

## DIGESTIVE ENZYMES IN AFRICAN GIANT LAND SNAIL (*ARCHACHATINA MARGINATA*) DURING AESTIVATION

### ENZIMAS DIGESTIVOS DEL CARACOL GIGANTE AFRICANO (*ARCHACHATINA MARGINATA*), DURANTE LA ESTIVACIÓN

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#### ADDITIONAL KEYWORDS

Gut regions.

#### PALABRAS CLAVE ADICIONALES

Tracto digestivo.

#### SUMMARY

The activities of digestive enzymes in the gut regions of African giant land snail, *Archachatina marginata* were investigated during aestivation induced in the laboratory by the withdrawal of water and food for three weeks. All studied enzymes, amylase,  $\alpha$ -glucosidase, cellulase, lipase, and protease, were detected on the gut regions (oesophagus, crop, stomach and intestine). Aestivated snails had significantly lower enzyme activities in all the gut regions than the active snails. Furthermore the stomach recorded the highest enzyme activities of all the gut regions.

#### RESUMEN

Se investigaron las actividades de las enzimas del tracto digestivo del caracol gigante africano *Archachatina marginata* durante la estivación, inducida en laboratorio mediante retirada del agua y alimento durante tres semanas. Todas las enzimas estudiadas, amilasa,  $\alpha$ -glucosidasa, celulasa, lipasa y proteasa fueron encontradas en las regiones del tracto digestivo (estómago, intestino, esófago y bulbo oral). Los caracoles en estivación tienen, en todas las regiones del tracto digestivo, actividades enzimáticas significativamente menores que los caracoles activos. En el estómago, se registró la mayor tasa de actividad enzimática de todo el tracto digestivo.

#### INTRODUCTION

The African giant land snails are regarded as delicacies and important source

of animal protein. In many developing countries meat production from domestic livestock is not sufficient to meet the high demands for animal protein (Adedire *et al.*, 1999). Revitalization of some wild animal species like snail could assist in combating this challenge (Ajayi and Tewe, 1985).

However, raising of snails in captivity is faced with problem of feeding and nutrition. Ademosun and Imevbore (1988) opined that commercial snail farming should use food stuffs which are not competed for by other livestock. Ademolu *et al.* (2007) similarly observed that snails consumed poultry dropping based diet and recorded a better growth performance than snails fed with conventional feeds. Furthermore, snails have been reported to consume household wastes which were converted and utilized for their growth and egg production (Amusan and Omidiji, 1999; Ademolu *et al.*, 2011).

In Nigeria, snails aestivate during the dry season months of November-March. During this period, the mouth aperture is temporarily closed by a whitish calcium material called epiphragm (Odaibo, 2003). This process is adaptive in nature as it assists the snails to survive the adverse atmospheric condition when water and food are partially not available. Ademolu *et al.*

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(2009) recently reported that the activities of glycosidases in the foot muscles *Archachatina marginata* reduced significantly during aestivation.

The digestive tracts of giant land snails are endowed with multiples of enzymes like cellulase, trypsin, lipase,  $\alpha$ -glucosidase and protease (Adedire *et al.*, 1999). However, so far in literature, no attention has been paid to the digestive enzymes of snails during aestivation like had been done for snails during active periods. This study therefore examines the digestive enzymes activities in the gut of *A. marginata* during aestivation. This will help to understand their digestion biology and utilization of nutrients during this period.

#### MATERIALS AND METHODS

Forty individuals of *A. marginata* ( $130 \pm 0.02$  g; obtained from the Department of Forestry and Wildlife Management, University of Agriculture, Abeokuta, Nigeria), were randomly divided into two groups of twenty individuals: active snails and aestivated snails. Each group was further divided into 4 baskets (45 x 20 x 21 cm) containing 5 snails each to avoid over crowding.

Active snails were fed with pawpaw leaves *ad libitum* and drinking water was also provided daily. Aestivated snails were denied water and food and thus formed epiphragm in the mouth aperture after three weeks. The two groups were observed for three months.

The snails were dissected using the methods of Segun (1975). The various regions of alimentary canal (oesophagus, crop, stomach and intestine) were carefully dissected out and each region homogenized separately in 20 mL of phosphate buffer (PH 7.0). The homogenate was centrifuged at 4000 r.p.m. for 30 min. The sediment was discarded while the supernatant was used as enzyme extract.

Cellulase,  $\alpha$ -glucosidase, amylase, protease and lipase activities were deter-

mined following the methods described by Adedire *et al.* (1999) and Ademolu *et al.* (2009). All the enzyme assays were done in triplicates.

The data from the experiment were subjected to analysis of variance (ANOVA), separation of significant means was done by Student-Newman Kuel (SNK).

#### RESULTS

The result of amylase assay in the gut region shows that active snails recorded significantly higher activities ( $p < 0.05$ ) in all the gut regions than aestivated snails (**table I**). Stomach has the highest amylase activity followed by the crop.

The  $\alpha$ -glucosidase activities in the gut regions of the snails are shown in **table II**. Active snails recorded a significantly higher  $\alpha$ -glucosidase activity in all the gut regions except the oesophagus. The stomach followed by the crop recorded highest  $\alpha$ -glucosidase activity.

The cellulase activity is found to be statistically higher in active snails than the aestivated ones. The stomach similarly recorded the highest activity while the intestinal recorded the least (**table III**).

**Table IV** shows the result of protease activity in the gut regions of both active and aestivated snails. Gut regions of active snails

**Table I.** Activity of amylase (Abs/min) in the gut regions of *Archachatina marginata* snails. (Actividad de la amilasa (Abs/min) en las regiones del tracto digestivo de caracoles *Archachatina marginata*).

Gut regions	Active snails	Aestivated snails
Intestine	7.0 $\pm$ 0.1 <sup>a</sup>	5.0 $\pm$ 0.02 <sup>b</sup>
Oesophagus	5.0 $\pm$ 0.2 <sup>a</sup>	3.0 $\pm$ 0.1 <sup>b</sup>
Stomach	11.0 $\pm$ 0.11 <sup>a</sup>	9.0 $\pm$ 0.2 <sup>b</sup>
Crop	9.0 $\pm$ 0.01 <sup>a</sup>	7.0 $\pm$ 0.15 <sup>b</sup>

<sup>ab</sup>Mean values in the same row with different superscript are significantly different ( $p < 0.05$ ).

## DIGESTIVE ENZYMES IN AFRICAN GIANT LAND SNAIL DURING AESTIVATION

**Table II.** Activity of  $\alpha$ -glucosidase (Abs/min) in the gut regions of *Archachatina marginata* snails. (Actividad de la  $\alpha$ -glucosidase (Abs/min) en las regiones del tracto digestivo de caracoles *Archachatina marginata*).

Gut regions	Active snails	Aestivated snails
Intestine	21.0±0.1 <sup>a</sup>	18.0±0.13 <sup>b</sup>
Oesophagus	18.0±0.16 <sup>a</sup>	24.0±0.04 <sup>a</sup>
Stomach	41.0±0.23 <sup>a</sup>	27.0±0.32 <sup>b</sup>
Crop	32.0±0.15 <sup>a</sup>	22.0±0.22 <sup>b</sup>

<sup>ab</sup>Mean values in the same row with different superscript are significantly different (p<0.05).

**Table IV.** Activity of protease (Abs/min) in the gut regions of the experimental snails. (Actividad de la proteasa (Abs/min) en las regiones del tracto digestivo de caracoles *Archachatina marginata*).

Gut regions	Active snails	Aestivated snails
Intestine	44.0±0.41 <sup>a</sup>	32.0±0.14 <sup>b</sup>
Oesophagus	29.0±0.21 <sup>a</sup>	15.0±0.22 <sup>b</sup>
Stomach	59.0±0.15 <sup>a</sup>	26.0±0.11 <sup>b</sup>
Crop	38.0±0.10 <sup>a</sup>	29.0±0.30 <sup>b</sup>

<sup>ab</sup>Mean values in the same row with different superscript are significantly different (p<0.05).

had a higher protease activity than the aestivated snails. However, intestine recorded highest activity out of all the regions.

Aestivated snails had a significantly (p<0.05) lower lipase activity in their gut regions than active snails (**table V**). Intestine recorded the least activity while stomach has the highest activity.

### DISCUSSION

African giant land snail is well equipped with various enzymes to cater for its multivariuous feeding habit (Yoloye, 1994). This study confirms this as protease, lipase,  $\alpha$ -glucosidase, amylase and cellulase were

present in all the gut regions of the alimentary canal of *A. marginata* during periods of activity and aestivation. The various carbohydrases detected in the gut of the experimental snails are highly needed for digestion and utilization of various carbohydrates consumed by snails. Amylase presence in the gut was not surprising as it is needed to hydrolyze starch which is the main component of pawpaw leaves consumed by the snails. The high activity of  $\alpha$ -glucosidase was not unexpected as it is responsible for the breakdown of cellobiose, the product of cellulose hydrolysis which is the common part of plant cell (Pigman and Horton, 1970). Higher  $\alpha$ -glucosidase activity

**Table III.** Activity of cellulase (Abs/min) in gut regions of the experimental snails. (Actividad de la celulasa (Abs/min) en las regiones del tracto digestivo de caracoles *Archachatina marginata*).

Gut regions	Active snails	Aestivated snails
Intestine	15.0±0.04 <sup>a</sup>	11.0±0.08 <sup>b</sup>
Oesophagus	18.0±0.02 <sup>a</sup>	15.0±0.18 <sup>b</sup>
Stomach	36.0±0.3 <sup>a</sup>	32.0±0.02 <sup>b</sup>
Crop	24.0±0.19 <sup>a</sup>	21.0±0.1 <sup>b</sup>

<sup>ab</sup>Mean values in the same row with different superscript are significantly different (p<0.05).

**Table V.** Activity of lipase (Abs/min) in the gut regions of the experimental snails. (Actividad de la lipasa (Abs/min) en las regiones del tracto digestivo de caracoles *Archachatina marginata*).

Gut regions	Active snails	Aestivated snails
Intestine	8.0±0.17 <sup>a</sup>	5.0±0.1 <sup>b</sup>
Oesophagus	14.0±0.80 <sup>a</sup>	9.0±0.20 <sup>b</sup>
Stomach	19.0±0.12 <sup>a</sup>	15.0±0.1 <sup>b</sup>
Crop	12.0±0.01 <sup>a</sup>	8.0±0.01 <sup>b</sup>

<sup>ab</sup>Mean values in the same row with different superscript are significantly different (p<0.05).

was observed in the gut regions of both active and aestivated snails. This parallels the report of Umezurike (1976) where a high  $\alpha$ -glucosidase activity was found in the gut of land snail *Achatina achatina*. This reveals that the major diet of the experimental snails is plants based and thus are more herbivorous. The presence of cellulase in the gut of the experimental snails is noteworthy. This is not synthesized by animals and its presence in these snails suggests a symbiotic relationship between microorganisms and the snails. Microbes help in digestion of snails by either secreting enzymes or supply of vitamins (Akinnusi, 2002; Idowu *et al.*, 2008).

The presence of protease and lipase in the gut regions suggests that the experimental snails consumed fatty and proteic food substance along with their normal carbohydrate diets. Amusan and Omidiji (1999) had earlier observed that earthworms, ants and mushroom formed parts of snails feed while in captivity and the wild.

Enzymatic activities in the gut regions of active snails were significantly higher than those of aestivated snails. During aestivation the mouth aperture of the snails is closed and feeding is thus impossible unlike active snails that were eating and drinking continuously. Secretion of enzymes is in direct response to the presence of nutrients (Terra *et al.*, 1996) and when there was no substrate to act upon, it resulted to low enzymes activities as observed in this study. Ademolu *et al.* (2009) similarly observed reduced activities of glycosidases

in the foot muscle of *A. marginata* during aestivation.

Stomach region recorded the highest activity for all the enzymes assayed. Stomach of snails is the central region of alimentary canal that receives most food substances for digestion (Segun, 1975). In a related experiment, Adedire *et al.* (1999) reported that hepatopancrease recorded the highest enzymatic activities in the gut of *A. marginata*. These higher enzymatic activities recorded for the stomach in this study might not be unconnected to the closeness of the two organs (stomach and hepatopancreas) and hence possible backward ciliary and muscular movement of enzymes into the stomach (Segun, 1975; South, 1992).

## CONCLUSION

Aestivation significantly reduces the activities of digestive enzymes of the snails, thereby lowering their nutrient uptake from the diet consumed. Snails cannot afford reduction in these enzymes as they are responsible for utilization of nutrients in their diets. Basically, reduced activities of amylase and cellulase mean low energy derivation from the diets which in turns leads to poor productivity.

Therefore, for maximum/optimum snail production in captivity, aestivation should be avoided in the dry season by continually moistening the habitat of the snails. Also, good management practices should be put in place like: regular cleaning, pest control and good stocking density.

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## DIGESTIVE ENZYMES IN AFRICAN GIANT LAND SNAIL DURING AESTIVATION

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