfall of 1,037 mm, an average temperature of 34.7°C and an imminent average relative humidity of 82% throughout the year.

Snails and their management

A total of 30 *Archachatinamarginata*weighing 130 -150g were used for this study. The snails were kept in plastic cages of dimension 24 x 30 x 40 cm. The plastic cages were clean prior to the commencement of the experiment, two weeks was set as a period of acclimatization. The snails were fed *ad libitum* with layers mash. Drinking water was provided daily *ad libitum* in drinking water troughs. Both feeding and water troughs were washed daily with clean water. All these were done before the period of aestivation. The experimented lasted for 8 weeks.

Experimental Procedure

Acclimatization

The cages were prepared and each cage was assigned a plastic trough, a drinker and a feeder. The weights of the snails (initial) were measured in grams using an electronic weighing scale (Mettler, Type BD 6000, Met-

tler- Toledo AG Switzerland). Feed (layers mash) and water were provided in the first two week to acclimatize the animal to the new environment different from their original abode.

The snails we

re randomly assigned into three (3) different treatments with 10 replicates for each treatment. Treatment allocation is shown below:
Treatment 1: Control (0 week)
Treatment 2: 3 weeks
Treatment 3: 6 weeks

Induction of aestivation

At the end of the two-week acclimatization period, feed and water were withdrawn from all the treatment snails to induce aestivation. Also, the control group was sacrificed immediately.

Epiphragm Formation

After withdrawal of feed and water within 7 -9 days, appearance of whitish calcareous substance was observed which sealed up the aperture of the shell. This was noticed to signal the commencement of aestivation. Care was also taken not to disrupt already formed epiphragm.

Reproductive tract dimension

Snails were weighed and dissected at week zero (control) 3 weeks and 6 weeks during the period of aestivation. Reproductive tract organs which include: Organ weight, Ovotestis weight, tract length and tract weight were weighed and measured. Organ weight was estimated as whole visceral organ after shell removal.

Gonado-somatic index

Gonado-somatic index(GSI) was evaluated according to Barber and Blake(1983) as ratio of gonad weight to total tissue weight. This is represented by the formulae below:

$$\textbf{GSI} = \frac{\text{Gonad weight}}{\text{Total tissue weight}} \times 100$$

Sperm concentration determination

Six snails per treatment were selected for spermatozoa concentration. A total of eighteen animals were used. After dissection, little hermaphrodite duct were removed and mashed in 1 ml of normal saline. A dilution of 1:19 was made with the aid of formalin – bi carbonate solution after which loading into improvedhaemocytometer was carried out and reading was carried out from the four squares. Thereafter, cell count was multiplied by a conversion factor of 50,000 to get the sperm concentration.

Histology of ovo-testis

Ovo-testis was harvested before and duringaestivation at 0, 3 and 6 weeks. Thereafter, dissected ovo-testis 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 analyses

The data generated from this study were subjected to least square analysis of variance using the SYSTAT Statistical Package (SYSTAT, 1992) in a completely randomized block design (CRBD). Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez 1985). The statistical model used was:

$Y_{IJ} = \mu + T_I + \sum_{IJ}$

Where

Y_{IJ} = dependent variable
μ = population mean
T_I = effect of aestivation condition (I = 1-3)
$\sum_{IJ}$ = random error

RESULTS

The result of analysis of variance showing the effect of duration of aestivation on organ weight, ovo-testis weight, tract length, tract weight, gonado-somatic index (GSI) and spermatozoa count of *Archachatinamarginata*during dry season is shown in Table 1. Aestivation duration had significant effect on organ weight (P<0.001), ovo-testis weight (P<0.01), tract weight (P<0.01) and spermatozoa count (P<0.01). However, tract weight and gonado-somatic index (GSI) were not significantly affected (P>0.05). For organ weight (Table 2), there was a decrease in weight as the duration of aestivation increased. Organ weight of snails under control group had the highest organ weight followed by those with three weeks of aestivation, while those groups that fall into six weeks aestivation period had the least organ weight.

Table 1: Analysis of variance showing the effect of duration of aestivation on organ weight,ovo-testis weight, tract length, tract weight, GSI and sperm count of *Archachatinamarginata*

Source	DF	Organ weight (g)	Ovo-testis weight (g)	Tract length (cm)	Tract weight (g)	GSI	Sperm count (x 106
AESTDUR	2	1445.39***	3.93**	30.94	95.68**	0.00	.250313E+15**
ERROR	15	58.19	0.75	9.49	7.48	0.00	.229613E+14

**P<0.01; ***P<0.001
AESTDUR: aestivation duration

Table 2: Effect of aestivation duration on Organ weight of *Archachatinamarginata*

Aestivation Duration (Weeks)	Internal organ weight (g)
0.00	90.88±3.114a
3.00	66.98±3.114b
6.00	61.77±3.114c

Means on the same column having different superscript differ significantly (P<0.001)

Effect of aestivation duration on reproductive tract dimension of *Archachatinamarginata*during dry season is shown in Table 3. For ovo-testis, the animal under control group were not significantly different (P>0.05) from those under three weeks aestivation in terms of ovo-testis weight but were different significantly from those under six weeks duration of aestivation. Considering the tract weight, those snails under the control group were significantly (P<0.05) higher compared to those under three and six weeks aestivation duration.

Table 3: Effect of aestivation duration on reproductive tract dimension of *Archachatinamarginata*during dry season

Aestivation Duration (weeks)	Reproductive apparatus dimension		
	Ovo-testisweight (g)	Tract length (cm)	Tract weight (g)
0.00	5.900±0.352a	21.583±1.258	16.917±1.117a
3.00	5.283±0.352ab	18.800±1.258	10.500±1.117b
6.00	4.295±0.352b	17.083±1.258	9.590±1.117b

Means on the same column having different superscript differ significantly (P<0.01; P<0.001)

Table 4: Effect of aestivation duration on gonado-somatic index and spermatozoa count of Giant African Land snail (*Archachatinamarginata*) during dry season

Aestivation Duration (weeks)	GSI	SPERM COUNT (x 106)
0.00	0.065±0.006	169875E+08a
3.00	0.080±0.006	8625000b
6.00	0.071±0.006	1175000c

Means on the same column having different superscript differ significantly (P<0.01)

Table 4 shows the effect of aestivation duration on gonado-somatic index and spermatozoa count of giant African land snail (*Archachatinamarginata)* during the dry season. It was obvious from the table that spermatozoa count decreased with increase in aestivation duration. The snails under control group had the highest spermatozoa count; followed by groups with three weeks aestivation duration while those group under six weeks aestivation duration had the least spermatozoa count. Figures 1, 2, and 3 showed histopathological effect of aestivation on Giant African Land snail (*Archachatinamarginata*). Figure 1 shows the normal architecture of ovo-testis for the control group (0, aestivation duration) with normal spermatogenic activities in the acini of the ovo-testis. For those groups of snails undergoing three weeks (3 wks) aestivation, degenerated spermatocytes were observed in the acini of the ovo-testis (Figure 2). Figure 3 shows section of the acini of Ovo-testis of snails undergoing six weeks (6 wks) aestivation. Hypoplasia of the germinal cells was observed in the acini of the ovo-testis. Degenerated spermatocyte seen in the acini of the ovo-testis of snails undergoing three weeks aestivation and hypoplasia of the germinal cells observed among snails undergoing six weeks aestivation were clear indication that spermatogenesis were disrupted during extended period of aestivation with increase in levels of severity of negative effect caused by this process.

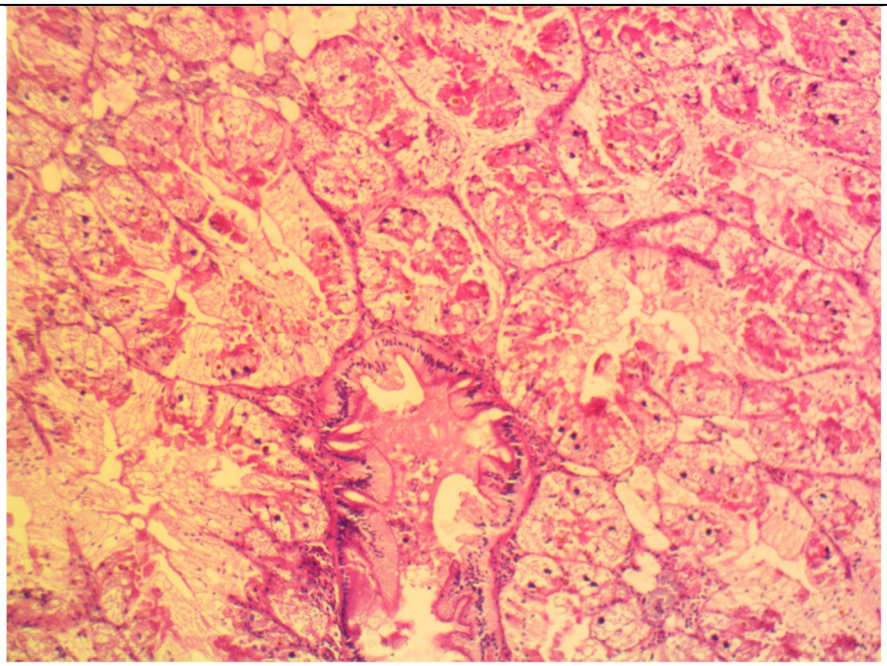

Figure 1: Section of the Ovo-testis of snail from control (none aestivated) showing normal spermatogenesis in the acini of the ovo-testis (x100; H&E)

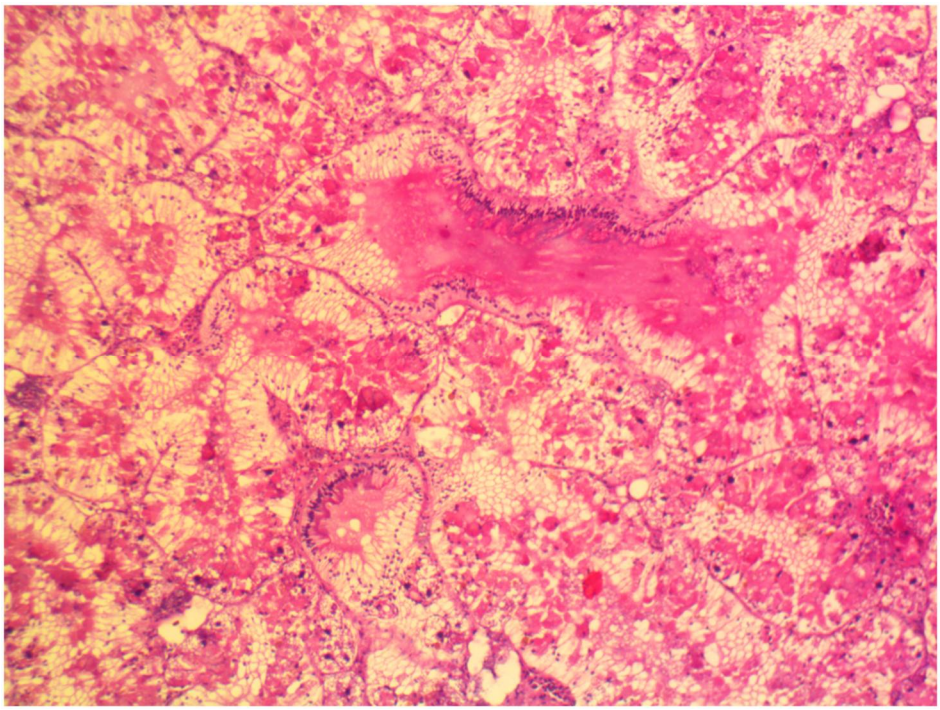

Figure 2: Section of the Ovo-testis of snail undergoing threeweeks aestivation showing degenerated spermatocyte in the acini of the ovo-testis (x100; H & E)

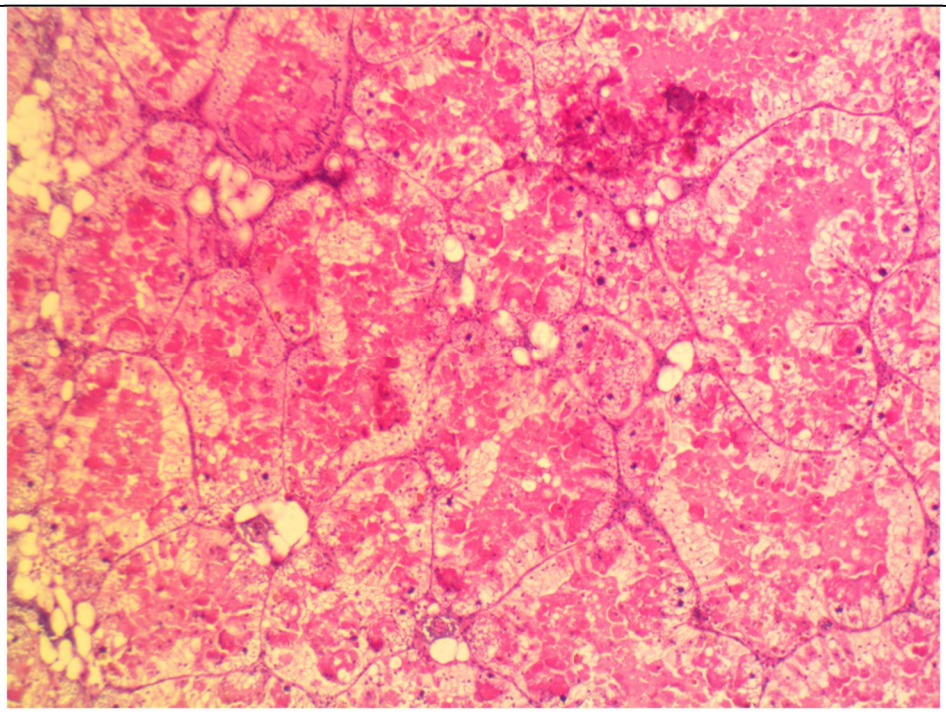

Figure 3: Section of the acini of Ovo-testis region of snail undergoing six weeks aestivationshowing marked hypoplasia of the germinal cells (arrow) (x100; H & E)

DISCUSSION

Decrease in organ weight of snail after the zero week of aestivation through three weeks to six weeks is a reflection of body weight loss which is a common phenomenon in aestivation. Rahman and Raut (2012) opined that body weight of snail reduced as high as 50.33 percent during aestivation. The result of this study showed differences in organ weight as week progressed. The extents of weight loss depend on the period of aestivation (Omoyakhi and Osinowo, 2010). Reduction in body weight will eventually lead to reduction in organ weight since viscera organs are subset of body weight of the animal. Reduction in the weight of ovo-testis at six weeks of aestivation compared to the control and three weeks of aestivation is an indication that food and water deprivation during this period had led to reduction in weight of this vital organ which is responsible for both spermatozoa and ova production. Tamburi and Martin (2011) had also established negative effect of starvation/aestivation on reproductive output in which ovo-testis is the key organ. This key organ is also known to reduce in weight during starvation (Vega *et al*., 2006). Similarly, reduction in reproductive tract length may also be linked to occurrenceof reproductive tracts involution during this period as reported by Marijk (1973). Marijk (1973) also reported that albumen gland, spermduct and prostate gland showed more pronounced decrease in weight among other organs of the reproductive tract. Histological observation of various organ of the reproductive tract during aestivation further confirmed reduction in epithelial heights of the accessory sex glands and ultrastructural ob-

servations (Marijk, 1973). Spermatozoa count during period of aestivation in this study decreased as aestivation period increased. The control was shown to have the highest count with subsequent decrease as period of aestivation increased.

The reason for this observation is that aestivation/starvation is known to affect reproductive function as nutrition supplies the metabolites required to produce spermatozoa (Cameron and Nosbisch, 1991; Rodjmark, 1987). It was also reported that hormones regulating reproduction and growth especially those of the hypothalamic -pituitary-gonadal axis are seriously affected (Steiner *et al*., 2003) during periods of starvation.

Reduction in sperm count explains the previous decrease seen in ovo-testis weight which is an indication that aestivation/ starvation inhibit spermatogenesis. This observation is line with the findings of Gonzale, *et al*,(2004) which showed that starvation inhibit gonadotropins. Similarly, starvation had also been reported to increase sperm abnormality and decrease volume of semen in goat (Olusola*et al*., 2003). Marc (1984) also reported that starvation limit spermatogenesis in *Biomphalariapfeifferi* and *B. glabrata*i.e two snail species. It was further revealed that further developmental stages were prevented, Spermatogenesis is limited to spermatogoniain *B.glabrata* while they were limited to spermatocytes in *B.pfeifferi* (March, 1984). Arrest of germ cell growth and hypoplasia observed in this study may be as a result of cell injury due to oxidative stress (Christian *et al*., 2014).

CONCLUSION

Aestivation as a condition of metabolic suspension is known to be a useful process in the transportation of the animal for commercial purpose, however, prolonged periods of this process may compromise the development of organ weight, reproductive tract dimension and spermatozoa production. However, it therefore becomes important to provide enabling environment to break this process during dry season in order to boost production of this animal especially when breeding is targeted very close to this period.

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