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SHORT COMMUNICATION

ACTIVITIES OF GLYCOSIDASES IN THE FOOT MUSCLES OF AFRICAN GIANT LAND SNAIL, *ARCHACHATINA MARGINATA* DURING AESTIVATION

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ABSTRACT: The growth performance and the activities of glycosidases (amylase, cellulase and α-glucosidase) in the foot muscle of giant land snail, *Archachatna marginata* were examined during aestivation. Aestivation significantly affected the growth performance of the snails as active snails gained 16.4±0.02g while the aestivated snails lost 15.5±0.1g. Three glycosidases were detected in the foot muscle of the snails at varying levels: 33-37 Abs/min (α-glucosidase), 11-15 Abs/min (amylase) and 28-31 Abs/min (cellulase) with the active snails having significantly higher activities (Abs/min) in α-glucosidase and cellulase. Aestivation thus significantly affects the foot muscle activities of *A.marginata*.

Key words/phrases: Aestivation, Glycosidases, Growth, Snail.

INTRODUCTION

Snail farming (Heliculture) is becoming popular due to its numerous nutritional, medicinal and economical benefits (Akinnusi, 2002; Amusan and Omidiji, 1998). Snails are ectotherms (Yoloye, 1994) thus they are affected by the nature of the environment. Feed types (Ademolu *et al.*, 2007) and stocking density (Akegbejo Samson and Akinnusi, 2000; Ademolu *et al.*, 2006) are some of the factors that influenced the growth and development of snails in captivity.

Aestivation is a state of inactivity in snails induced by high temperature and low moisture availability during the dry season. During this state, the aperture of the snail is covered with epiphragm, a thin whitish membrane formed by calcified slime (Yoloye, 1994). Aestivation tends to reduce the much anticipated benefits of snail rearing, for during the process the snails are physiologically weak as both growth and metabolic rates are reduced maximally. Aestivation occurs mainly as an adaptation to seasonal drought and is an effective mechanism for surviving periods when food and water are scarce (Hughes, 1974).

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Since the metabolic activities are affected during aestivation and feeding is virtually halted, it will have implications on the enzymatic reactions and processes of the snail tissues. The focus of this study was to examine the glycosidases activities of the foot muscle during this phase in the life history of the land snail, *A. marginata*.

MATERIALS AND METHODS

Experiment site

The study was conducted in the Animal House of the Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria (7^0 10^1N and 2^1E) during the months of September to December, 2007.

Experimental snails

Twenty (20) individual snails (*A. marginata*) with average weight of 126.7±0.01g were purchased from the Snail Pen of the Department of Forestry and Wildlife Management of University of Agriculture, Abeokuta, Nigeria. These snails were randomly divided into two groups of ten (10) each. Group A (Active snails) were fed *ad libitum* with pawpaw leaves (*Carica papaya*) and drinking water was also provided with continuous sprinkling of water while Group B (Aestivated snails) were giving same treatment except for the sprinkling of water which made them experience aestivation evidenced by the production of epiphragm in the mouth aperture. The two groups of snails were observed for 12 weeks.

Live weight measurements

The live body weight of the snails in the two groups was assessed by the use of a sensitive electronic weighing scale (Mettler-PM 11-K, UK).

Preparation of snail for enzyme analysis

The snails were dissected according to the methods described by Segun (1975). The foot muscles were carefully separated from the visceral parts with aid of sharp scissors. Two gram of the foot muscle was homogenized in 20ml of phosphate buffer (pH 7.0) and the homogenate was centrifuged at 400 Rev/min for 30 minutes. The sediment was discarded while the supernatant was used for the enzyme analysis.

Enzyme analysis

The activities of glycosidases (Cellulase, amylase and α-glycosidase) were determined following the protocol described by Adedire *et al.* (1999). These were estimated quantitatively by Dinitrosalicylic acid reagent (DNSA). The amount of reducing sugar (glucose) produced at the end of incubation period was determined calorimetrically at 550nm. A standard glucose calibration curve was prepared in order to determine the amount of glucose in the reaction. Each reaction mixture comprised of 0.2ml of 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 1 hour. The experiment was repeated three times.

Statistical analysis

The data obtained from the study were analyzed using one way analysis of variance (ANOVA) and where significant differences existed, means were separated using Duncan's multiple range test.

RESULTS

Aestivation significantly (P<0.05) affected the growth performance of the snails. While active snails (group A) gained significant weight (16.4±0.02g), aestivated snails (Group B) lost significant weight (15.6±0.1g) (Table 1).

Table 1. [a]Growth performance of active and aestivated snails (g) (mean ± SEM)

	Group A (Active) n=10	Group B (Aestivated) n=10
Initial weight (g)	126.9±0.2	128.2±0.14
Final weight (g)	142.5±0.1^a	102.6±0.3^b
Weight gain (g)	16.4±0.02^a	-15.6±0.1^b

[a] Mean values in each row with the different superscript are significantly different (p<0.05)

Result of enzymes analysis of the snail foot muscles revealed the presence of glycosidases like amylase, α– glycosidase and cellulase (Table 2).

Aestivated snail had significantly lower (P<0.05) α-glucosidase and cellulase activities than the active ones (Table 2). However, no significant difference was found in the activity of amylase of the two groups. Similarly, amylase activities in the foot muscles of experimental snails were lower compared to α-glycosidase and cellulase activities.

Table 2. [a]Glycosidases activities of the snail foot muscles (abs/min) (mean ±SEM)

	α- Glucosidase n=10	Amylase n=10	Cellulase n=10
Group A (Active snails)	37±0.02^a	13±0.04	31±0.01^a
Group B (Aestivated snails)	33±0.01^b	11±0.03	28±0.02^b

[a] Mean values in each column with different superscript are significantly different (p<0.05)

DISCUSSION

The result of this study on the snail growth performance during aestivation showed that the aestivated snails experienced weight loss while the active snails gained significant weight. This is not unexpected as the aestivated snails did not consume any food during the process of aestivation as the mouth was covered by the epiphragm, rather the food reserves were depleted. Odaibo (2003) similarly reported that there was little or no growth during aestivation in *A. marginata*.

Glycosidases like amylase, cellulase and α-glucosidase were detected in the foot muscles of *A. marginata*. This correlates well with the high carbohydrate content of pawpaw leaves which the snails were fed with. These enzymes had likewise been found in other tissues of animals like the liver, kidney and intestine (Umezurike, 1976). The presence of these enzymes reflects the presence of their substrates in the foot muscle for energy utilization. Adedire *et al.* (1999) detected similar enzymes in the alimentary canal of *A. marginata* suggesting that gut enzymes are similarly present in foot muscles. This correlates well with the report of Ademolu *et al.* (2009) on the femoral muscles of variegated grasshopper, *Zonocerus variegatus* (l).

The detection of cellulase in the foot muscle of *A.marginata* is noteworthy. Animals are known for not synthesizing cellulase which is needed for digesting cellulose present in their food plants. However, the presence of symbiotic microorganisms like *Pseudomonas* and *Bacillus* in the gut made cellulose utilization possible (Idowu *et al.*, 2008).

Secretion of enzymes is in direct response to the presence of nutrients (Terra *et al.*, 1996). The present study supports this as glycosidases activities were lower in aestivated snails than the active ones. This might be due to low availability of substrates to act upon. During aestivation, snails stop feeding and thus can not get enough fuel for enzyme utilization. Also, respiratory activities and heart beat in snails decrease (Odaibo, 2003). Umezurike (1976) observed that high molecular weight β-glucosidase was found in the gut of active snails whereas low molecular weight β-glucosidase was detected in that of aestivated snails due to absence of enough ATP. Thus, it can be inferred that the nutritional state of snails determines the enzyme (glycosidases) activities of the foot muscles at any time.

Cellulase and α-glucosidase activities in the foot muscle of snails were very close and significantly higher than amylase. Cellulose is the major source of carbohydrate derived from the pawpaw leaves, the food plant of snails, thus it is not surprising that α-glucosidase and cellulase (enzymes with cellulolytic properties) have high activities in the foot muscle. Thus, it can be inferred that α-glucosidase plays significant role in production of monosaccharides used by the foot muscles.

CONCLUSION

Aestivation significantly affects the growth and muscular activities of *A. marginata*. Hence, efforts must be made to avoid it or reduce its impact, especially during the dry season and cold weather of harmattan. This can be achieved by continuous sprinkling with adequate amount of water during these periods.

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