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EFFECT OF SHELL INJURY ON HAEMOCYTE CONCENTRATION AND SHELL REGROWTH OF GIANT AFRICAN LAND SNAIL (*ARCHACHATINA MARGINATA*)

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Abstract

The effect of shell injury on growth and haemocyte concentration were evaluated in this study. Thirty-two (32) snails between 130-180g were randomly divided into four (4) treatments with eight (8) replicate each. The four treatments include: T1 (control), T2, (1 cm shell damage) T3 (2 cm shell damage) and T4 (3 cm shell damage). Haemolymph was collected on weekly basis for four weeks. Parameters monitored were total haemocyte count and shell growth. Result showed that shell injury/damage had significant effect (P<0.001) on total haemocyte count and shell growth. It can be concluded from this study that shell injury had influence on immune response of the animal, although compensatory growth was recorded after week four of the experiment. It can be recommended from this study that irrespective of level of shell damage used in this study, adequate attention should be given not to kill the animal as the process compromise the total haemocyte count which is responsible for immune defense of the animal. It is therefore recommended that adequate care and proper hygiene must be maintained in other not allow opportunistic infection since immune cells (haemocytes) are compromised in other not cause economic loss due to unforeseen mortality.

Key words*: Archachatina marginata, haemocyte, Land Snail, Shell growth, Shell injury.*

INTRODUCTION

Land snails generally have shell which protects them from physical damage, predators and dehydration (Ademolu et al., 2015). Similarly, the shell housed the animal especially during unfavorable condition. The shells are twisted into spiral level known as whorls. The whorls are largest at the base and each one gets progressively smaller as it gets to the tip, known as the apex. The snail shell has a large opening called aperture (Adamowicz and Bolaczek, 2003). Due to current trend of intensive rearing of snails to meet up with demand, there is need for cage culture or semi-intensive rearing of this animal. During intensive rearing, snails at times try to escape from their rearing vicinity and thus fall off from some height and as such break their shell. This occurrence put snails at a great danger depending on the site of injury. It could also lead to haemolymph loss which may result to death of this animal if such injury is much. In most occasions, the damage to shell calls for the process of healing which require regrowth of the damaged part and this may be energy demanding and costly (Jonathan, 1990). Studies have also shown that wound healing process requires the activity of macrophages which promote angiogenesis and collagen formation (Leibovich and Ross, 1975; Polverini et al., 1977; Hunt et al., 1984; Kovacs and DiPietro, 1994).

For invertebrate like mollusks, shell formation is known to be a complex process which involves deposition of both organic and inorganic materials (Wilbur, 1983).

The shell formation process comprises of shell mineralization known to be in succession of compartments (Crenshaw, 1972: Saleuddin and Petit, 1983).

The first to be reckoned with is the mantle cavity which secrete the molecules that form the shell, followed by periostracum (with mostly organic layer) and the extrapallial cavity into which the outer fold epithelium secretes calcifying mixture of proteins, glycoproteins and calcium carbonate ($CaCO_3$) (Mutvei, 1980; Fenget al., 2000; Marin and Luquet, 2004; Dalbeck et al., 2006; Marie et al., 2011; Marin et al., 2012).The longitudinal section of a shell is made up of a multilayer of calcium carbonate in two or more concentric layers, which are usually covered by an external layer (Saleuddin

and Petit, 1983). Below the periostracum is an inner nacreous layer, followed by inner primastic (Marie et al., 2011).

During rearing of snail under intensive system, damages in shell do occur due to climbing of housing facility by this animal and such may lead to economic loose due to mortality. It therefore becomes very important to understand the influence of this damage on immune status of this animal within specific period of time and to monitor recovery period depending on the level of damage. The aim of this study is to evaluate the effect of shell injury on haemocyte concentration and shell regrowth of Giant African Land snail (*Archachatina marginata*).

MATERIALS AND METHODS

Experimental Site

The research was carried out at the Snail Research Unit of the College of Animal Science and Livestock Production (COLANIM), Federal University of Agriculture, Abeokuta, Ogun State. Abeokuta lies between the rain forest vegetation zone of Western Nigeria on latitude $7^0$10'N, longitude $3^0$2'E and altitude 76m above sea level. The climate is humid with a mean annual rainfall of 1,037mm, an average temperature of 34.7^{0}C and an imminent average humidity of 82% throughout the year (Google earth 2017).

Materials

A total of thirty-two (32) snails (*Archachatina marginata)* between 130-180 g were purchased from local market. The snails were kept in plastic cages (30cm by 40cm by 24cm). Feeding trough, watering trough, sensitive scale, plier, eppend of tube, syringe and needle (5 ml), ruler, Vernier caliper and concentrate feed were used during this study. Marker and masking tape was also used for proper identification.

Snails and their management

The plastic cages along with the plastic feeders and drinkers were cleaned before the arrival of the snails and the commencement of the experiment. Feed and water were also provided *ad libitum* throughout the period of the experiment. Four weeks was set aside for the acclimatization of the snails before the commencement of the experiment. The experiment lasted for six (6) weeks.

Experimental Design

Thirty-two snails used for this experiment were randomly assigned into four (4) different treatments with 8 replicates for each treatment.

Treatment 1: No shell damage (control)
Treatment 2: 1 cm shell damage
Treatment 3: 2 cm shell damage
Treatment 4: 3 cm shell damage

All snails in both groups were treated equally in terms of feeding and drinking water provision. Composition of feed used was given in Table 1.

Table 1. Composition of experimental diets (g/100g)

Ingredients	Quantity (g)
Maize	50
Wheat offal	27.5
Groundnut cake	12.25
Soy bean meal	4
Bone meal	3
Oyster shell	3
Salt	0.25
Total	100

Shell damage/Injury

The snails were cleaned with damp foam in order to remove the dirt on them. The snails were weighed on a sensitive scale before the damaged of the shells. The snails in each treatment (1, 2, 3, 4) were brought out of cages, a ruler was placed on the tip of shell and white board marker was used to mark out the part to be damaged as 0 cm, 1 cm, 2 cm and 3 cm. After marking out, a plier was used to cut out the part as marked to be damaged. Shell growth was measured weekly for six weeks using Vernier caliper (Figure 1).

Collection of Haemolymph

Haemolymph was collected from the anterior portion of the head region after full extension of the foot muscle with the aid of syringe and needle. Haemolymph was collected from the control group and other treatment levels (1 cm, 2 cm and 3 cm) immediately after shell damage and stored in eppendof tube for haemocyte count. Haemolymph collection was also carried out on weekly basis.

Total Hemocyte Count

Haemolymph from eight snails per treatment were selected from the four groups of snails with damaged shell (control, 1, 2, and 3 cm). A dilution of 1:19 was made with the aid of 5% eosin solution which was loaded into improved haemocytometer. Haemocyte found in the four squares were counted. Thereafter, numbers of cells counted were multiplied by a conversion factor (50,000) to obtain the total haemocyte count.

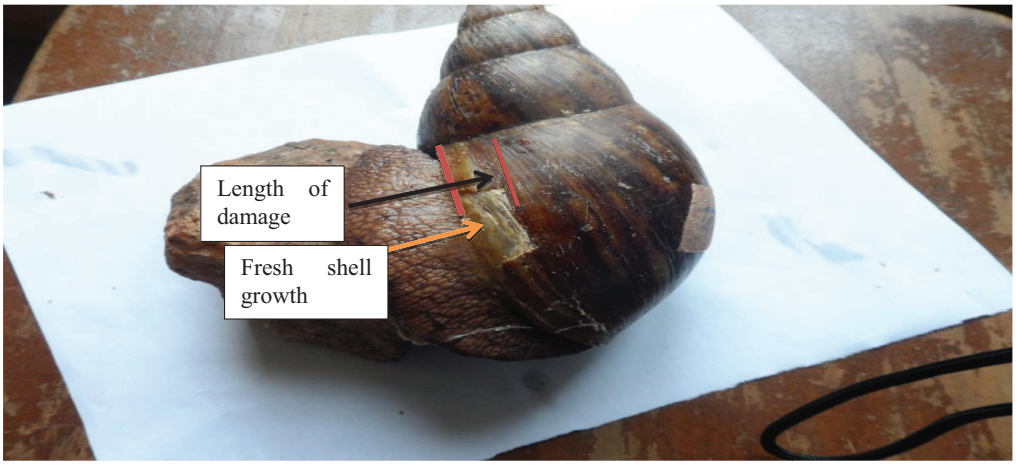

Figure 1. Portion of shell damage and regrowth

Statistical Analysis

The data generated from this experiment was subjected to a least square analysis of variance using the SYSTAT Statistical package (SYSTAT, 1992) in randomized complete block design (RCBD). Significant treatment means were separated using Duncan multiple range test (Gomez and Gomez, 1984). Model used for this experiment is stated below.

$Yij = \mu + T_{i} + W_{ij} + (TW)_{ij} + \sum ij$

Where,

Yij = Dependent Variables

μ = Population mean

Tí = effect of levels of shell damage (I = 1-4)

$W_{[j}$ = effect of weeks of haemolymph collection (I = 1-4)

∑ij = random error

RESULTS AND DISCUSSIONS

Result of summary of analysis of variance showing the effect of shell injury on haemocyte count of Giant African Land snail is shown in Table 2. Different levels of shell damage had significant effect on haemocyte count (P<0.001), while effect of week on haemocyte count during the shell damage was not significant (P>0.05).

Significant effect seen in haemocyte count is as a result of anti-inflammatory responses which are very common during injury in many animal models. Allograft inflammatory factor-1 (AIF-1) which is an interferon inducible calcium-binding cytokine has been associated with inflammatory response in mollusks (Liet al., 2013).

Table 2. Analysis of variance (ANOVA) showing the effect of shell injury on haemocyte count of Giant African Land snail (*Archachatina marginata*)

Source	Degree of freedom	Mean square
Treatment	3	330945.650***
Week	3	2425.117NS
Error	73	399960543

P< 0.001***

Studies had also shown that macrophages which facilitate wound healing, angiogenesis and collagen formation are found at the site of injury (Leibovich and Ross, 1975; Polverini et al., 1977; Hunt et al., 1984; Kovacs and DiPietro, 1994). Inflammatory response is vital to body injury, wound repair and immune response (Ottaviani et al., 2010). In mollusk, especially in snails, haemocytes are the analogue of various types of immune cells

found in vertebrate and as such, they are known to be released whenever there are challenges in the system of this animal.

Table 3 shows the least square means of effect of shell damage on haemocyte count of Giant African Land snail.

The control group had the highest number of means compared to other levels which were not significantly different from each other.

This observation is an indication that damages of shell at any magnitude compromise immune status of this animal which is largely represented by total haemocyte population.

Table 3. Least square means showing the effect of shell injury on haemocyte count of Giant African Land snail (*Archachatina marginata*)

Parameter	Lease square means($\times 10^6/mm^3$)	S.E.M (±)
Control(undamaged shell)	345.200^a	44.719
1 CM Shell damage	107.000^b	44.719
2 CM Shell damage	109.400^b	44.719
3 CM Shell damage	114.500^b	44.719

Legend: CM: Centimeter

Means within the same column having different superscript differs significantly (P<0.001).

Haemocytea are known to be the chief immune effect or cells which perform diverse immunological activities such as phagocytosis, encapsulation and cytotoxicity (Ray et al., 2013). If damages to shell could affect the population of these cells, then it means that any other challenge at this moment of injury may be very dangerous to the survival of the animal. Jonathan (1990) reported that experimentally shell-damaged snails had higher rate of mortality than did uninjured snails. Also, Ray et al.(2013) reported that exposure of two species of snails (*B. begalensis* and *L. marginalis*) to cypermethrin and fenvalerate lead to haemocyte density shift and morphological damage. All these reports are testifying to the fact that both physical and chemical damage could compromise the population of haemocytes which are known to be responsible for immune activities in the system of this animal.

Table 4 shows least square means showing effect of shell injury on weekly haemocyte count of Giant Africa Land snail (*A. marginata*). Result showed that haemocyte count was not significantly different (P>0.05) across the three weeks of collection.

The implication of this observation is that quick adjustment within the system of the animal had taken place thus nullifying effect of the damage within the three weeks of the study. Least mean square showing effect of different levels of shell damage on growth after damage is shown in Table 5.

Table 4. Least square means showing the effect of shell injury on weekly haemocyte count of Giant African Land snail (*Archachatina marginata*)

Week	Lease square means($\times 10^6/mm^3$)	S.E.M (±)
0	149.400	44.719
1	172.400	44.719
2	152.600	44.719
3	149.700	44.719

Table 5. Least square means showing the effect of different levels of shell growth after injury

Parameter	Lease square means	S.E.M (±)
Control(no shell damage)	0.175^c	0.057
1 cm shell damage	0.241^{bc}	0.057
2 cm shell damage	0.347^{ab}	0.057
3 cm shell damage	0.444^a	0.057

Means within the same column having different superscript differs significantly (P<0.001).

It was obvious that snails with 3 cm shell damage had the highest regrowth of 0.444 cm, followed by 1 cm and 2 cm shell damage which were not significantly different from each other (0.241 vs 0.347 cm) while the control had the least growth (0.175 cm).

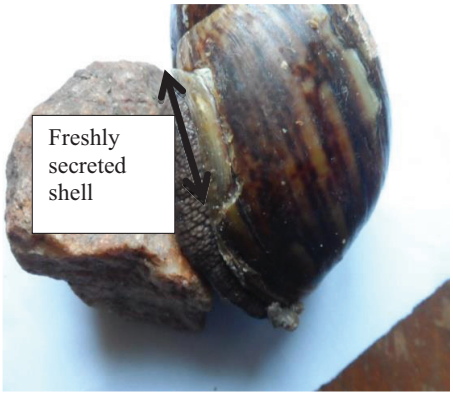

Figure 2. Freshly secreted shell after shell damage

Figure 2 shows the freshly secreted shell after shell damage.

The observation made in this study may be as a result of calcium and phosphorous mobilization from the body of the animal to compensate for the losses that occur during shell damage procedure.

According to Jonathan (1990), this process of shell repair is highly energy demanding.

It was also reported that experimentally damaged shells grew significantly more new shell than the undamaged ones (Jonathan, 1990).

This assertion is in line with the observation made in this study. Mollusks shell formation has been reported to be complex and involves deposition of calcium carbonate ($CaCO_3$) which is known to be an inorganic material mixed with organic material (Hare, 1963; Wilbur, 1983).

CONCLUSIONS

This study has shown that shell injury had significant effect on haemocyte concentration. Irrespective of the level of shell damaged used in this study, total haemocyte count reduced compared to the control group.

This observation is an evidence of immunosuppression and this call for adequate care during this period of shell injury. If adequate care is not taken during this period of injury, opportunistic infection may kill the animal as haemocyte play crucial role in the immune defense of this animal.

The implication of this study is that snail farmers should maintain hygienic environment with adequate care during any eventuality of shell damage under intensive method of production.

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