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Digestion and Utilization of Protein in *Gesonula punctifrons* Stal. (Acrididae : Orthoptera)

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Protein percentage of the host plant *(Eichhornia crassipes)* leaves and its utilization by the acridid, *Gesonula punctifrons* Stal. were calculated. The activity of the necessary enzyme, protease and its regional distribution in the gut were simultaneously investigated. Characterization of the enzyme was also done with respect to the effect of *p*H, substrate concentration, temperature and starvation. Protease is presumed to be secreted by a secretagogue model.

Key Words : Gesonula punctifrons, Host-leaf protein, Utilization, Protease activity

Introduction

Nutritional needs of grasshoppers are similar to those of most insects and include optimal level of proteins, carbohydrates, lipids, some water-soluble vitamins and organic salts at low concentration (Bernays & Chapman 1978). Among the nutrients, protein fulfils a major part in dietary requirements both from quantitative (Schroeder 1986) and qualitative aspects (Broadway & Duffey 1988) and its proper digestion is very much essential for an insect. Broadly speaking, digestive enzymes of an insect are adapted to the diet on which the species feeds (Pilk & Kabacik-Wasylik 1989). Broadway and Duffey (1986) demonstrated in the beet armyworm, *Spodoptera exigua* (Hübner) that quantity of dietary protein not only significantly influences the growth of the larva but also is directly correlated with the level of tryptic activity in the gut. In case of grasshopper *Oxya fuscovittata* (Marshall), the activity of midgut protease was found to be influenced by the host plant protein and the enzyme has an effective role on the growth and development of the grasshopper (Partho et al. 1993). The purpose of the present investigation was to study the activity and characteristics of digestive protease of the grasshopper *Gesonula punctifrons* Stal. and its potential in utilization of the dietary protein.

[1]The experiment was initiated under Late Dr R K Sur, who deceased on 8.1.92.

Materials and Methods

Insect Culture

Adult females of *Gesonula punctifrons*, measuring 2-2.1 cm in length were collected and acclimatized to the laboratory condition in glass containers (13.5" × 10.5" × 11") and offered same sized fresh leaves of water hyacinth (*Eichhornia crassipes*) everyday.

Analysis of Host-leaf Protein

Water soluble protein in the dry weight of leaf tissue was extracted in 0.05 M phosphate buffer (*p*H6.8) and precipitated with 40% tricarboxylic acid (TCA). The precipitate was dissolved in 0.1N NaOH and total protein was estimated following the method of Lowry et al. (1951). Alkali-soluble protein in the leaf material was estimated following the method of Lowry et al. (1951) after extraction in 80% ethanol and alkali hydrolysis of the protein in 0.3 N NaOH. Total protein of the tissues was expressed in terms of dry weight percentage.

Utilization of Host-leaf Protein

Each animal was maintained in separate glass jar and offered same size leaves everyday. Area of the leaf ingested was found out from graph paper and thus the fresh weight of the leaf consumed was calculated from the standard curve of area vs weight (Santos et al. 1983). Dry weight and total protein of the leaf consumed were obtained from the value of the moisture content and percentage of total protein of the host-leaves respectively. Total water and alkali-soluble proteins of the excreta (dry weight) pooled from three specimens at every 24 hr were measured following Lowry et al. (1951). Total protein of the excreta expelled by an individual insect was obtained by multiplying the dry weight of the excreta expelled out/day with the mg of total protein/mg dry weight of excreta / day. Percentage of utilization of leaf protein was calculated from the formula of digestive coefficient after Waldbauer (1968).

$$\frac{\text{Protein ingested} - \text{Protein expelled out}}{\text{Protein ingested from the leaf}} \times 100$$

Enzymology

The enzyme was extracted from homogenized tissues (oesophagus, crop, gizzard, midgut, hind gut and caeca) with 3 ml of distilled water for 30 min. at 4°C at 5000 rpm for 5 min. and the supernatant was stored frozen (–20°C) until needed, usually within 2 days.

Optimum *p*H for enzyme activity was determined within the *p*H range from 2 to 9.5 using "acetate-HCl" and "double phosphate" buffers as required.

Protease was assayed at optimum *p*H of the enzyme following Ichishima (1970) using 2% vitamin free casein (Nutritional Biochemicals Corporation, USA) as substrate. For determination of enzyme activity 1ml of substrate and buffer was mixed with 1 ml of enzyme extract and incubated at 37°C for 1 hr. 2 ml of 6% TCA was added and centrifuged at 5000 rpm for 10 min. The activity was measured in terms of tyrosine equivalent at 625 nm from 1 ml of the aliquot after developing colour with 4% Na_2CO_3 and Folin reagent. Enzyme blank was prepared by addition of TCA prior to the addition of the extract.

Total protein of the extract was assayed following Lowry et al. (1951) and the enzyme activity was expressed in terms of tyrosine equivalent / 10 mg of total protein. Michaelis Menten constant was determined using different substrate concentrations as required. Optimum temperature for the enzyme activity was determined following

incubation at different temperature (27° to 67°C with an interval of 10°C) chambers.

Effect of starvation was studied only in the whole gut and caeca of the insects starved for 24 hours (at intervals 3, 6, 12 and 24 hr) after feeding commenced. The data were analyzed following students '*t*' test (Snedecoer & Cochran 1967) after comparing the data with 'control' (i.e. the insects fed *ad lib*).

Results

Host-leaf Protein

The data on percentage of total protein of the host-leaves (dry weight) and its utilization by the insect are given in tables 1 and 2 respectively.

Table 1 *Total protein of the leaves of water hyacinth, Eichhornia crassipes, dry weight %*

Total Protein	
Water soluble	Alkali soluble
0.221 ± 0.005	13.856 ± 0.319

Mean ± S.E, n = 5

Enzymology

Effect of *p*H on protease (figure 1) indicates that optimum *p*H of activity was noticed at *p*H 8.5 though at *p*H 4 the enzyme showed a small peak.

Zonal distribution of the protease (*p*H 8.5) showed that the region of oesophagus and crop was the chief site of activity (figure 2)

Michaelis Menten Constant and optimum temperature of the enzyme concerned are given in the table 3 and figure 3.

During starvation, protease remained unchanged in both the whole gut and the caeca with the exception of significant elevation of the specific activity ($P < 0.05$) by

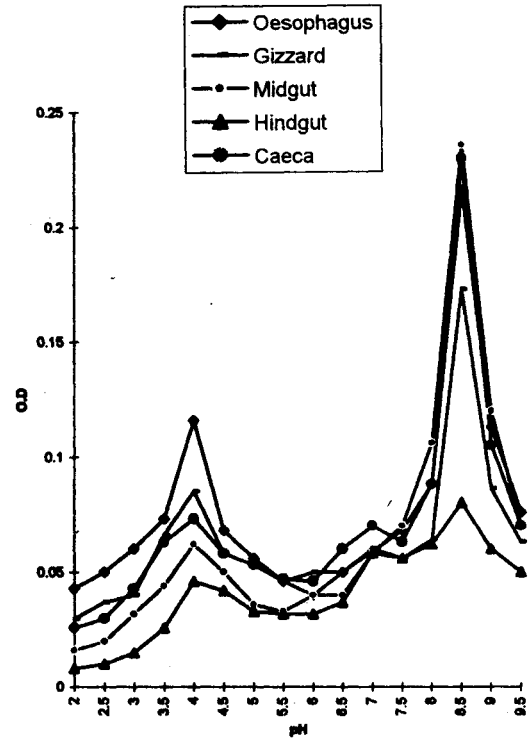

Figure 1 Effect of *p*H on protease activity in different portions of the gut of *G. punctifrons* Stal. (*OC*, Oesophagus + Crop, *Gi*, Gizzard, *Mg*, Mid gut, *Hg*, Hind gut and *Ca*, Caeca) n = 4

63.69% and 68.45% in the whole gut at 3rd and 6th hr of fasting respectively. In caeca, at the respective durations there was insignificant increase of total activity (table 4).

Discussion

Water and alkali soluble proteins of the host leaf altogether amount to 14.077% or 140.77 mg/g and total consumption of protein is 0.4335 mg/individual/day. The value of protein consumption is slightly less than that of the host *Coix lachryma* and slightly greater than that of the host *Cyperus rotundus* for the acridid grasshopper *Oxya fuscovittata*

Table 2 *Percentage of utilization of host protein by G. punctifrons* Stal.

(a) Water soluble protein

Area of the leaf eaten/day	Fresh weight of the leaf consumed/day	% of water in the host leaf	Dry weight of the leaf consumed by the insect/day	Total protein of the host leaf (dry weight)	Total protein of the leaf consumed (dry weight) by the insect/day	Dry weight of the excreta expelled/day	Total protein fo the excreta (dry weight)	Total protein of the excreta (dry weight) expelled out/day	% of utilization of host protein
cm^2/day	mg/day	%± S.E	mg/day	mg tyrosine eqv./mg d.w	mg tyrosine eqv./day	mg/day	mg tyrosine eqv./mg d.w	mg tyrosine eqv./day	%
124.33 ± 34.93	25.76 ± 7.18	86.76 ± 0.776	3.41 ± 0.947	0.00221	0.0075 ± 0.002	2.74 ± 0.402	0.002	0.0065 ± 0.002	13.75 ± 4.47
(b) Alkali soluble protein									
112.33 ± 15.39	23.38 ± 3.18	86.76 ± 0.776	3.09 ± 0.421	0.138	0.426 ± 0.058	3.5 ± 0.834	0.1385	0.487 ± 0.116	1.49 ± 1.48

% of utilization of total protein = 7.62 ± 2.97%; Mean ± S.E., n = 3

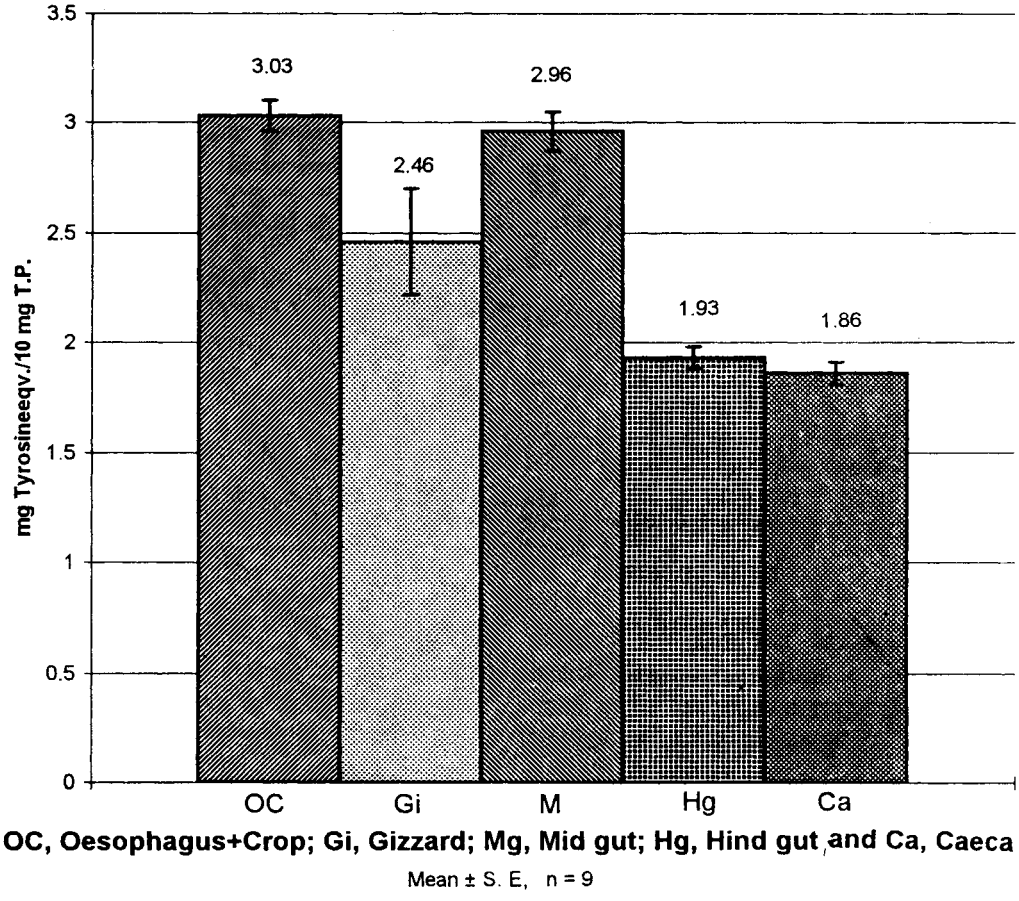

Figure 2 Zonal distribution of the activities of protease in *G. punctifrons*. (*OC*, Oesophagus + Crop, *Gi*, Gizzard, *Mg*, Mid gut, *Hg*, Hind gut and *Ca*, Caeca) n = 9

(Partho et al. 1993). With the experiments of artifical diets it was found that 20 - 25% of protein (as casein and yeast protein) allows 90% of the first instar *Melanoplus* to reach the adulthood (Kreasky 1962). A minimum of 20% to the maximum of 40% protein could be used by the *Schistocerca* and *Locusta* without any problem, however, the satisfactory synthetic diet comprised 27% protein (Dadd 1960 a, b, 1961). Food intake in the insects generally change in relation to their reproductive activities. It was postulated that utilization of protein in the ovary is poorer in the unmated females of *O. fuscovittata* than that of the mated females when both are reared on the same host leaf *C. lachryma* (Partho et al. 1993). In the present investigation, utilization of protein is only 7.62% which may be an indication of the absence of reproductive phase and the unutilized protein thus probably is excreted out. However, the larvae of silkworm digest 62% protein of the mulberry leaves to get sufficient protein for high growth rate (Trager 1953). In case of cut worm, *Agrotis segetum* (Schiff) the requirement of nitrogen by the

larvae decreases gradually during the last few instars (Gong et al. 1991). Protease, in the present investigation is maximally active in the oesophagus and crop and it may reach there by the process of regurgitation from ventriculus and caeca (Teo & Woodring 1988, 1994, Ferreira et al. 1990).

Table 3 *The km values of protease in different portions of the gut of G. punctifrons*

Zones	Km
Oesophagus + Crop	0.238 ± 0.030%
Gizzard	0.292 ± 0.104%
Midgut	0.700 ± 0.067%
Hindgut	0.142 ± 0.028%
Caeca	0.186 ± 0.090%

Mean ± S.E. n = 3

The two *p*H optima, *p*H 4.0 and 8.5 of the protease may be due to two types of proteolytic enzymes, cathepsin and trypsin (Mitra et al. 1970). However, the gut *p*H of *Gesonula* is slightly acidic (Chattopadhyay 1995); thus the activity of protease is partly inhibited in the gut of the same. Likewise, the trypsin like, chymotrypsin like and caseinolytic enzymes show optimal turnover at *p*H values above 7 in the midgut of *Apis mellifera* L. (Berta & Crailsheim 1987). The Michaelis Menten Constants of protease in the different regions of the gut are very low, indicating that protease has maximum activity at a low substrate concentration.

In the present investigation, optimum temperature for protease activity is found to be comparatively high i.e. 57°C. It is 60°C for *Vespa orientalis* (Hagenmaier 1971) and 45°C in *A. domesticus* (Teo & Woodring 1988). This refers to the physiological

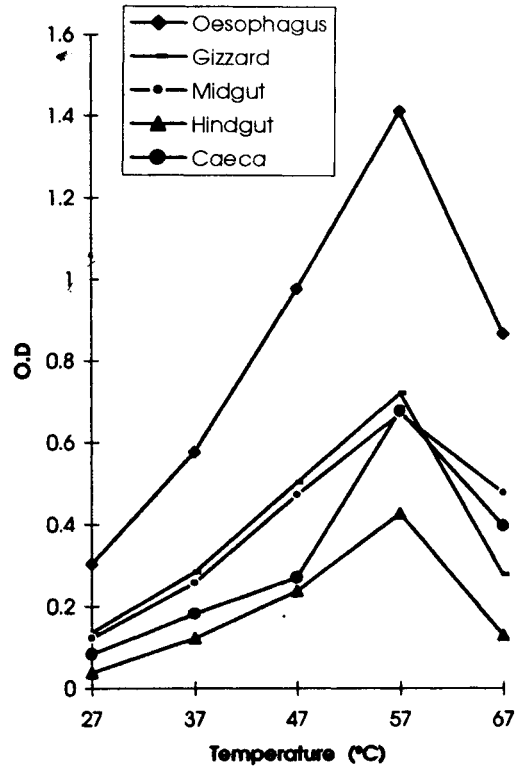

Figure 3 Effect of temperature on the activity of protease in different portions of the gut of *G. punctifrons*. (*OC*, Oesophagus + Crop, *Gi*, Gizzard, *Mg*, Mid gut, *Hg*, Hind gut and *Ca*, Caeca) n = 4

adaptation of the enzyme at higher temperature liberating more of water molecules for utilization (Mishra 1991).

G. punctifrons cannot tolerate starvation beyond 24 hr due to water loss by the processes of excretion, expiration through spiracles or evaporation through cuticle (Uvarov 1966). During 24hr protease shows no significant change though in the whole gut, the specific activity gets significantly elevated at 3rd and 6th hr of fasting. In the caeca, total activity rises slightly at the respective durations. Secretagogue model of

Table 4 *Effect of starvation on protease of the whole gut and caeca of G. punctifrons*

Period of testing (Hour)	Whole gut		Caeca	
	Total activity (mg tyrosine equivalent/gut)	Specific activity (mg tyrosine equivalent/10 mg total protein)	Total activity (mg tyrosine equivalent/caeca)	Specific activity (mg tyrosine equivalent/10 mg total protein)
0	0.391 ± 0.08	1.68 ± 0.232	0.208 ± 0.09	1.97 ± 0.515
3	0.385 ± 0.204	2.75* ± 0.272	0.294 ± 0.064	2.21 ± 0.530
6	0.423 ± 0.011	2.83* ± 0.204	0.282 ± 0.019	2.21 ± 0.163
12	0.203 ± 0.061	1.798 ± 0.210	0.155 ± 0.035	1.954 ± 0.284
24	0.349 ± 0.109	1.97 ± 0.227	0.162 ± 0.110	2.19 ± 0.767

Mean ± S.E, n = 5
*means significant, $P < 0.05$

proteinase production can be proposed in this case where some component of ingested meal is responsible for proteinase prduction (Houseman & Downe 1986). Similar activity curve was observed for the aminopeptidase in the whole gut of *Melanoplus sanguinipes* (Hinks et al. 1991) and also in the midgut of *Anopheles stephensi* Liston (Billingsley & Hecker 1991).

Thus, like other insects, protease of *Gesonula* may be secreted by secretagogue model, its activity is partly inhibited in the gut, though presence/absence of protease inhibitor in the host leaf has not been investigated. However, utilization of protein is found to be low, that may be due to higher dependence of the insect to the carbohydrate diet.

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References

Bernays E A and Chapman R F 1978 Plant chemistry and acridoid feeding behaviour; in *Biochemical Aspects of Plant and Animal Co-evolution* - Annual Proceedings of Phytochemical Society of Europe pp 99-141 ed. J B Harborne (London: Academic press)

Berta M and Crailsheim K 1987 Physiology of protein digestion in the midgut of the honey bee (*Apis mellifera* L.); *J. Insect Physiol.* 33 923-932

Billingsley P F and Hecker H 1991 Blood digestion in the mosquito, *Anopheles stephensi* Liston (Diptera : Culicidae) : activity and distribution of trypsin, aminopeptidase and α-glucosidase in the midgut; *J. Med. Entomol.* 28 865-871

Broadway R M and Duffey S S 1986 The effect of dietary protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*; *J. Insect Physiol.* 32 673-680

—— and —— 1988 The effect of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors; *J. Insect Physiol.* **34** 1111-1117

Chattopadhyay D 1995 *Food, feeding habit and digestion of two grasshoppers*; Ph.D. Thesis (submitted). Dept. of Zoology, Calcutta University, India

Dadd R H 1960a The nutritional requirements of locusts. I. Development of synthetic diets and lipid requirements; *J. Insect Physiol.* **4** 319-347

—— 1960b Observations on the palatability and utilization of food by locusts with particular reference to the interpretation of performances in growth trials using synthetic diets; *Ent. exp. appl.* **3** 283-308

—— 1961 The nutritional requirements of locusts. IV. Requirements for vitamins of the B complex; *J. Insect Physiol.* **6** 1-12

Ferreria C, Olivera M C and Terra W R 1990 Compartmentalization of digestive process in *Abracris flavolineata* (Orthoptera: Acrididae) adults; *Insect Biochem.* **20** 267-274

Gong P, Wu K and Li X 1991 The utilization of food and dietary nitrogen by the cut worm *Agrotis segetum* (Schiff.); *Sinozoologia* O 117-126

Hagenmaier H E 1971 Purification and characterization of a trypsin like proteinase from the midgut of the larva of the hornet, *Vespa orientalis; J. Insect Physiol.* **17** 1995-2004

Hinks C F, Cheeseman M T, Erlandson M A. Olfert O and Westcott N D 1991 The effects of kochia, wheat and oats on digestive proteinases and the protein economy of adult grasshoppers, *Melanoplus sanguinipes; J. Insect Physiol.* **37** 417-430

Houseman J G and Downe A E R 1986 Methods of measuring blood meal size and proteinase activity for determining the effects of mated state on digestive process of female *Aedes aegypti* (L.) (Diptera : Culicidae); *Can. Entomol.* **118** 241-248

Ichishima E 1970 Purification and mode of assay for acid proteinase of *Aspergillus saitoi* : in *Methods in Enzymology*, Vol XIX pp 397-399 eds S P Colowick and N O Kaplan (New York: Academic press)

Kreasky J B 1962 A growth factor in romaine lettuce for the grasshoppers *Melanoplus bilituratus* (Wlk.) and *M. bivittatus* (Say); *J. Insect Physiol.* **8** 493-504

Lowry O H, Rosebrough N J, Farr A L and Randall R S 1951 Protein measurement with the folin phenol reagent; *J. Biol. Chem.* **193** 265-268

Mishra S C 1991 Effect of temerature and relative humidity on consumption and digestion of food (bark) by the larvae of *Indarbela quadrinotata* Wlk (Lepidoptera : Indarbelidae); *J. ent. Res.* **15** 287-293

Mitra D, Banerjee H and Raychaudhuri D N 1970 Protease activity in normal and aposymbiotic gut of the grasshopper, *Gesonula punctifrons* Stal.; *Proc. Zool. Soc.*, **23** 155-160

Partho P D, Sanjayan K P and Muralirangan M C 1993 Nutrition modulated proteolytic activity during oocyte development in *Oxya fuscovittata* (Marshall) (Orthoptera : Acrididae); *Proc. Indian Nat. Sci. Acad.* **B59** 139-145

Pilk L and Kabacik-Wasylik D 1989 Activity of amylase and proteinase digestive enzymes at four species of field carabidae; *Pol. ecol. Stud.* **15** 119-125

Santos C D, Ferreira C and Terra W R 1983 Consumption of food and spatial organization of digestion in the cassava hornworm, *Erinnyis ello; J. Insect Physiol.* **29** 707-714

Schroeder L A 1986 Protein limitation of a tree feeding lepidopteran; *Ent. exp. appl.* **41** 115-120

Snedecoer G W and Cochran W G 1967 *Statistical Method* 6 edn. Iowa State University Pr. Ames, Iowa

Teo L H and Woodring J P 1988 The digestive protease and lipase in the house cricket *Acheta domesticus; Insect Biochem.* **18** 363-367

—— and —— 1994 Comparative total activities of digestive enzymes in different gut regions of the house cricket, *Acheta domesticus* L. (Orthoptera : Gryllidae); *Ann. Entomol. Soc. Am.* **87** 886-890

Trager W 1953 Nutrition; in *Insect Physiology*. pp 350-386 ed. K D Roeder (New York: Wiley)

Uvarov B P 1966 *Grasshoppers and Locusts*; Vol 1 (Cambridge, U.K.; Cambridge University Press)

Waldbauer G P 1968 The consumption and utilization of food by insects; in *Advances in Insects Physiology*. Vol V pp 229-282 eds J E L Beament, J E Treherne and V B Wigglesworth (London: Academic Press)