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Composition, Enzymes Analysis and Retraction Time of Columellar Muscles of Giant African Land Snail (*Archachatina marginata*) in Response to External Stimuli

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Target Audience: Animal Physiologists, snail farmers, enzymologists, pharmacologists

Abstract

With the aid of columellar muscle, snails retract the soft part into the shell when disturbed. The response time of three Giant African Land Snail (GALS) species: Archachatina marginata, Achatina achatina and Achatina fulica to touch and sodium chloride (NaCl) solution was examined. Chemical composition (protein, glucose, lipids, K^+, Na^+) and enzyme activities (amylase, cellulase, α glucosidase and lipase) in the columellar muscle of the three snail species were also investigated. Flame photometry was used to analyze the ions while spectrophomerty methods were employed for enzymes assay. A.achatina responded significantly faster (6.00 seconds) than other two snail species when stimulated by touch. The columellar muscle of A. achatina had the highest concentration of glucose and lipids (8.3mg/dl and 46.9mg/dl respectively) while A. marginata had the least. Na^+ and K^+ concentrations of A. achatina columellar muscle were lower than those of other two species. The four enzymes assayed were detected in the columellar muscles of the three snails at varying levels. A. achatina recorded higher α glucosidase (0.71 Abs /min) and cellulase (1.28 Abs/min) activities than other two species. It can be concluded that the quicker response of A.achatina to environmental factors lies in its columellar properties.

Keywords: giant African Land Snails; enzyme activities; sensory physiology

Description of the Problem

The body of Giant African Land Snails (GALS) can be divided into three parts; the head, muscular foot and visceral mass enclosed by a mantle cavity. The body parts are attached to the shell by columellar muscles (1).

The head of snails bears two pairs of tentacles that are retractable. The posterior pair bears the small black eyes at the tip, while the anterior pair is olfactory in function (2). In coiled gastropods, the muscle is responsible for retraction. The columellar muscle originates on the columellar of the shell and extends into the foot where it terminate into the operculum (3). Hodasi (4) studied the behavior of

Achatina achatina and reported that they are nocturnal by nature mostly coming out at night. Furthermore, Ademolu *et al.* (5) found that the peak of the feeding activity for *Archachatina marginata* and *A. achatina* was 24:00 hour while lipid concentration in the haemolymph of the two snail species was significantly higher at 24:00hour than other periods of the day, suggesting supply of high energy source.

However, there have been reports of variations in responses of snails to environmental factors. Ademolu *et al* (5) reported that *A. marginata* travelled farther and spend more time feeding than *A. achatina*. Similarly, *A. marginata* consumed more food than *A. achatina* in the same management system (6).

At rest, the head and foot of the snails are withdrawn into the shell by the contraction of the columellar muscles (2). The motion of the muscle is determined by muscle fibres orientation and by the attachment to the shell which is along the upper edge of the muscle (7). The emergence of the head and foot from the shell is a gradual process caused by squeezing of blood from the sinuses into the foot which emerges first followed by the head (2). The columellar muscle functions as a muscular hydrostat controlling protraction from the shell and it is composed of muscle fibres that are oriented longitudinally and obliquely with respect to the long axis of the columellar muscle (3). Thus columellar muscle controls its own twisting, shortening and elongation in addition to protraction and retraction (7). The tactile sense (sense of touch) is well developed in snails so much that on a slight touch it shrinks and on repetition the body withdraws into the shell (1).The aim of this study is to investigate the responses of three snail species to two external stimuli and to also examine the enzyme activities in the columellar muscles of these snails.

Materials and Methods

Experimental Site

The study was carried out in the laboratory of Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria

Experimental Snails

Forty five (45) adult snails (15 snails for each species: *A. marginata, A. achatina* and *A. fulica*) used for this study were purchased from Kuto market, Abeokuta, Nigeria.

Experimental Procedures

The snails were made to come out fully from their shell by putting them in water as described by Segun (1975). The responses of the snails to touch and salt solution were tested by gently touching the snails' feet with needle (2cm long). Similarly, 2ml of 0.02mg/ml solution of NaCl was shot at the feet of snails using empty syringe. The time taken for the snails to retract into the shell was measured using stop watch.

Chemical Analysis

The snails were killed by the method described by Segun (8). The columellar muscle was carefully cut off from the shell and 2g of it was homogenized in 0.05M KCl and centrifuged at 500rpm for 30 minutes at 5^0C. The supernatant (enzyme extract) was decanted into 30ml centrifuge tube and kept in the

freezer for further analysis.

Enzymes Analysis

Activities of cellulase, amylase, α – glucosidase and lipase were determined by protocol described by Adedire *et al.,* (9). They were estimated quantitatively by Dinitrosalycyclic acid reagent (DNSA). The amount of reducing sugar (glucose) produced at the end of incubation period was determined calorimetrically at 550 nm. Each reaction mixture composed of 0.2ml enzyme extract, 0.2ml of phosphate buffer (pH 7.0) and 0.4ml of the substrate. The reaction mixtures were incubated at 37^0C for 1hour.Lipase activity was determined by adding 0.4g of sodium taurocholate to the enzyme extract and incubated at 35^0C. The absorbance of the sample was read at 415 nm.

Chemical Composition of the Columellar Muscle

The protein content of the columellar muscle was determined by Biuret method (10) while glucose content was determined by colorimetric method (11). The protocol described by Grant (12) was adopted for determination of lipids. Sodium (Na^+) and potassium (K^+) concentration in the columellar muscle were determined by flame photometer (Corning UK model 405) by A.O.A.C. (13) procedures.

Statistical Analysis

Data obtained experiments were subjected to one – way analysis of variance (ANOVA) using SPSS and separation of means was done by Student – Newman – Keuls (SNK) test**.**

Results

The time taken for snails to retract back into the shell in response to the external stimuli (touch and NaCl) is presented in Table 1. *A. achatina* retracted significantly faster (more quickly) than other two snail species taking only 6.00 seconds to withdraw into the shell followed by *A. fulica* (8.75 seconds), while it took *A. marginata* 19.00 seconds.

The time taken to respond to NaCl (osmotic) by the three snail species was not significantly different, but the quickest response was recorded by *A. marginata*, followed by *A. fulica* while *A. achatina* had the least response time (Table 1).

It can be observed from Table 1 that snails responded more quickly to NaCl than touch. Response time to stimulus by NaCl varied from 4.75 – 6.50 seconds while 6.00 – 19.00 seconds were spent by the snails before fully withdrawing into their shell when stimulated by touch

Table 1: *Response time of three snail species to external stimuli (seconds)

	Archachatina marginata	*Achatina achatina*	*Achatina fulica*
Touch	19.00 ± 0.3^a	6.00 ± 0.01^c	8.75^b
NaCl solution	4.75±0.1	6.50±0.01	5.25±0.2

*Mean values in the same row having different superscript are significantly different ($p<0.05$) (SNK).

Table 2 shows the organic and inorganic composition of the columellar muscle of the three snail species. The concentration of glucose ranged from 7.00mg/dl – 8.3mg/dl. The columellar muscle of *A. achatina* had the highest glucose concentration followed by *A. fulica* while the least value was recorded

by *A.marginata*. A similar pattern was observed in the lipid composition of the three snail species. It was also observed that lipids had the highest concentration out of the three organic substances measured in the columellar muscles.

Na^+ and K^+ were detected in the columellar muscle of the three snail species in varying levels that are not significantly different from one another. However, *A. achatina* had the least concentrations of Na^+ and K^+ in its columellar muscle.

Table 2: *Composition of the columellar muscle of three snail species (mg/dl)

	Glucose	Lipids	Protein	Na^+	K^+
Archachatina marginata	7.00±0.04[c]	35.8±0.11[c]	15.8±0.01[a]	3.00±0.10	3.50±0.03
Achatina achatina	8.3±0.01[a]	46.9±0.10[a]	13.00±0.01[b]	2.00±0.01	2.50±0.01
Achatina fulica	7.50±0.02[b]	43.6±0.24[b]	16.10±0.02[a]	2.50±0.20	3.00±0.01

*Mean values in the same column having different superscript are significantly different ($p<0.05$) (SNK).

The result of the enzyme activities in the columellar muscle of the three snail species is presented in Table 3. Lipase, α – glucosidase, amylase and cellulase were detected in the muscle of the snails at different levels. *A. achatina* had the highest α – glucosidase and cellulase activities while *A. marginata* had the least. The result also showed that α – glucosidase had the least activity in the columellar muscle while amylase had the highest activities.

Table 3: Enzymes activi ties in the columellar muscles of three snail species (Abs/min)

	Amylase	α – glucosidase	Cellulase	Lipase
Archachatina marginata	2.12±0.05	0.59±0.77	1.22±0.10	1.18±0.02
Achatina achatina	2.45±0.53	0.71±0.32	1.28±0.03	1.12±0.11
Achatina fulica	2.61±0.21	0.67±0.11	1.25±0.01	1.14±0.01

Discussion

A. achatina is highly sensitive and less adaptable to the environment (6). The results from the study support this fact as *A. achatina* withdrew quickly into its shell when touched unlike *A. marginata*. This shows that *A. achatina* is less tolerant to external stimuli which make its survival a bit difficult especially in the tropics with many adverse environmental factors. Adeyeye (14) and Ademolu *et al.,* (5) had earlier observed that *A. achatina* spent less time on feeding and travelled less distance than other snail species.

The three snail species responded significantly faster when stimulated with NaCl solution than with touch. Snails were reported to have been killed when fed with food with high NaCl concentration (1). Snails are ectothermic and their nocturnal behavior is adaptive nature (2) so as to prevent water loss during the day. Exposure of snails to high concentration of NaCl presents a hypertonic environment which through the process of osmosis withdraws water from the snails' tissue thereby rendering the cells plasmolysed. Water conservation and

availability are essential to the existence of giant African Land snails (GALS) which informed the type of habitats they are found in the wild, that is, cool and moist places like cocoa and plantain farms.

The columellar muscle of *A. achatina* had significantly higher concentration of glucose and lipids than other two species. Glucose and lipids are energy sources which supplies energy to the cells (15) and their higher concentration in the *A. achatina* columellar muscle might be responsible for its significantly quicker response to external stimulus (touch) than other snail species as the muscle has more energy substrates which assist in its contraction and withdrawal of the whole animals into the shell. The significantly higher concentration of lipid in the muscle than glucose is not unexpected as lipids supplies twice energy as glucose and columellar muscle requires high energy substrate in order to pull the whole animal into its shell.

Columellar muscles are part of excitable tissues that make up the snail's body. The contraction or excitability of the muscle is enhanced at low concentration of Na^+ and K^+ which causes the depolarization of the membrane and thus making communication or movement possible (16). The lower concentrations of Na^+ and K^+ in the columellar muscle of *A. achatina* caused depolarization of the muscle membrane resulting in action potential and thus better contraction. This observation might explain the sharp and less tolerance behavior of *A. achatina* as reported by Hodasi (4).

Glycosidases (amylase, α – glucosidase, cellulase) and lipase were detected in columellar muscle of the three snail species. Ademolu *et al.* (17) reported presence of similar enzymes in the femora muscle of *A. marginata*. The presence of these enzymes suggests that the columellar muscle is endowed with enzymes necessary for breaking down of food substrates in its cells.

Higher activities of α – glucosidase and cellulase in *A. achatina* muscles indicates that it will probably have more energy supply than other two species which is highly needed for contraction activities which as earlier mentioned might explain the reported behavior of *A. achatina*. Cellulase and α-glucosidase hydrolyze polysaccharide into lower monomers that are ready to be utilized by the cells.

Amylase is responsible for the conversion of starch to maltose by breaking the glycosidic bond (15). The higher activities of amylase in the muscle of snails in this study might not be unconnected to the diet of the snails. Snails generally feed on pawpaw leaves, high carbohydrate food which needs to be broken down further before it can be used.

Conclusion and Applications

The behavior of giant African land snails has physiological bases in their tissues, especially in the collumelar muscle that retracts the foot into the shell. The higher energy metabolites and enzymatic activities in *A.achatina* make it more active than other common land snails.

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