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Feeding pattern and gut enzymes activity of Giant African land snail (*Archachatina marginata*) during growth phases

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Additional keywords

Archachatina marginata.
Feeding pattern.
Growth phases.
Gut enzymes.

SUMMARY

Snails are multivarious feeders that eat different kinds of food present in their habitat. The feeding pattern, occurrence of gut microbes and gut enzymes (proteinase, lipase, cellulase, glucosidase and α-glucosidase) activity of the three stages of development of the Giant African land snail, *Archachatina marginata* were examined in this study. Results showed that the snails at all growth phases fed at night period (19:00 to 4:00 GMT), while the juvenile stage spent more time on feeding and had significantly higher feed intake than in the other two growth phases. The activities of the gut enzymes were significantly affected by the growth phase as the adult stage recorded the highest activity level for all the five enzymes followed by the juvenile stage, while the snailet stage recorded the lowest one. The stomach region of the gut had the highest enzymes activity while the oesophagus had the lowest. There was presence of many microbial species in the gut across the stages of development but, more bacteria than fungi species were isolated. These findings suggest that the adult stage of *A. marginata* is better equipped to hydrolyse its numerous diets.

Modelo alimenticio y actividad de los enzimas digestivos del Caracol Africano Gigante (*Archachatina marginata*) durante las fases de crecimiento

Palabras clave adicionales

Modelo alimenticio.
Enzimas digestivos.
Archachatina marginata.
Fases de crecimiento.

RESUMEN

Los caracoles comen diferentes clases de alimentos presentes en su hábitat. En este trabajo, se analizan el modelo de alimentación, la presencia de microbios en el tracto digestivo y las actividades de los enzimas digestivos (proteinasa, lipasa, celulasa, glucosidasa y α-glucosidasa) del Caracol Africano Gigante (*Archachatina marginata*) en tres etapas de crecimiento. Los resultados ponen de manifiesto que que los caracoles en todas las fases de crecimiento, se alimentan durante el periodo nocturno (19:00 a 4:00 GMT), por otro lado, los caracoles en la fase juvenil dedican más tiempo a la alimentación e ingieren mayor cantidad de alimento que en las otras dos fases de crecimiento. Las actividades de los enzimas digestivos fueron afectadas significativamente por la fase de crecimiento, siendo en la fase adulta cuando se registra la máxima actividad de todos los enzimas estudiados, seguida por la fase juvenil, registrándose la actividad mínima en la etapa inicial. La región estomacal es la parte del tracto digestivo con mayor actividad enzimática, registrándose los valores mínimos en el esófago. Se identificó la presencia de muchas especies microbianas en el tubo digestivo a lo largo de las etapas de crecimiento, siendo mayor la presencia de bacterias que la de hongos. Estos hallazgos sugieren que *A.marginata* en la fase adulta, está mejor equipada para hidrolizar sus numerosas dietas.

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INTRODUCTION

The numerous benefits derived from giant African land snails have necessitated increased raising in captivity. Shells, mucin and flesh have all been isolated and researched and found to be rich raw materials for industries (Akinnusi, 2014). In captivity, three stages of development are found namely: Snailet, juvenile and adult (South, 1992).

According to Ademolu *et al* (2013a) the juvenile stage of *Archachatina marginata* laid average of 9.3 eggs followed by the adult stage that laid 4.7 eggs, while no egg was laid by the snailet stage. Similarly, Idokogi and Osinowo (1998) observed that the reproductive tract of *A. marginata* increased in size as the age increased.

Previous works on snails revealed that the three stages of development have different circulatory physiology. The juvenile stage has highest haemolymph biochemical values while the adult recorded the least values (Ademolu *et al*, 2009). In a related study, Abiona *et al*, (2007) observed that juvenile phase produced highest number of spermatozoa and ova suggesting their higher sexual activity than the two other developmental phases.

The success of any animal husbandry depends on how the animals can convert and utilize food substances given to it. This factor depends majorly among others on the presence and activities of the enzymes in the gut. Snails are good converter of various household wastes into good body weight (Akinnusi, 2014). They are able to do this effectively because they are equipped with necessary enzymes required to breakdown these foods (Yoloye, 1994). Lipase, proteinase and carbohydrases were earlier detected in the gut regions of *A. marginata* at varying levels during aestivation (Ademolu *et al*, 2013b).

However, unlike studies done on reproductive tract of *A. marginata* there has not been a comparative study on its digestive physiology across the three development phases. This knowledge will reveal its nutrients utilization and feeding behaviour which are prerequisite in rearing animals in captivity.

MATERIALS AND METHODS

Experimental site

This study was conducted in the animal house of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria ($7^0$10′N$3^0$2′E).

Experimental animals

A total of sixty snails (*A. marginata*) where purchased from Kuto market in Abeokuta, Nigeria (7^0 2′N 3^0 4′ E). They were bought based on their weight and number of whorls (20 individuals per stage of development) as earlier described by Idokogi and Osinowo (1998).

Dissection procedures

Snails were dissected following the methods described by Segun (1975) and modified by Ademolu *et al* (2013b). The alimentary canal was carefully separated from the common hermaphroditic duct and was totally removed using flamed forceps and scissors. The isolated alimentary canal was cut out into various sections: Oesophagus, Stomach and intestines. The gut contents of each section were then emptied into separate labelled sterile petri dishes for further analysis.

Morphometrics of the alimentary canal

The length and width of the gut regions were measured using calibrated tape rule and the weight of the stomach was taken with aid of a sensitive weighing balance (PM-Mettler-K)

Feed intake and feeding pattern

The amount of feed consumed by the experimental snails was determined by subtracting the weight of food left (uneaten) from the weight of food given.

Feed Intake= food given - food uneaten

Feeding period was determined as the time spent by individual snail at the feeding place (Grimm and Schaumberger, 2002).

Gut microbes estimation

The methods described by Idowu *et al* (2008) were followed for gut microbial estimation. The bacterial enumeration and identification were done by methods of Sneath *et al* (1986). Identification of fungi was done by Bernett and Hunter (1972) techniques.

Enzymes activities assay

Each gut region of the experimental snails was homogenized separately in 20ml of phosphate buffer (ph 7.0). The homogenate was centrifuged at 4,000 rpm for 30 minutes. The sediment was thrown away, while the supernatant was used as the enzyme extract.

Cellulase, α-glucosidase, amylase, proteinase and lipase activities were determined following methods of Adedire *et al* (1999). 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 550nm. 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 415nm. All enzymes assays were done in triplicates.

Statistical analysis

All data from the above experiments were analysed by one-way analysis of variance (ANOVA) and where there were significant differences, means were separated using Student Newman- Kuel Test (SNK)

RESULTS

The measurements of the alimentary canal of *A. marginata* across the three growth phases are shown in **table I**. There was a significant difference in the measu-

Table I. Measurements of the gut regions of *A.marginata* at the three growth phases (Dimensiones de las regions del tubo digestivo de *A.marginata* en varias etapas de crecimiento)*

Growth phases	Length of stomach (cm)	Width of stomach (cm)	Length of intestine (cm)	Length of oesophagus (cm)	Weight of stomach (g)
Snailet	1.25^a	0.83^c	1.97^c	1.13^c	1.1^b
Juvenile	1.17^c	0.97^b	2.87^b	1.48^b	1.2^b
Adult	1.50^a	1.33^a	2.93^a	1.97^a	2.5^a

* Mean values in each column with the same superscripts are not significantly different (p>0.5).

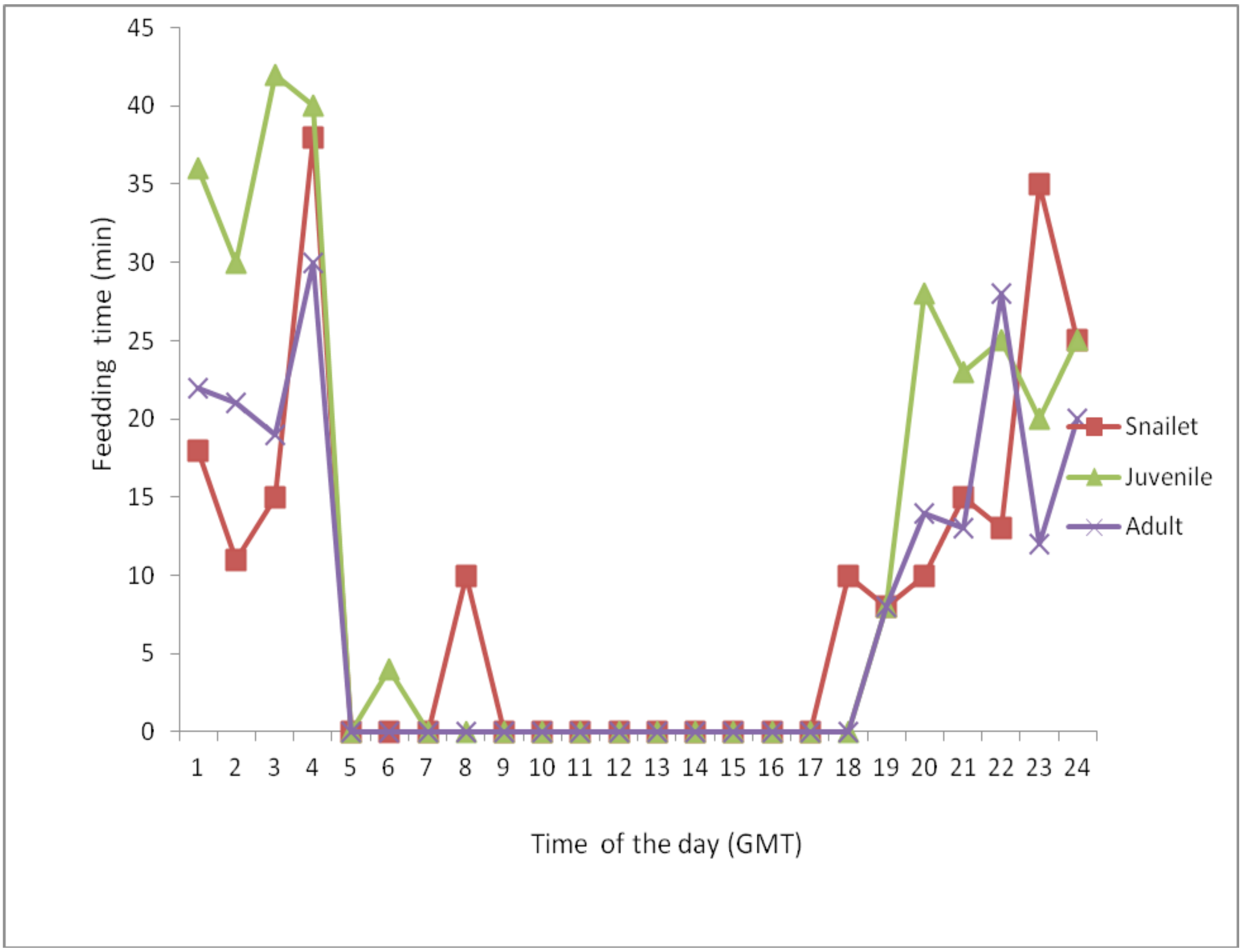

Figure 1. **Feeding pattern of the three (3) growth phases of** *A. marginata* (Modelo alimenticio de *A. marginata* durante tres fases de crecimiento)

rements of the gut sections of *A. marginata* across the growth phases, with the adult stage having the highest size.

Figure 1 showed the feeding pattern of the three (3) growth phases of *A. marginata*. During the snailet stage, two peaks of feeding time were observed at 4:00 and 23:00 GMT, while there was no feeding between the hours of 9:00 and 17:00 GMT. The juvenile stage fed between the hours of 19:00 and 4:00 GMT. However, highest feeding activity was noticed at 3:00 GMT. No feeding occurred between 7:00 and 18:00 GMT. The adult stage of *A. marginata* did not feed between the hours of 5:00 and 18:00 GMT, but commenced feeding at 19:00 to 4:00 GMT. During this feeding period, juvenile stage spent more time than other two stages.

Table II. **Total feed intake of** *A.marginata* **at the three growth phases** (Ingesta total de alimento por *A.marginata* durante tres fases de crecimiento)*

Growth phases	Feed intake (g)	Average feed intake/day (g)
Snailet	78.8[c]	5.63[c]
Juvenile	129.8[a]	9.27[a]
Adult	86.1[b]	6.15[b]

*Mean values in the column with the same superscripts are not significantly different (p>0.5)

Table II showed the feed intake of the experimental snails. The juvenile stage had the highest feed intake (129.8g) followed by the adult stage while the least feed intake was recorded by the snailet stage (78.8g).

The enzyme activities in the oesophagus of the 3 growth phases of *A. marginata* are shown in **table III**. The snails in adult stage recorded the highest enzyme activities (except lipase) while the snailet stage recorded the least activity.

Table IV described the enzyme activities in the intestine of *A. marginata* during different growth phases. Adult stage similarly had the highest activities while the snailet stage had the least activity. Amylase activity was significantly ($p<0.05$) higher than other enzymes activities in the intestine. Similar observation was noticed in the stomach region of the gut **(table V)**. Amylase and α-glucosidase recorded the highest and lowest enzyme activities respectively in the stomach region of the snails. Comparison of means showed that the stomach region had the highest enzyme activities than the other gut regions.

Table IV. **Enzymes activity in the intestine of *A.marginata* at the three growth phases (mg/g)** (Actividades enzimáticas en el intestino de *A.marginata* durante tres fases de crecimiento (mg/g))*

Stages of development	Amylase	Lipase	Cellulase	Proteinase	α-glucosidase	Total activities
Snailet	13.80^b	6.60^b	10.60^b	9.50^b	3.70^c	44.20
Juvenile	14.20^b	8.40^b	11.70^b	10.80^b	5.30^b	50.40
Adult	18.40^a	9.30^a	13.20^a	13.00^a	7.50^a	61.40
Total	46.40	24.30	33.50	33.30	16.50	156.00

*Mean values in the column with different superscript are significantly different (p<0.05)

Table V. **Enzymes activity in the stomach of *A.marginata* at the three growth phases (mg/g)** (Actividades enzimáticas en el estómago de *A.marginata* durante tres fases de crecimiento (mg/g))*

Stages of development	Amylase	Lipase	Cellulase	Proteinase	α-glucosidase	Total activities
Snailet	11.60^c	11.20^c	8.40^c	8.40^c	4.80^b	44.70
Juvenile	13.80^b	13.20^b	10.20^b	10.20^b	5.10^b	51.90
Adult	15.20^a	15.80^a	12.50^a	12.50^a	8.30^a	60.40
Total	40.60	40.20	31.10	31.10	18.20	157.00

*mean values in the same column with different superscript are significantly different (p<0.05).

Table VI. **Microflora and load (cfu) of the gut regions of *A. marginata* at the three growth phases** (Microflora y carga microbiana (ufc) en las regiones del tracto digestivo de *A. marginata* durante tres fases de crecimiento)

Stages of development	Oesophagus	Stomach	Intestine
Snailet	*Staphyloccus aureus,* *Penicillium* sp ($4.0x10^5$,$3x10^5$)	*Baccillus subtilis* *Staphyloccus aureus,* *Streptobacillus* sp, *Aspergillus niger,* *Penicillium* sp ($6.85x10^6$,$1.0x10^6$)	*Baccillus subtilis,,* *Streptobacillus* sp, *Aspergillus niger,* ($3.35x10^6$,$2.0x10^5$)
Juvenile	*Baccillus subtilis,* *Streptococcus aureus* ($5.0x10^5$,nil)	*Escherichia coli,* *Baccillus subtilis,* *Streptococcus sp* ($6.55x10^6$,$3.5x10^5$)	*Penicillium* sp, (nil,$7.0x10^5$)
Adult	*Streptobacillus* sp, *Staphyloccus aureus,* *Baccillus subtilis,* *Penicillium* sp *Aspergillus niger* ($5.0x10^6$,$6.5x10^5$)	*Penicillium* sp, *Aspergillus niger,* (nil, $2.0x10^5$)	*Escherichia coli,* *Penicillium* sp ($2.0x10^6$, $1.45x10^6$)

() cfu of bacteria and fungi isolates respectively

There were presence of both bacteria and fungi species in the gut regions of the experimental snails **(table 6)**. However, bacterial species were more present than the fungi. Also, the stomach region (juvenile and snailet stages) had highest bacterial colony forming units while the intestine region had the least.

DISCUSSION

Age has significant effects on the size of giant African land snails. The older the snails the bigger their sizes (Okon and Ibom, 2012). In this study, the adult stage recorded the highest measurements of the gut regions. This parallels the report of Idokogi and Osinowo (1998) that as the snail age increased the reproductive tract size also increased, thus the adult stage had the biggest size.

Juvenile stage is the most active phase in the life of snails (South, 1992). Juvenile stage in this study recorded the highest feed intake while the snailet recorded the least. The juvenile phase requires high energy substrate for its growth and activities like oviposition and movement which are highly reduced in the adult phase due to big size and age. Ademolu *et al* (2009) had earlier observed that snails in juvenile phase had significantly higher glucose and lipids concentration in their haemolymph than other two growth phases.

Results showed that the experimental snails across the three growth phases fed in the dark period of the day (19:00 and 4:00 GMT), while no feeding occurred in the day time. Snails are nocturnal animals that feed, walk and mate during the night hours (Hodasi, 1982; Okon and Ibom, 2012). Ademolu *et al* (2011) and Ogbu *et al* (2014) had earlier reported that *A. marginata* and *A. achatina* fed during the night from 20:30 to 2:00 GMT. According to Yoloye (1994), the nocturnal nature or behaviour of snails is adaptive in function as there are fewer predators to attack them in the night. Similarly, the cool environment helps to reduce water loss due to evaporation.

Juvenile stage spent comparatively more time feeding than other stages of development. This likely relates to their active nature and need for more nutrients. The adult stage on the other hand has reached their peak growth stage thus requiring less food.

Snails are equipped with adequate enzymes necessary for the digestion or hydrolysis of their multi various food items (Adedire *et al* 1999). Lipase, α-glucosidase, amylase, cellulose and proteinase were detected in the gut regions of the experimental snails which enabled them to hydrolyse various food items in their diet. The presence of lipase and proteinase in the gut confirmed the omnivorous nature of snails as earlier suggested by Ademosun and Imevbore (1988). Snails not only feed on plant based food but on earthworms, ants and mushroom (Amusan and Omidiji, 1999).

Phase of growth had a significant effect on the gut enzymes activities as the adult stage had the highest enzymes activities followed by the juvenile phase while the snailet had the least. Maha *et al* (2009) reported that steroids hormones were more abundant in the tissues of adult water snail *Biomphalaria alexandrina* than juvenile snails. The size of the snail gut might be responsible for this observation. Big snails (adults) have more cells in the gut which release or synthesize more enzyme than the young snails with fewer cells. Bundit (2000) observed that as the gut size of Nile Tilapia, *Oreochromis niloticus* increased, the enzymes activities of the intestinal tract increased. Le Francois *et al* (2000) similarly observed that the activities of glycolytic enzymes increased with the fish mass. The adult stage of snails had a bigger size and thus contained more food mass which consequently will stimulate secretion of relevant enzymes

Amylase is needed for the hydrolysis of starch; hence, the presence of amylase in the gut of the experimental snails at a significantly higher activity than other enzymes is not unexpected. The main constituent of pawpaw leaves consumed by these snails is starch. Hildalgo *et al*, (1999) reported that herbivorous and omnivorous fishes have high amylase activity because it is used to break down the polysaccharide in the diet.

Comparison of means revealed that the stomach region of the snail gut recorded the highest enzyme activities. This conforms with the report of Ademolu *et al* (2013b) and Adedire *et al* (1999) that the stomach and hepato pancrease are the main sites of food digestion as earlier mentioned by Segun (1975).

Many microorganisms were found in the gut regions of the snails, however, there were more bacteria species than fungi species. Adedire *et al* (1999) similarly detected no fungi in the gut of *A. marginata* while various bacteria species were isolated. The nature of food consumed by the snails might account for this occurrence. Snails prefer moist cool environment and feed on decaying plant materials which support the growth of bacterial species more than fungi species.

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