Gut content analysis of *Achatinafulica*withdigestive enzymes- a comparative study

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Received: June 24 , 2018 Accepted: August 02, 2018

ABSTRACT *Achatinafulica is an arboreal and terrestrial mollusc,are maintaining low metabolic activities in anaerobic condition during aestivation. The major metabolic changes of two periods of activities of A. fulica - dormant and active were identified with special reference to gut physiology. The physico-chemical parameters of gut contents were investigatedand changes in dormancy and active periods were compared. Assays were performed to determine digestive enzymes produced by the gut regions. All studied enzymes were detected in the gut regions in varying quantities in both dormant and active periods. These enzyme activities were found to enhance the process of digestion thereby causing a decrease in the gut contents from oesophagus to rectum. Aestivated snails had negligible amount of enzymes in all the gut regions than active snails. Furthermore, the intestine showed highest quantities of enzymes than other regions of gut. The study clearly evinced that the digestion efficiency ofA. fulica is aided by the presence of rich amount of enzymes and also by the various biochemical adaptations with respect to the allied environmental conditions. Moreover, the above adaptations thriving for its better performance and making them one of the most important alien pests worldwide.*

Keywords: *Achatinafulica, gut contents, sustainable environmental conditions.*

1. Introduction

The Giant African Land Snail *Achatinafulica*(GALS) is considered as one of the world's most damaging invasive alien species. These snails are polyphagous and are vectors for plant pathogens, causing severe damage to agricultural crops and native plants. It feeds voraciously and is competitors of native snails as well as habitat modifiers. Their importance in agricultural systems, gardens and as agents for the reduction of biological diversity is increasing at an alarming rate making them one of the 100 most important pests worldwide (USDA, 2007; Zanol*et al*., 2010).

Land snails arehighly adapted to the changes in environmental conditions by a series of changes in the physiology, which enables them to survive from the adverse stressful conditions. Aestivation is such an adaptation during periods of hot and dry conditions that exhibits remarkable metabolic changes in *A. fulica*, especially in the gut. The gastro-intestinal tract of giant land snails are endued with enzymes such as cellulase, trypsin, lipase, α-glucosidase and protease (Adedire*et al*., 1999). Eventhough, the biochemical aspects of molluscan metabolism have attracted the attention of many scientists (Livingstone and de Zwaan, 1983), the reports on metabolic changes during aestivation of pulmonates are scanty (Umezurike and Eke, 1983; Umezurike and Iheanacho, 1983; Wang *et al*., 1992).

Since the dormancy and active periods of *A. fulica* exhibits specialized modes of metabolismwith respect to the prevailing environmental conditions, the present study intented to investigate and correlate the biochemical parameters of *A. fulica* during the dormancy and active periods with special reference to digestive physiology. This will help to understand their digestion biology and the implication of the digestive enzymes in the adaptive physiology of *A. fulica* during allied environmental conditions.

2. Materials and methods

Medium sized, healthy, terrestrial snail, *A. fulica*were collected from Kochuveli (Lat. 9º96683´N & Long. 76º25´08E), Thiruvananthapuram. The collected specimens were brought to the laboratory, sorted them and were acclimatized to suite the parameters of laboratory conditions. The medium sized specimens were selected, fed with *Papaya* leaves, weighed and sacrificed for further analysis.

Animals were anaesthetized using chloroform and menthol. The adult animals (33.58±0.144 gm.) were selected and their shells were carefully broken using a bone cutter to expose soft body. Animals were then aseptically dissected using 70% ethanol to separate whole gastro-

intestinal tract. From the whole GI tract, oesophagus, stomach, intestine, and rectum were separated and the isolated gut contents were homogenized in an isotonic saline solution for gut content analysis and the gut tissue of the respective regions were separated and used for enzyme assays.

Gut content analysis

Moisture content and ash were determined by the method of Pearson (1976) and James (1995). pH and temperatures of each gut regions (Oesophagus, Stomach, Intestine and Rectum) were measured separately using pH meter and digital thermometer respectively.

The quantitative estimation of gut contents (Protein, Lipid, Carbohydrate and Cellulase) were performed in quadruplicate from freshly homogenized gut contents, using the following methods and expressed in %. Protein was measured by the method of Lowry *et al*., (1951). Lipid was measured by the method of Folch (1957) and Carbohydrate and Cellulose was estimated by using the method of DuBois *et al*., (1956).

Biochemical enzyme assays

The enzyme assays were also performed in quadruplicate according to the following methods and expressed as U/ml. The protease activity using casein as the substrate was assayed as per the method of Birk*et al*., (1962). Amylases were determined by the dinitrosalicylate (DNS) methods described by Noelting and Bernfeld (1948) and the endoglucanase property of cellulases - CMCase was done by using carboxymethyl cellulose (Mandels, 1976).

Data analysis

Data analysis was done by ANOVA. The differences in mean were tested by using Duncon (1995) analysis. Significant level used was p ≤0.05. All the statistical analysis was performed using the software SPSS 22.0 for windows.

3. Results and discussion

The physiological changes occurred in the gut during the dormancy and active periods were analysed by observing the biochemical parameters in the gut and gut contents. Compared to the active stage, dormancy period showed higher temperature along the gut regions ranging from 29.100±0.300 to 29.823±0.290 ^{0}C. The active stage showed highest temperature in stomach (24.800±0.122 ^{0}C) and lowest in the intestine (23.925±0.433 ^{0}C) (Fig. 1). During dormancy, pH observed to be in between 6.588±0.08 to 7.475±0.03 (Fig. 2), but during the active stage, it was in between 5.837±0.06 to 8.655±0.02. An acidic pH was noted in oesophagus and stomach, whereas a basic pH was noted in intestine and rectum.

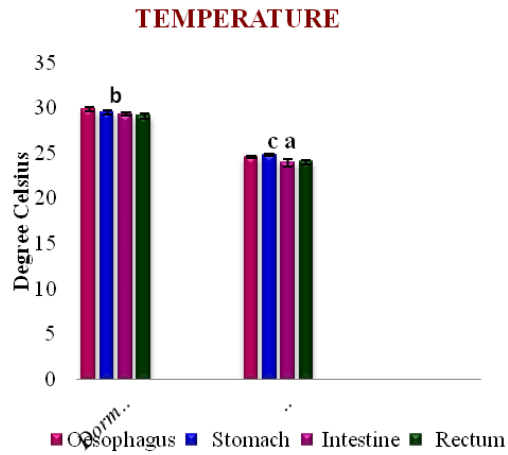

Figure 1- Temperature in different regions of gut content during dormancy and active periods (In degree Celsius). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

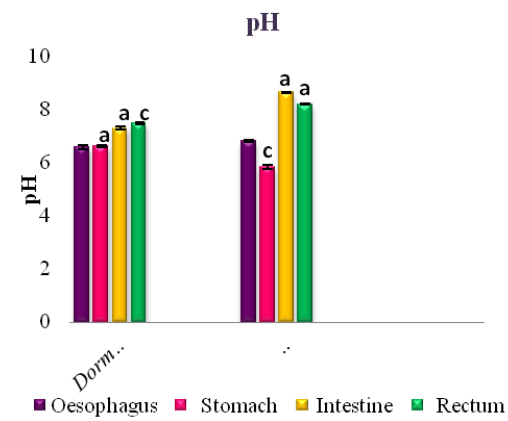

Figure 2- pH in different regions of gut content during dormancy and active periods. 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

Significant changes were observed in gut contents with respect to the moisture content (Fig. 3). During dormancy stage, moisture in the gut content was below 0.588±0.040%, whereas in active period, moisture content showed significant changes as the gut contents moved from anterior to posterior regions. The highest moisture content (88.400±0.381%) was observed in oesophageal content while lowest was seen in rectal content (62.197±0.924%). It may be due to the uptake of *Papaya* leaves that contains 89.560±0.06% moisture content. A very negligible amount (below 0.005±0.001%) of ash was observed during the dormancy period (Fig. 4), but in active period, the ash content was found in between 1.175±0.159% to 2.292±0.086%,

while, the ash content of *Papaya* was observed as 2.274±0.32%.

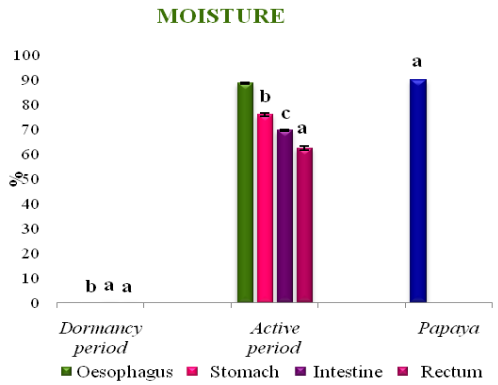

Figure 3- Moisture content in different regions of gut content during dormancy and active periods (In % tissue). 'a' significant p≤0.001,'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

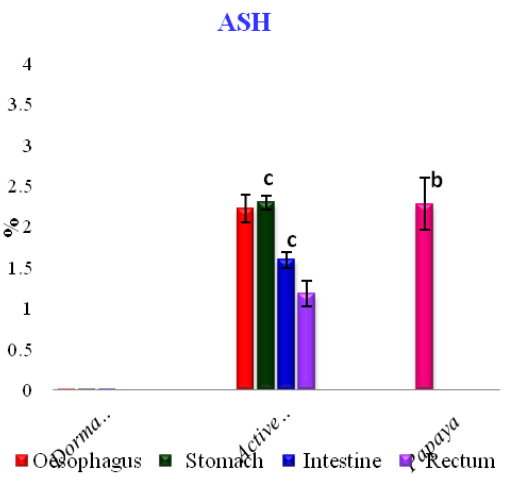

Figure 4- Ash content in different regions of gut content during dormancy and active periods (in % tissue). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

The enzymatic and biomolecular analyses of different regions of gut and gut contents showed specific patterns of the digestion during aestivation and active periods. During the dormancy period, biomolecular components (carbohydrates, protein, lipid and cellulose) in different parts of gut were not present in significant quantity, because, during aestivation, the mouth aperture of the snails is closed and feeding is arrested. 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 in low enzymes activities as observed in the present study. The above enzymes could also have even been found in herbivorous molluscs - *Aplysia* and*Limnaea* (Carriker, 1946). It has been claimed that esterase may be present in the saliva of *Helix* (at least in the crop juice), but it is possible that esterase reached the stomach, but, it is not secreted by the salivary glands (unknown reasons). The present study clearly showed that there was a significantly low level of oesophageal content in *Achatinafulica* when food was supplied(papaya). This is in compromise and favour the conclusions of Carriker (1946).

African giant land snail is well equipped with various enzymes to cater for its multivarious feeding habit (Yoloye, 1994). The present study confirms this as all the studied enzymes were present in all the mentioned gut regions irrespective of dormancy and active periods. The concentration of protease (Fig. 6) has been increased along the gut that resulted in a significant decrease of protein content from oesophagus to rectum (Fig. 5). Since, pH of stomach (5.837±0.06) in gastropod molluscs is more acidic than other GI tract regions (Kumud and Minakshi., 1994), a significant protein digestion was observed in the stomach (5.129±0.065 %) as the food moved from oesophagus (6.552±0.028 %). The presence of protease in the gut suggests that the experimental snails consumed proteic food substance along with their normal carbohydrate diets. Even in the absence of sufficient amount of protease enzyme (0.574±0.015 U/ml), a significant protein digestion was observed in the rectum, microbial flora which aid in protein digestion.x The findings was agreed with the observations of Pawar*et al*., (2012) reported the presence of γ-Proteobacteria in rectum of *A. fulica*.

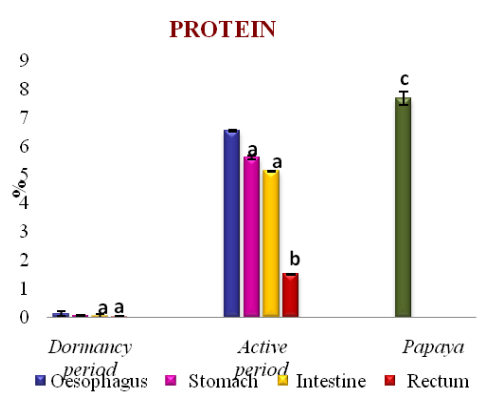

Figure 5- Total protein in different regions of gut content during dormancy and active periods (in % tissue). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

e ISSN 2348 –1269, Print ISSN 2349-5138
Cosmos Impact Factor 4.236

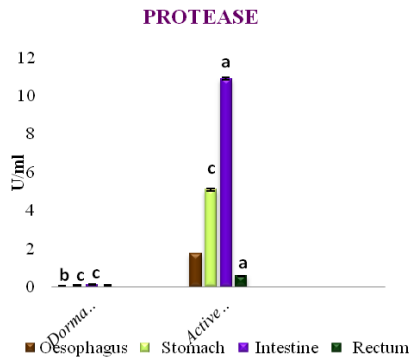

Figure 6- Total protease in different regions of gut during dormancy and active periods (in U/ml). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

The enzyme amylase is needed to hydrolyze starch which is the main component of Papaya leaves. Therefore, it was found in much higher quantities than other enzymes present in the gut (Fig. 8). Highest enzyme concentration was observed in the intestine (10.110±0.014 U/ml) as the pH of 8.6 with a temperature of 23.9 ^{0}C and the lowest was observed in the rectum (2.333±0.041 U/ml) at the pH of 8.2 with 24.5 ^{0}C temperature. Ghose (1961) reported the presence of amylases in the intestine, digestive gland, stomach and rectum of *A. fulica*. Thus, the study suggests the intestine as the major site of carbohydrate digestion (Fig. 7). In *A. fulica,* carbohydrate and the protein in the gut content had been decreased drastically. This may be due to high quantity of carbohydrases, proteases (including peptidases and perhaps in certain cases also dipeptidases as extracellular enzymes), and esterase (lipase) enzymes produced by the midgut gland. The amount of enzyme produced by this gland seems to depend more or less on the natural diet and on the amount of food available. These enzymes may be of bacterial origin. But Mansour (1946) found protease, peptidases and esterase in the stomach juice of *Tridacnapinctada* and*Unioprasedens.*

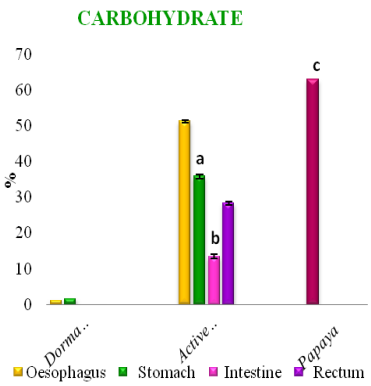

Figure 7- Total carbohydrate in different regions of gut content during dormancy and active periods (in % tissue). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

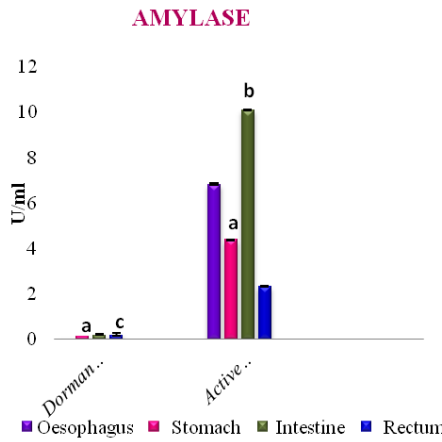

Figure 8- Total amylase in different regions of gut during dormancy and active periods (in U/ml). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

The enzyme cellulase is responsible for the breakdown of cellobiose, the product of cellulose hydrolysis which is the common part of plant cell (Horton and Pigman, 1970). The optimum activity of cellulase enzyme was observed in the intestine of both the snails in a basic pH of 8.6 and with a mild temperature of 23.9 ^{0}C. Compared to proteases and amylases, cellulases were present in very small quantities in the gut (below 3.515± 0.234 U/ml) (Fig. 10). The significant reduction in the cellulose content was observed in the intestine (Fig. 9) even in the absence of sufficient enzyme concentrations (3.515±0.234 U/ml), suggests the activity of cellulose digesting microbes in the intestine as a result of the symbiotic relationship between microorganisms and the snails. This is in compromise with the findings of Aravind*et al*., (2017) on the cellulose digesting enzyme production of cellulolytic bacteria isolated from the gastrointestinal tract of *A. fulica* that showed the optimum growth of cellulose digesting bacteria in the intestine where the value of pH was in between 8.57 and 9. 07. The present study also showed the optimum cellulase activity at the pH ranging between 8.6 and 8.8. The studies of Ghose (1961), also confirms the presence of cellulolytic bacteria in the intestine of *A. fulica*.

The lipid content of gut in *A. fulica* during the dormancy period was found to be in between 0.004±0.001 to 0.026±0.006%. The

active period showed much higher lipid content ranging in between 5.897±0.371 to 12.348±0.672%. Eventhough, significant amount of lipid content was observed from oesophagus to rectum, a very low lipid digestion was observed (Fig. 11).

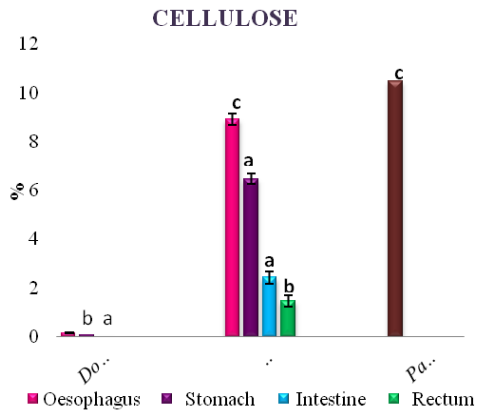

Figure 9- Total cellulose in different regions of gut content during dormancy and active periods (in % tissue). 'a' significant p≤0.001, 'b' significant p≤0.01 'c' significant p≤0.05., ±S.E.

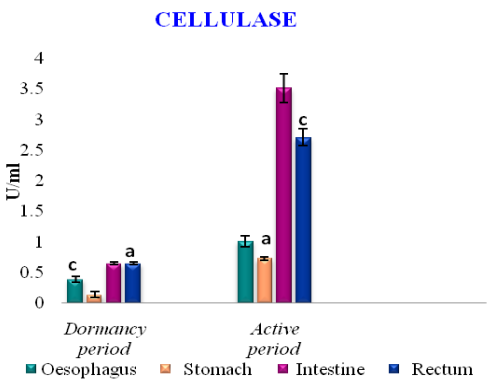

Figure 10- Total cellulase in different regions of gut of during dormancy and active periods (in U/ml). 'a' significant p≤0.001, 'b' significant p≤0.01, 'c' significant p≤0.05., ±S.E.

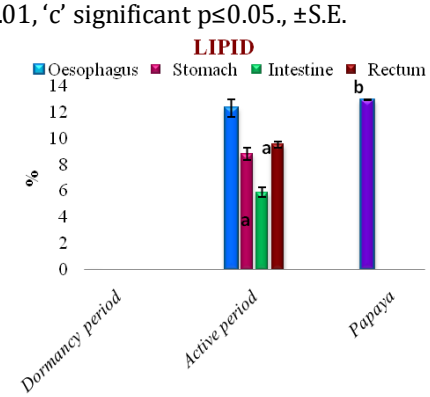

Figure 11- Total lipid in different regions of gut content during dormancy and active periods (in %). 'a' significant p≤0.001, 'b' significant p≤0.01,'c' significant p≤0.05., ±S.E.

4. Conclusion

Significant differences were observed in the gut contents during the dormancy and active periods and a disproportionate digestion rate was observed in relation to the concentration of digestive enzymes. The present study inferred that the animals can adjust its gut physiology by accommodating the physical and biochemical characteristics in the gut contents during aestivation and active period, in order to sustain their life activities as a pest species.

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