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Research Article

STUDY OF ENZYME ACTIVITY IN THE LAND SNAIL *CRYPTOZONA BISTRIALIS* IN NAGAPATTINAM, TAMIL NADU INDIA

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ABSTRACT

Enzyme assay was made to amylase, protease, lipase, cellulase, acid phosphatase and alkaline phosphatase in different zones of the gastrointestinal tract and digestive gland of the land snail *Cryptozona bistrialis* in summer and monsoon season. The amylase activity ranged from 2.46 to 8.42 IU/mg/protein. Protease varied from 1.69 to 3.90 µ mole tyrosine /mg/ protein/ hr. Lipase activity ranged from 1.01 to 5.01 µ mole tyrosine/ mg/ protein/ hr. The total activity of cellulase ranged between 0.74 and 13.12 µ mole tyrosine/mg/protein/hr. The level of acid phosphatase ranged from 1.7 to 4.03 µ mole p-nitrophenol/ mg/ protein /hr and alkaline phosphatase level ranged from 3.27 to 4.90 µ mole/ p-nitrophenol/ mg/ protein/ hr. The highest activity of enzymes were noticed in the digestive gland and lowest activity was found in stomach in both seasons. It was also observed that the land sail *C.bistrialis* showed strong amylase activity followed by alkaline phosphatase. Seasonally the maximum activity of enzymes were found in monsoon and minimum was in summer.

Keywords: Enzyme activity, Gastrointestional tract, Digestive gland, *Cryptozona bistrialis*, Seasonal changes.

INTRODUCTION

Enzymes are protein made by living cells to promote specific metabolic reactions. They are necessary to properly digest and absorb all nutrients in order to give the body what it needs to function. Metabolic enzymes run all the body organs and system by performing various chemical reactions within the body cells. In Land snail, nutrition is influenced by various factors such as pH, digestive enzymes, buffering mechanisms etc., in addition to food selection and feeding mechanism. The digestive tract of *Helix pomatia* is known to contain many digestive enzymes. Holden and Tracey (1950) list a wide variety of substrates which are attacked by Helix specific digestive juices.

Investigations on proteolytic and lipolytic enzymes of gastropods are few. Extracellular proteases have been recorded in carnivorous snails. Ferreri and Ducato (1959) have extensively studied the lipases and esterases of gastropods. A number of investigations have been carried out on food and feeding habits and anatomy of the digestive tract of the gastropods (Purchon,1977; Balaparameswara Kamala,1983). Correlation between digestive enzymes and feeding habit in molluscs has also drawn the attention of Yonge (1932), Biswas and Ghose (1968) and Das *et al.* (1982). The present investigation was made to study the enzymatic activity in the different zones of the gastrointestional tract and digestive gland of land snail, *Cryptozona bistrialis.*

MATERIAL AND METHODS

Preparation of an enzymatically active extract from *C.bistrialis*

Land snail, *C.bistrialis* were collected from the Kadambadi area of Nagapattinam. For enzyme assay 20 snails at each season (summer and monsoon season from April-2007 to December 2007) were collected. The shells were removed and dissected and removed the digestive tract. The organs like oesophagus, stomach and digestive gland were separated from the snails. The different organs of the digestive tracts of the snails were weighed accurately to the nearest 0.1mg and rinsed with saline. These tissues were homogenized with 6.8 pH phosphate buffer in a homogenizer. Each homogenate was centrifuged at 3000

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rpm for 20-30 mts and the clear brown supernatant was filtered through a muslin cloth. The filtrate thus obtained was used in the enzyme assay for amylase, protease, lipase, cellulase, acid phosphatase and alkaline phosphatase.

Estimation of enzymes

Amylase enzyme was estimated by Caraway method (1959). Protease enzyme was estimated by Snell and Snell (1971). Lipase enzyme was estimated by Oser (1965). Acid phosphatase activity was determined by Barret (1972). Alkaline phosphatase activity was determined by Barret (1972). VI) Cellulase enzyme was estimated by Miller (1959).

RESULTS

The results of the present study on the activity of enzymes viz., amylase, protease, lipase, acid phosphatase and alkaline phosphatase in the different zones of the gastro intestinal tract and digestive gland in the summer and monsoon seasons are given in Table 1.

a) Amylase

The total activity of amylase ranged from 2.46 to 8.42 IU/mg/protein. The amylase activity was greater in the oesophagus in the monsoon and digestive gland in summer. Stomach recorded the minimum activity in the both seasons. The highest activity was noticed in the digestive galnd. Seasonally the maximum amylase activity was found in monsoon.

b)Protease

The total activity of protease varied form 1.69 to 3.9 µ mole tyrosine/mg/protein/hr. The highest and lowest activities were recorded in the digestive gland and oesophagus in both seasons. Proteolytic activity was strong in monsoon season and weak in summer season.

c) Lipase

The total activity of lipase ranged between 1.01 and 5.01 µ mole tyrosine/ mg/ protein/ hr. The high level of lipase recorded in the digestive gland and low level of lipase in the oesophagus. It could be also noticed that the lipase activity was pronounced in summer and weak activity was in the monsoon season.

d) Cellulase

The total activity of cellulase ranged between 0.74 and 13.12 µ mole tyrosine/mg/protein/hr. The high level of cellulose recorded in the digestive gland and low level of cellulase in the stomach. The highest and lowest activities were recorded in the stomach and oesophagus in both seasons.Cellulase activity found in the oesophagus was scanty in both summer and monsoon seasons. It could be also noticed that the cellulose activity was pronounced in monsoon season (25.65 µ mole tyrosine/mg/protein/hr) and weak activity was in the summer (9.94 µ mole tyrosine/mg/protein/hr).

e) Acid Phosphatase

The total activity of acid phosphatase varied from 1.7 to 4.03 µ mole p- nitrophenol/ mg/ protein/ hr. The highest activity was recorded in the digestive gland and lowest activity in the stomach. The maximum activity was observed in the summer season and minimum was recorded in the monsoon season.

f) Alkaline Phosphatase

The total activity of alkaline phosphatase ranged between 3.27 and 4.90 µ mole p-nitrophenol/mg/protein/hr. The alkaline phosphatase activity was greater in the digestive gland and lesser in the oesophagus. The maximum activity of this enzyme was found in summer season and minimum in monsoon.

Table 1. Enzyme activity (IU/mg) in the different zones of the gastrointestinal tract and digestive gland of *Cryptozona bistrialis* in summer and monsoon season.

Enzyme	Summer			Total enzyme activity	Monsoon			Total enzyme activity
	Oeso-phagus	Stomach	Digestive gland		Oeso-phagus	Stomach	Digestive gland	
Amylase	4.82	2.46	6.25	13.53	8.42	5.23	8.12	21.77
Protease	1.69	2.79	3.2	7.68	2.1	2.9	3.9	8.9
Lipase	1.28	3.85	5.01	10.14	1.01	1.64	2.06	4.71
Cellulase	0.74	6.52	2.68	9.94	5.38	13.12	7.15	25.65
Acid phosphatase	3.5	1.7	4.03	9.23	2.15	2.98	3.05	8.18
Alkaline phosphatase	3.27	3.52	4.68	11.47	3.84	4.25	4.90	12.99
Total enzyme activity	15.3	20.84	25.85	61.99	22.9	30.12	29.18	82.2

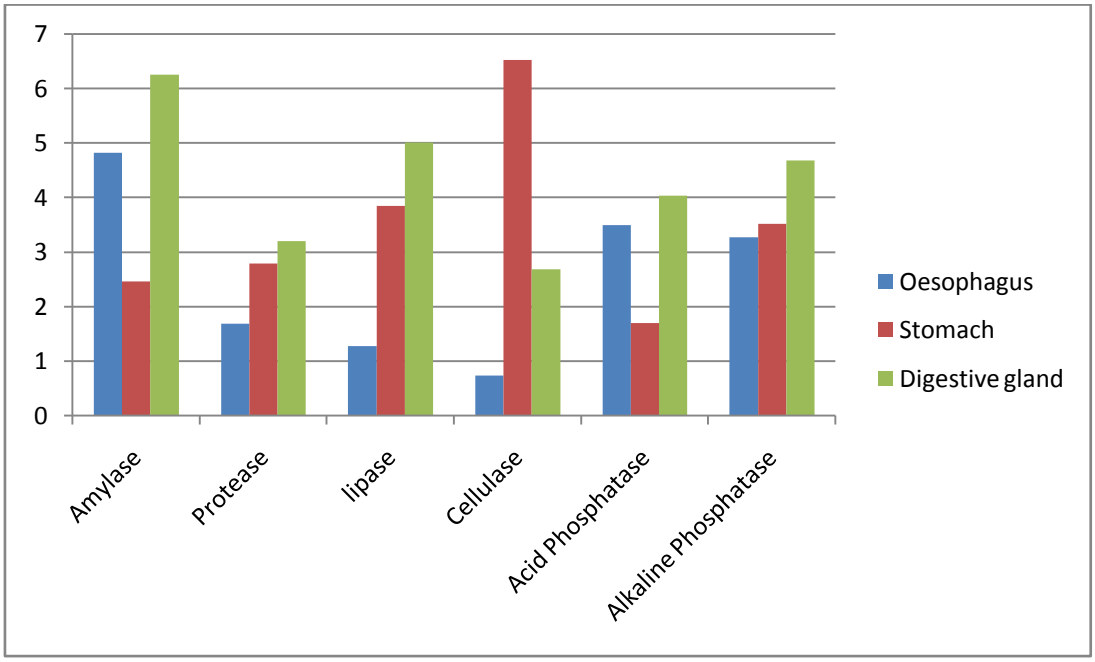

Figure 1. Enzyme activity (IU/mg) in the different zones of the gastrointestinal tract and digestive gland of *C.bistrialis* in summer season.

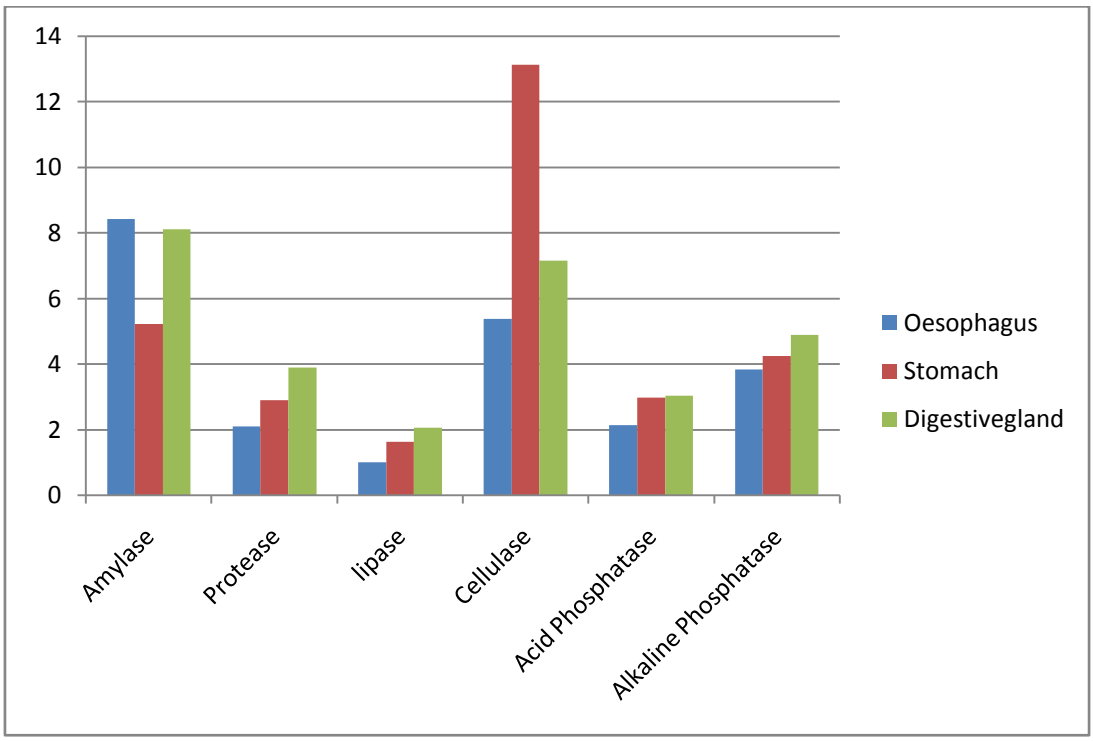

Figure 2. Enzyme activity (IU/mg) in the different zones of the gastrointestinal tract and digestive gland of *C.bistrialis* in Monsoon season.

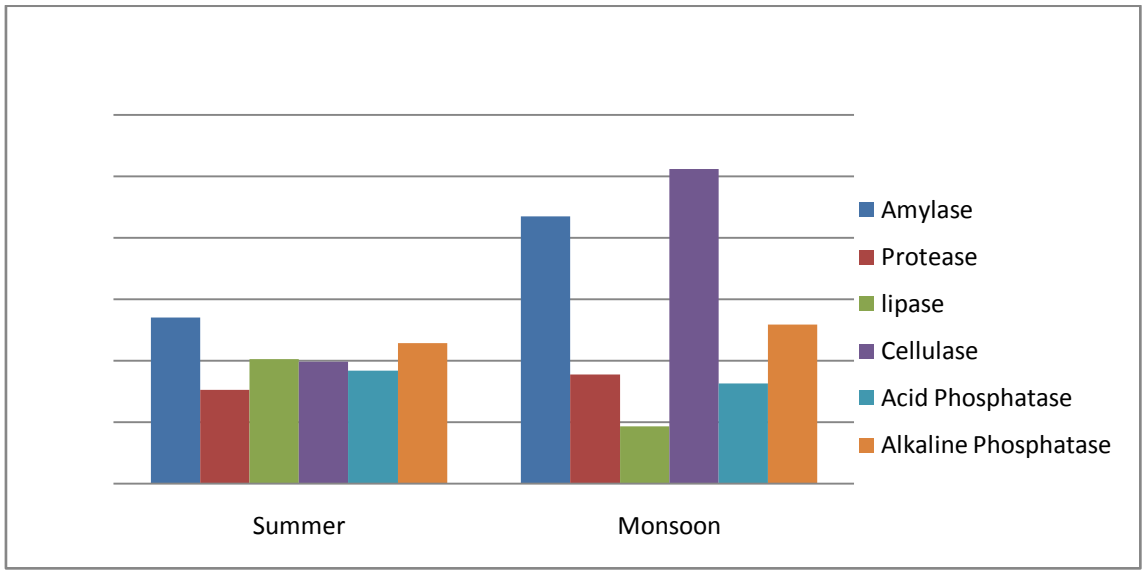

Figure 3. Enzyme activity (IU/mg) in the different zones of the gastrointestinal tract and digestive gland of C.bistrialis in summer and monsoon season**.**

DISCUSSION

Gastropods feed on a wide variety of food for their substinance (Purchon, 1977). An extreme variation in the secretion of hydrolysing enzymes of gastrointestinal tract and associated organs of gastropods has been reported by a number of authors (Ghose, 1961; Ghose *et al.* 1982; Chaki *et al.*, 1983).

The carbohydrases are the predominent enzymes showing high activity in pulmonate snails. Among the carbohydrases, the amylase activity is high in the midgut and the digestive gland in the some snails. Purchon (1977) stated that some gastropods possessed glandular oesophageal pouches which might supply the amylase, as confirmed by the present study.

The digestive gland of *C.bistrialis* showed maximum activity of the amylase in monsoon and summer seasons. These findings support the previous ones on *C. radiata* (Balaparameswara Rao,1975a) and Euchelus (Kamala, 1983). Similarly, high amylase activity in the stomach and digestive gland was recorded by Stone and Morton (1958) in *P.vulgata*.

In *M.lineata*, Stone and Morton (1958) observed that the stomach and the digestive gland released more sugar than the foregut. Maximum amylase activity in the midgut of *T. funebralis* has been recorded by Galli and Giese (1959) as in the present investigation except stomach region. The present findings clearly showed that the amylase activity showed marked difference in summer and monsoon season may be due to landsnail, *C.bistrialis* was very active in the monsoon than summer as it was aestivate, present analysis inferred that greatest activity in the digestive gland and followed by oesophagus.

Protease is generally wanting from the secretion of the foregut glands but is usually present in more posterior regions of the digestive tract, this findings was not aggreed with the present study. The presence of proteolytic activity in the stomach and digestive gland of *C.bistrialis* was supported by the finding of Krukenberg (1882). He found protease present in the crop and the stomach fluids of various terrestrial pulmonates. In the present investigation the activity of protease was weak noted in the oesophagus of *C.bistrialis*.

The present study showed that the lipase activity was minimum in the oesophagus and pronounced lipase actvity in the digestive gland and stomach of *C.bistrialis* is an aggrement with the studies on *E.asper* by Kamala (1983). Acid phosphatase activity was mainly localized in the lysosomes in all the regions of the outer epithelium. It is postulated that the acid phosphatase in the mantle epithelium is responsible for conferring calcifiability to the organic matrix of the shell (Joyce Chan and Saleuddin, 1973).

The invertebrate mollusc, African giant snail (*Archachatina marginata*), feeds on any edible plant or animal matter. These range from succulent fruits and vegetables ,decaying organic material and grains (Wosu, 2001). The nature of their food will require an effective cellulase system for degradation and digestion. Some have reported possible endogenous enzyme sources are the hepatopancreas, gastric teeth and crystalline styles (Whitaker *et al.*, 1963). The present work report the presence of a cellulolytic enzyme from the stomach and

digestive gland of gastrointestinal tract of the C.bistrialis snail. Seasonally cellulase was predominant during monsoon season than the summer season.The occurrence of a cellulolytic enzyme capable of hydrolysing soluble cellulose mainly may be because of the adaptation of the snail to majorly a vegetarian diet. Thus, it enables the animal to grow on the moist leafy vegetables, which form its major food sources.

During shell regeneration, there is a speeding up of calcification process due to cellular activities and alteration of the number of organelles in the cell of the mantle epithelium. These activities are governed by mainly alkaline and acid phosphatases (Saleuddin, 1970; Tse and Salenddin,1971). The present study revealed that the activity of acid phosphatase was found to be greater in the digestive gland and lesser activity in the stomach. Seasonally maximum activity is noted in the summer season. The high activity of acid phosphatase in hepatopancreas of land snails has also been reported earlier (Baldwin,1938) could further support the present investigation.

Alkaline phosphatase was exhibited higher activity in hepatopancreas of Pila globosa than foot (Srinivasa Reddy *et al.,* 1978) suggesting that hepatopancreas is a labile store of the various phosphate esters. The enzyme activity was found to decrease in the aestivated snail. During aestivation, the snails would be in state of suspended animation, where in, the locomotor, reproductive and digestive function cease to operate (Srinivasa Reddy and Swami, 1976). These finding on enzyme activity seems to be agreement with the present study.

The results indicated the predominant role of the digestive gland in the secretion of the enzyme responsible for the degradation of the most of the food intake and calcification for shell regeneration. Oesophagus secreted the enzymes but in amounts lower than secreted by the digestive gland. Thus the enzymatic activity fluctuated seasonally and also depends more on the nature of the diet, amount of food available, physiological status and seasonal variation.

CONCLUSION

The land snail *C.bistrialis* is the paramount sourse of digestive enzymes.The activity of the enzyme is greately influenced by season and availability of food. The present finding will imminent resercher to work more on the importance of enzymes in various biological reactions and application of enzymes in several field.

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REFERENCES

Balaparameswara Rao, M., 1975. Some observations on feeding,anatomy,histology of the digestive tract and digestive enzymes in the limpet *Cellana radiata* (Born) (Gastropoda: Prosobranchia). *Proc. Malac. Soc. Lond.,* 41, 309-320.

Baldwin, E., 1938. On the respiratory metabolism of *Helix pomatia*. *Biochem. J.,* 82, 1225.

Barrett, A.J., 1972. In Lysosomes, A Laboratory Handbook (Dingle, J.T., ed.), North Holland, Amsterdam and London. p. 46-135.

Biswas, A.K. and Ghose, K.C., 1968. Elobration of enzymes in the digestive gland of *Belamya bengalonsis* (Mollusca: Gastropoda) during the first hours of digestion. *J. Zool. Lond.,* 156, 325-332.

Caraway, W.T., 1959. A stable starch substrate for the determination of amylase in serum and other body fluids. *Am. J. Clin. Path.,* 32, 97-99.

Chaki, K., Sur, R, and Ghose, K.C., 1983. Localization activity and characterstics of α- amylase in active and estivating *Achatina fulica*. *Indian Biologist,* 10, 22-24.

Das, S., Ghose, U.R., Sur, R. and Ghose, K.C., 1982. Comparative studies on food and digestion. In *Betamya bengalinsis*, A crostonia variable ND *Telescopient telescopium* (Prosobranch: Gastropoda: Molusca) *Comp. Physical. Biol.,* 3, 183-187.

Ferreri, E. and Ducato, L. 1959. Verglichende biochemische and histochemiche untersuchungen uber die lipolytische tatigkeit des Darkanal epitheliums von Zellforsch. *Mikroskop. Anat.,* 51-57.

Galli, D.R and A.C. Giese, 1959. Carbohydrate digestion in a heribivorous snail, Tegula funebralis. *J. Exp. Zool.,* 140, 415-440.

Ghose., K.C. 1961. Observation on the digestive enzymes and cellulolytic bacteria of the gaint land snail. *Achatina fulica* their occurrence in the gastropoda. *Proc. Zool. Soc. Lond.,* 137, 127-133.

Holden, M. and Tracey, M.V., 1950. A study of enzymes that can break down tobacco-leaf components. 2. Digestive juice of *Helix* on defined substrates. *Biochem. J.,* 47, 407-414.

Joyce Chan, F.Y. and Saleuddin, A.S.M., 1973. Acid phosphatase in the mantle of the shell regenerating snail *Helisoma duryi duryi* calcified. *Tissue. Res.,* 5, 213-220.

Kamala, B., 1983. Studies on some aspect of the biology of the top shell *Euchelus asper* (Gmelin) (Gastropoda, prosobranchia) of the palm beach shingles of the Visakhapatnam coast. Ph.D. Thesis, Andra university, Waltair.

Krukenberg, C.F.W., 1882. Vergleichend-physiologische studien an den K and ten der Andria. *Experimentelle Untersuchungen,* 2, 143-147.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chem.,* 31(3), 426.428.

Oser, B.L., 1965. Hawk's Physiological Chemistry. 14th edn. McGraw-Hill Co, New York.

Purchon, R.D., 1977. The biology of the Mollusca (11th Ed). Pergamon press, Oxford.

Saleuddin, A.S.M., 1970. Electron microscopic study of the mantle of normal and regenerating *Helix*. *Can. J. Zool.*, 48, 409-416.

Snell, F.D. and Snell, C.T., 1971. Colometric methods of analysis Van Nostrane Reinhold. Co., New York, p.7-145.

Srinivasa Reddy, Y. and Swami, K.S., 1976. Some metabolic effects of aestivation on glycolysis in *Pila globosa* (Swainson). *Indian J. Exp. Biol.*, 14, 191-193.

Srinivasa Reddy, Y., Subba Reddy, S. and Swami, K.S., 1978. Alkaline phosphatase activity in the tissues of active and aestivated *Pila globosa*. *Curr. Sci.,* 47,138-139.

Stone, B.A. and Morton, J.E., 1958. The distribution of cellulase and related enzyme in Mollusca. *Proc. Malac. Soc. Loc.*, 33, 127-141.

Tse, V. and Saleuddin, A.S.M., 1971. Studies on shell regeneration in *Helisoma duryi*. *Proc. Can. Fed. Biol. Soc.*, 14, 82.

Whitaker, D.R., Hanson, K.R. and Datta, P.K., 1963. Improved procedures or preparation and characterization of Myrothecium cellulase. P art 2. Purification procedures. *Can. J. Biochem. Physiol.*, 41, 671-696.

Wosu, L.O., 2003. Commercial snail farming West Africa: A Guide. Ap Express Publishers, Limited, Nsukka, Nigeria.

Yonge, C.M., 1932. Notes on feeding and digestion in Pterocera and Vermetus with a discussion on the occurrence of the crystalline style in the Gastropoda. *Sci. Rept. Gt. Barrier Reef Exped.* 1, 259-281.