Nutritional effects of different diets on the on digestive enzymes in *periplaneta americana* (l.)

Sidharth D Pagare

Department of Zoology, Karmveer Abasaheb Alias N. M. Sonawane Art's, Commerce & Science College, Satana. 423301. Maharashtra

E.mail : sidharthpagare@gmail.com

ABSTRACT

The present study was studied with the effect of different Diets i.e. Diet-C, Diet-G and Diet-T were studied with the fore gut, mid gut and Hind gut. The lipase activity is highest in the fore gut with Diet-C and lowest in the Hind gut of the Diet-T. The Proteinase activity of is similar in the fore gut, Mid gut and Hind gut with the three Diets. The amylase activity was highest in the fore gut with Diet-G and lowest in the hind gut with Diet-T. The Protein concentration was lowest in the Mid gut and higher in the Hind gut.

Keywords: *Periplaneta americana* (L.), Proteinase assay, Amylase assay, Lipase.

Introduction: Cockroaches (Blattodea) are among the most primitive and successful insects. Their omnivorous, detritophagous feeding habit and association with symbiotic bacteria (Cruden and Markovetz, 1987) obviously contributed to their survival for at least 350 million years (Thorne and Carpenter, 1992). Many modern species have retained the omnivorous nature of their ancestors and this has no doubt contributed to their success as obnoxious pests of humanity (Cruden and Markovetz, 1987). Today there are 3,500 species of cockroaches found on every continent except Antarctica. Truly representing the diversity of insects, the cockroach family provides excellent models for anatomical and physiological investigations. The American cockroach, *Periplaneta americana* (L.) (Blattidae), is the largest of the cockroaches measuring an average 4 cm in length. Adults are reddish-brown in appearance with a pale-brown or yellow band around the edge of the pronotum. The cockroach is easily, albeit unintentionally and regretfully, spread by human commerce and the species is currently cosmopolitan in distribution. It is found mainly in basements, sewers, steam tunnels and drainage systems (Rust *et al*., 1991) making it difficult to control. *Periplaneta americana* is a voracious omnivore that feasts on almost anything such as paper, boots, hair, bread, fish, fruit, peanuts, old rice, the soft part on the inside of animal

hides, dead insects and cloth, thereby causing economic loss (Bell and Adiyodi, 1981). American cockroaches can become a public health problem due to their association with human waste and disease, and their ability to move from sewers into homes and commercial establishments. At least 22 species of pathogenic human bacteria, virus, fungi, and protozoans, as well as five species of helminthic worms, have been isolated from field-collected cockroaches (Rust *et al.*, 1991).

The digestive system functions to break down and absorb nutrients for maintenance, survival and reproduction in insects. The long and somewhat coiled digestive tube in *P. americana* could be divided into three regions: the foregut which includes the mouth, salivary glands, esophagus, crop, and the proventriculus or the gizzard; the midgut which includes the ventriculus, gastric caeca and malphigian tubules; and the hindgut comprising ileum, colon and rectum. The occurrence of a multitude of digestive enzymes in the gut of cockroaches is consistent with their omnivory and feeding adaptability. Digestive tract of *P. americana* harbours xylanase, laminaribiase, cellobiase, maltase, sucrose, α- and β-glucosidase, α- and β-glycosidase, β-fucosidase, chitinase and N-acetyl-β-glucosaminidase that attack various polysaccharides including those in the plant and fungal cell walls (Genta *et al.*, 2003). Similar versatility in the digestion of proteinaceous substrates is indicated by the presence of 11 proteinases in the gut of *P. americana* (Hivrale *et al.*, 2011). Vinokurov *et al*. (2007) recorded high proteolytic and amylolytic activities in the midgut of *P. americana* with a moderate activity in the crop. Cockroaches have been the most popular group of insects for studies of lipid digestion, and a number of early studies indicated lipolytic activity in the fore- and midgut. It was also demonstrated that lipase originates in the epithelial cells of the midgut and caecae. Thus the presence of lipolytic activity in the foregut results from regurgitation of midgut contents into the foregut (Downer, 1978).

A large number of previous studies have been devoted to determining enzyme activity in the fore- and midgut. This has been as a result of the assumption that little or no digestion (but absorption) takes place in the hindgut. The present work was, therefore, designed to quantify activities of enzymes in the three gut regions of adult male and female *P. americana*. Enzymes hydrolyzing three major classes of food (carbohydrate [α-amylase, β-amylase, γ-amylase], protein [proteinases] and fat [lipases] were assayed. Information about digestive enzymes is of fundamental importance in understanding the digestive processes, food and feeding habits of insects. Such information could also assist in formulating sustainable and effective control strategies tainance, survival and reproduction in insect the long and somewhat coiled digestive tube in *Periplanata americana* could be divided, in to three region the forgut, midgut and hindgut .the occurance of a multitude of

digestive protein in the gut of cockroaches is consistent with their omnivory and feeding adaptibility

Digestive system of cockroach

Foregut : Foregut consists of the mouth surrounded by the mouthparts. The mouth cavity is called the pharynx. It continues as the oesophagus that is short, narrow and thin-walled. The canal then enlarges into the crop, which is also thin-walled. The crop opens into a short, muscular organ, the gizzard or the proventriculus. Outside and lying below the crop are a pair of salivary glands.

Each salivary gland is branched, the secretions of all the branches being poured into a common duct. For either pair of salivary glands there is a thin walled salivary receptacle or reservoir which is like a bladder. It stores the salivary secretions. The receptacles of either side have a common receptacular duct which opens into the common salivary duct. This common salivary duct opens into the mouth cavity at the labium. The entire foregut is lined with chitin. In the gizzard, the chitin forms proventricular teeth and the plate to facilitate grinding of the food.

Midgut : Midgut forms the true gut or the mesenteron and consists entirely of stomach or ventriculus. At the junction of the gizzard and stomach are six pairs of gastric caecae ('gastric' means pertaining to stomach). These are pouch-like structures arranged in a ring-like manner around the anterior end of the stomach. The anterior lobe of each pair of the caecae extends over the proventriculus and the posterior lobe extends over the ventriculus. The caecae secrete digestive juices and pour them into the stomach. The midgut is not lined by chitin or cuticle but by a peritrophic membrane. This membrane protects the stomach wall from abrasions and is fully permeable to enzymes and digested food.

Hindgut: Hindgut is a coiled structure consisting of anterior ileum, middle colon and posterior rectum. The rectum opens to the exterior through the anus. The hindgut is lined by cuticle. At the junction of the stomach and ileum are attached numerous long tubules called the Malpighian tubules.

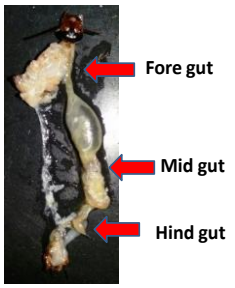
Insect have been prosed as a high quality, efficient and sustainable dietary protein source. The present study evaluated the protein quality of a selection of insect species .The cockroaches were relatively high in protein but the indispensable AA (Amino acid) contents, AA scores and the in Vitro digestibility values were relatively low. The gut of the insects favours the development of microorganisms because of the extended surface of their intestinal lumen and the availability of nutrients .Though insects comprise one of the most divers taxa of life, little is known about the

implication of insect associated bacteria on human health (Allen et al., 2009). A vector is an organism capable of mechanically or biologically transferring a pathogen from one organism to another. Two group of non biting insects most frequently screened for food-borne pathogens are houseflies and cockroaches (Ahmad et al., 2011).These insects have been implicated as mechanical or biological vectors for bacterial pathogens. The bacterial flora of insects gut appears to the fortuitous contamination of the environment and often reflects its feeding habits (Hunt and Charnley, 1981). Ahmad et al., (2011) observed enterococci as very common in the gut content of cockroaches suggesting the bacterial species as the predominant commensal in the insects intestine. *P. americana* as a carrier of human pathogen.

According to World Health Organisation report, cockroaches have been in existence for about 360 million years and during the intervening time the insect has gone very little structural change (Cochran, 1982). *P. americana* (the American cockroach) is widely distributed throughout the temperate tropical and subtropical regions of the world. Invertebrates are an important food source of animals and humans and as a result the nutritional values of many species of invertebrate have been published (Ademolu et al., 2004, Banjo 2006; Barker et al, 1998; Bernard and Allen, 1997; Finke, 2002; Finke, 2007 and Omotoso and Adedire, 2007; Ooninor and Vander poel, 2009; Raksakantong et al., 2009 and Ramos-Elordury et al., 1997). This literature review seek to establish what is known about the nutritional composition of invertebrate in to context with the nutritional requirement of amphibians .It also seeks to ascertain the most appropriate and commonly used analytical methods used to determine proximate composition of dry matter, protein, fat etc of invertebrates. Protein content of sample is an estimate of protein based on the nitrogen content of a sample, protein is calculated by multiplying the nitrogen content by a conversion factor. The most commonly used method for nitrogen determination among the literature for invertebrate proximate analysis determines the total protein and food content of a food sample, given as the percentage composition of the samole (self 2005). proximal analysis is the Kjeldahl method. The universality of the Kjeldahl method make it the most widely used method for nitrogen determination (James,1999). For invertebrate studies the conversion factor is usually 6.25 (protein=nitrogen×6.25). (Finke, 2002; Finke, 2007; Raksakantong et al., 2009). Some authors however do not calculate the protein but instead give the value for total nitrogen (Barker et al., 1998; Bernard and Allen, 1997; Hatt el. al, 2003). This is because it is widely thought that large amounts of non –protein nitrogen may be contributed by Chitin (amino-cellulose).As such estimating protein by nitrogen ×6.25 may result in an overestimate of the true protein content .Finke (2007) analysed a number of invertebrate species to estimate the amount of

chitin they contained. It was found that the quantity of chitin nitrogen (as a percentage of total nitrogen) is actually small and that a conversion factor of nitrogen ×6.25 does provide a reasonable estimate of total protein in invertebrates. Nitrogen content will be determined using the Kjeldahl method and protein calculated using conversion factor of 6.25.the crude protein content of the Argentinean cockroach was 54.25% DMB (Bernard and Allen, 1997).

Periplanata americana (L.)



Digestive system of Cockroach *Periplanata americana* (L.)

Materials and Methods : The *P. americana* were collected natural habitat and maintained in Laboratory at 26 ± 2 ℃ and 73 ± 3% RH. for the present study both the sexes were used. The different region of gut viz. Fore gut, Mid gut and Hind gut were dissected in insect ringer and homogenized in phosphate buffer 0.1mM, pH 7.2. the homogenate was centrifuged for 10 min at 4ºC after centrifugation the supernatant was used for protein and enzyme estimation.Casein (1%W/V), trichloroacetic acid, starch solutions, iodine mixture, Dinitrosalycylic acid and olive oil emulsion used were of A. R. Grade Phosphate and sodium acetate buffer, and Tris buffer from Rankem. Distilled water was prepared in the laboratory.

Protein Estimation by Lowry's method : Standard protein curve was prepared by using Standard BSA (1mg/100ml) Stock. Working BSA solution used was 100µg/ml. A procedure followed by described by Jayaraman. The sample protein estimation for fore gut, mid gut and hind gut carried out according to procedure by Jayaraman.

Estimation of reducing sugar by Dinitrosalicylic acid method :-Glucoose estimation is carried out by following method of 3-5 dinitro salicylic acid as described by Sadashivan.

Enzyme bioassay: Separate bioassays were constituted for male and female cockroaches to determine activities of digestive enzymes in the foregut, midgut and hindgut. The assays were replicated three times

Preparation of enzyme extract : Enzyme samples were prepared using the method described by Cohen (1993). The insects were starved for 24h before dissection to standardize them and to allow the accumulation of digestive enzymes. The insects were placed at -20 ℃ for 4 min and then dissected in ice-cold 0.9% NaCl solution under a dissecting microscope. The alimentary tract was removed by placing the scissors points between the junction of the third and second to the last tergites. Two incisions were made along each laterally arranged spiracle.Continuing through to the thorax. Once the tergites were freed from the underlying connective tissue they can be removed in one piece. By grabbing the head with a forceps and cutting the surrounding neck chitin, the entire digestive tract was removed by gently lifting the head and freeing the attached to the foregut,midgut and hindgut, and each gut region was kept in 1 ml ice-cold sodium phosphate buffer (pH 7.1). The tissues were homogenized and ultra-centrifuged at 16,000 rpm for 10 min at 4 ℃. The supernantant was placed in a centrifuge tube and kept at 4℃.

Proteinase assay : Proteinase activity was quantified spectrophotometrically as described by Morihara and Tsuzuki (1977) with slight modification. The reaction mixture consisted of 1 ml 1% (W/V) casein and 0.5 ml of the enzyme preparation. This was incubated in a water bath at 35 ℃ for 30 min. The reaction was terminated by the addition of 3 ml cold 10% (W/V) .Trichloroacetic acid (TCA).The mixture was allowed to stand at 4 ℃ for 30 min, centrifuged at 3000 rpm for 10 min and the supernantant was collected for the determination of non-precipitated TCA protein. This was done following the Folin-Ciocalteu's phenol reagent method of Lowry et al.(1951).One milliliter of the TCA protein was mixed with 5 ml of Lowery's reagent C, mixed thoroughly and incubated at room temperature for 10 min. Three fold diluted Folin-Ciocalteu's phenol reagent (0.5 ml) was added to the mixture with shaking, and incubated at room spectrophotometer. The amount of non-precipitated TCA protein was estimated as tyrosine from a standard curve of known concentrations of tyrosine. One unit of protease activity is defined as the quantity that is required to produce 100 µg of tyrosine in 1 ml of TCA filtrate under the above conditions.

Amylase assay : The reducing sugars produced by the action of α –amylase react with dinitrosalicylic acid and reduce it to a brown coloured product, nitroaminosalicylic acid.

Lipase assay : The assay method described by the Shihabi and Bishop (1971) was used with little modification. 3.0 ml of olive oil emulsion was pipette into a cuvette and warmed to 37℃. 0.1 ml of the homogenate was added and mixed by inverting the cuvette. The change in absorbance was recorded at 340 nm for 1-min intervals till it became consistent. Lipase activity (µmol triglyceride bonds broken /min /litre of homogenate) was calculated by multiplying change in absorbance per minute (ΔA/min) by the conversion factor (F).Based on olive oil being pure glyceryl trioleate (mol wt.880),the value of f was 5400.

Results and Discussion : Starch consist of two distinct fractions:amylase –linear α-1,4-linked glucans,and amylopectin –linear α-1,4-linked glucons branched with α-1,6linkages (Ball et al.,1996;Mouille et al., 1996),and the enzymes are responsible for hydrolysis of starch and related saccharides are called amylolytic enzymes or simply amylases. The three most known amylases (α-amylase,β-amylase and ϒ-amylase)were detected in the study.This is an adition to a number of carbohydrases that had been reported earlier in *P.americana* (Scrivener et al., 1998;Genta et al., 2003).Unlike α-and ϒ-amylases, β-amylase is not produced by animals although it may be present in microorganisms contained within the digestive tract (Adachi et al., 1998;Mikami et al., 1999).The detection of β- amylase in the presente study indicates that some symbiotic microorganisms are resident in the fore -,mid -,and hind gut of P .americana. Zurek and keddie (1996) reported that gut bacteria in *P. americana* played a functional roll in the devlopement and survival of the insect species .Cruden and Markovetz (1987) also reported a stoppage in the biosynthesis of cysteina and methionine and subsequent and reduction in fecundity. When gut bacteria eliminated from *P. americana.* The ditection of these amylases, proteinase and lipase in the digestive tract of *P.americana* shows that the species is well equipped for poly-phagous feeding habit.

The absence of lipase activity in the hind gut of females was preceded by a dramatic increase in the midgut. It is possible that female wasps made maximum srequirement. Lipids mostly triacylglycerol(TAG), smaller amount of phospholipids (PL) and cholesterol ,make up 30-40 of the dry weight of the insect oocyte (Kawooya and law, 1988;briegel,1990).Also lipids are the main source of the developing embryo (Beenakkers et al., 1981)and the PL is needed for the formation of membranes. Insect oocytes synthesize TAG using fatty acids (FA) (Lubzens et al.,1981;Ferenz,1985)but since the ability of oocytes to synthesize FA de novo is very limited,

Ziegler and Van Antwerpen (2006) concluded that all the lipids must be imported, especially from ingested food. This requirement might have necessitated maximal digestion of lipase substrate in the female midgut.

Generally, enzymatic activities in each sex of *P.americana* were, more or less, higher in the midgut followed by the foregut and hindgut in descending order. This supports previous reports identifying midgut as the principal site for enzyme secretion and food digestion in insects (Klowden, 2007; Nation, 2008). However, activity of α-amylase was relatively higher in the foregut of *P.americana.* This may be due to the fact that α-amylase is secreted by the salivery gland which are situated in the anterior part of the foregut. Apart from the location of secretory organs, prevailing conditions in different regions of insect gut such as redox potential and pH may also have differential effects on digestive enzyme (Vinokurov et al., 2007).This may explain in part why hydrolytic activity of each enzyme varied from one gut region to the other.

The general tendency of workers to pay little attention to the hindgut because of the assumption that negligible hydrolytic activities take place in the region may eventually rob the science world of some useful pieces of information. In the present work, a considerable level of proteinase and male lipase activities was detected in the hindgut of *P.americana* underlying the potentials of digestive enzymes in this region .Workers are encouraged to give adequate consideration to activities in the insect hindgut in future studies.

In the present study three different diet namely Diet-T, Diet-G, Diet-C were provided the proximate analysis are shown in table.

	Diet-T	**Diet-G**	**Diet-C**
Carbohydrates	76g	78.2g	72.0g
Suger	26g	25.5g	
Protein	7g	6.5g	12.0g
Fat	14g	12.5g	1.70g
Saturated Faty Acids	6.4g	6.5g	
Mono unsaturated fatty Acids	4.5g		
Trans fatty acids	0g	0g	
Poly Unsaturated Fatty Acids	1G		
Cholestrol	0g		
Energy	459Kcal	451Kcal	351.3Kcal
Iron	4mg		1.9mg

Folic Acid	18mg		
Dietary fiber		1.8g	

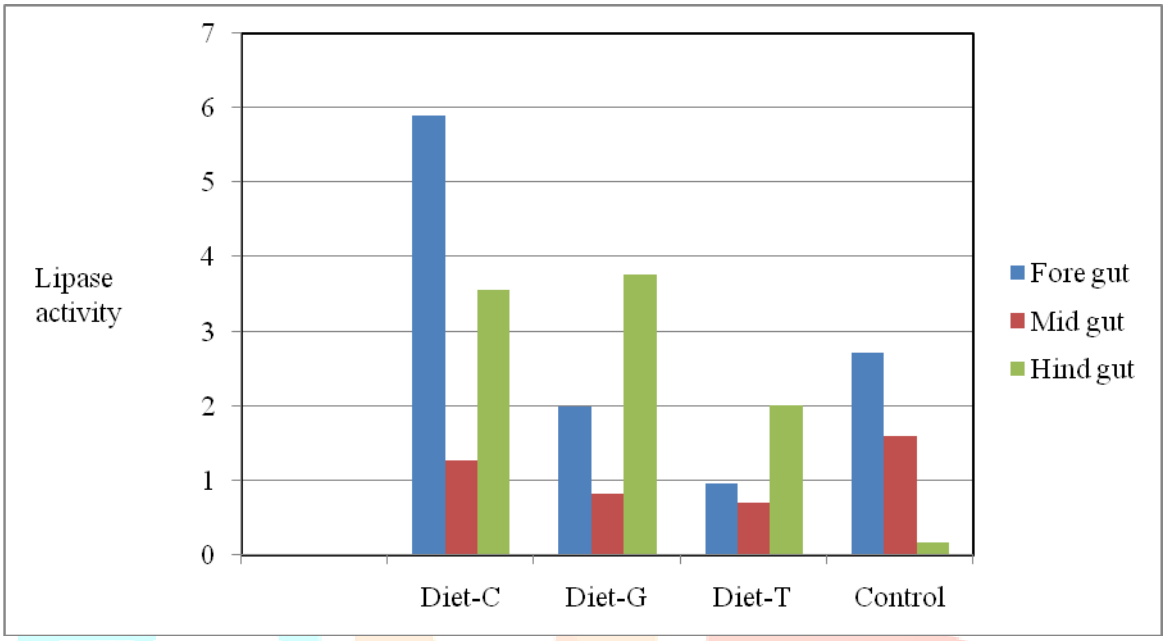


Fig.1: Activities of the lipase in the foregut, midgut and hindgut regions of *Periplaneta americana.((Lipase activity expressed in µmol triglyceride bonds broken /min /L of homogenate)*

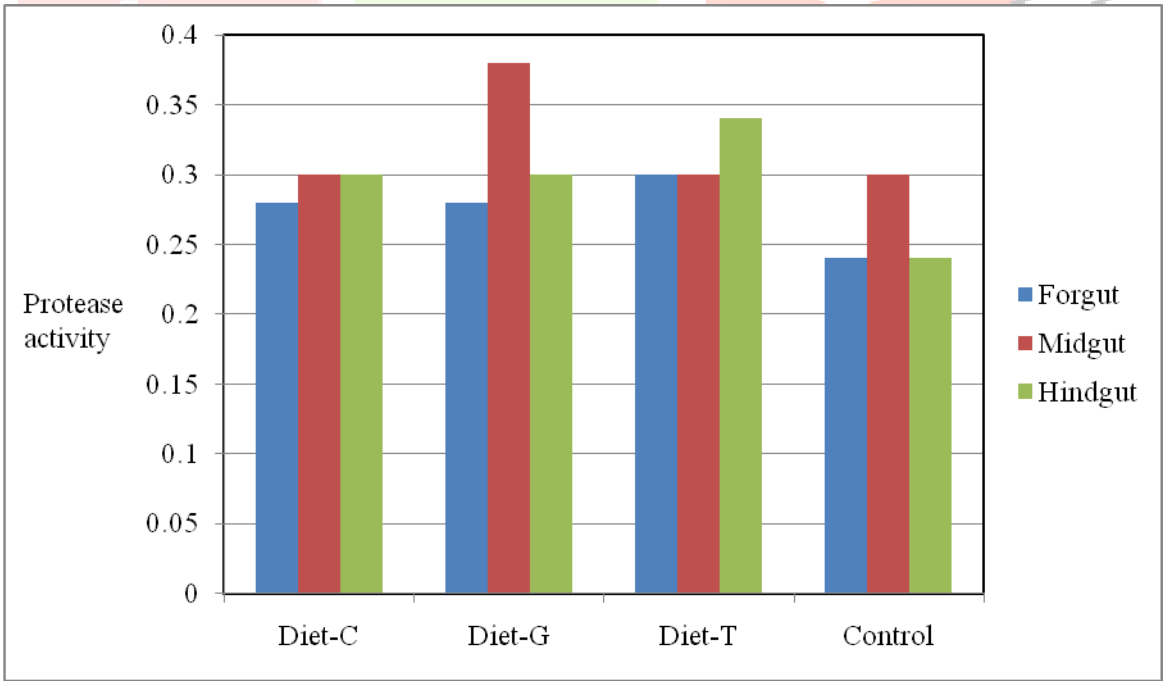


Fig.2: Activities of the Proteinase in the foregut, midgut and hindgut regions of *Periplaneta americana.*

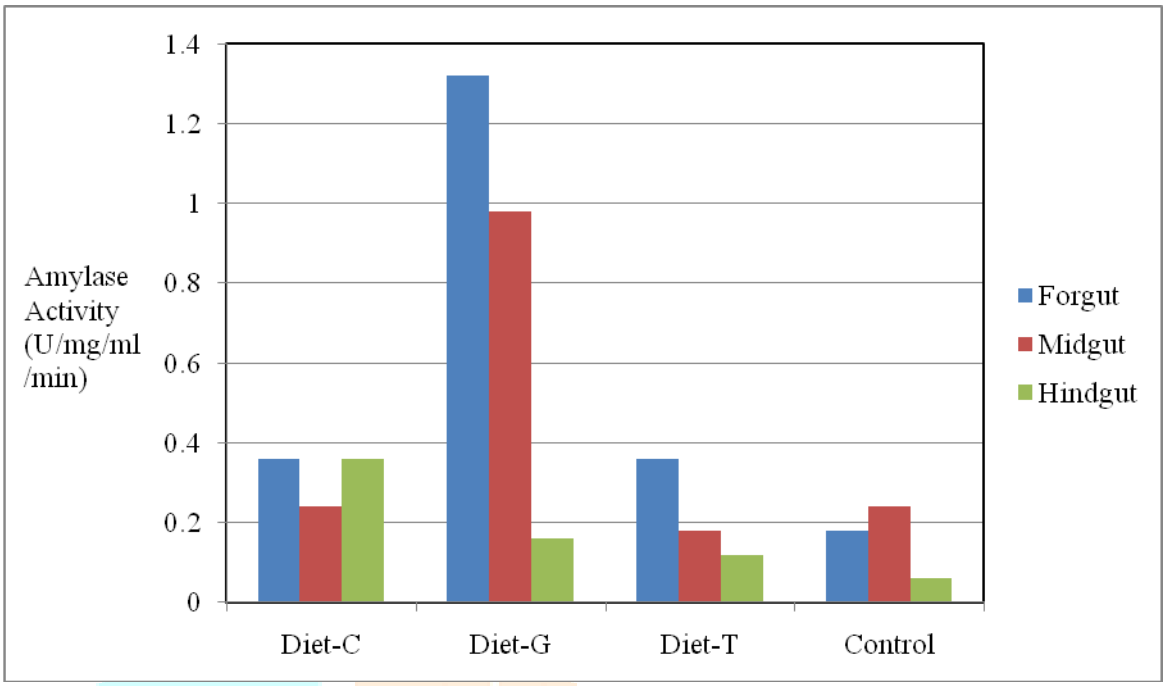


Fig.3: Activities of the amylases in the foregut, midgut and hindgut regions of *Periplaneta americana.*

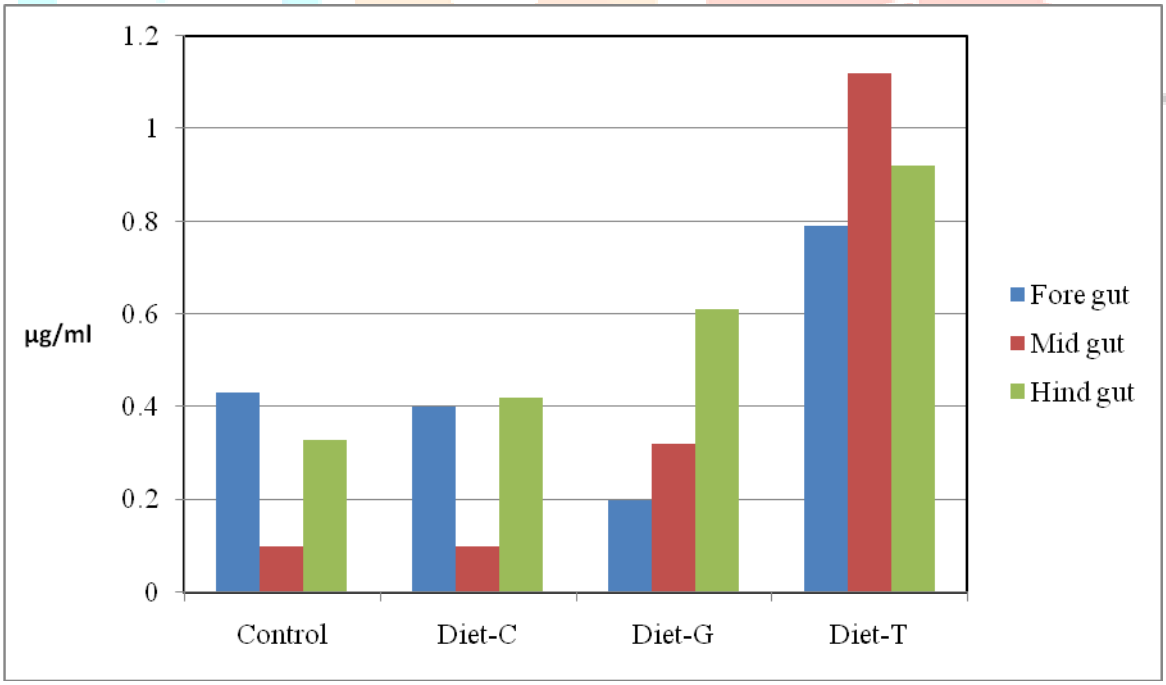


Fig.4: Protein concentration in the foregut, midgut and hindgut regions of *Periplaneta americana.*

Conclusion: The present study concludes that Diet-C is essential for the effective nutrition rather than the Diet-G and Diet-T.

Acknowledgement: Authors are thankful to the Principal of Karmveer Abasaheb Alias N.M.Sonawane for providing the necessary facilities.

REFERENCES:-

Ademolu, K.O., Idowu, A.B., Mafiana, C.F. and Osinowo, O.A (2004) Performance, proximate and mineral analyses of African giant land snail (Archachatina marginata) fed different nitrogen sources. African journal of Biotechnology.Vol.3(8), pp. 412-417.

Ahmad A, Ghosh A, Schal C, Zurek L. Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community. BMC Microbiol. 2011; 11:23.

Andrey Vinokurov, Daniel Geller, and Tamara L. Martin (2007). Translation as an Ecological Tool for Instrument Development. International Journal of Qualitative Methods 6 (2).

Barbara L. Thorne and Lames M. Carpenter (1992) Systematic Entomology. 17,253-268.

Barker ,D.Fitzpatrick,M.P and E.S.,Dierenfeld, 1998. Nutrient Composition of selected whole invertebrates. Zoo Biology.,Vol.17, PP.123-134.

Bell, WJ and KG Adiyodi. 1981. The American cockroach. Chapman and Hall, London.

Chris.E.Young-Proximate and mineral composition of selected whole invertebrates and Nutritional effects of different diets on the field cricket, Gryllus Bimaculatus.

Cohen, A.C., 1993. Organization of digestion and preliminary characterization of salivary trypsin-like enzymes in a predaceous heteropteran ,Zelus renardii.Journal of Insect physiology 39,823-829.

Cruden ,D.L.,Markovetz, A.J., 1987. Microbial ecology of the cockroach gut. Annual Review of Microbiology 41,617-643.

Cruden, D L and A J Markovetz(1987) Microbial ecology of Cockroach Gut.Annual Reviews 41: 617-643.

Downer, (1978) Environmental Physiology and Biochemistry of Insects.

Finke, M.D., and Winn,D.,2004 .Insects and Related Arthropods.A Nutritional primer for Rehabilitators. Journal of Wildlife Rehabilitation .Vol.27(3-4), PP.14-27.

Finke, M.D.2002, complete Nutrient composition of Commercially Raised Invertebrates Used as Food for Injectivores. ZOO Biology, Vol.21,pp.269-285.

Genta, S.B.; Cabrera, W.M.; Grau, A.; Sanchez, S.S. (2005). Subcronic 4-month oral toxicity study of dried Smallanthus sonchifolius (yacon) roots as a diet supplement in rats. Food Chem. Toxicol., Oxford, v.43, n.11, p.1657-1665.

Hivrale VK, Lomate PR, Kalve ND, Kachole MS: *Periplaneta americana* midgut proteases differentially expressed against dietary components from different plant seeds. Physiol Entomol. 2011, 36: 180-186. 10.1111/j.1365-3032.2011.00784.x.

Hivrale, V.K.,Chougule, N.P., Chhabda, P.J., Giri,A.P.,Kachole, M.S.,2005. Unraveling biochemical properties of cockroach *(Periplanata americana)* proteinases with a gel X-ray film contact print method. Comparative Biochemistry and Physiology B 141,261-266.

Hunt, J., and Charnley, A. K. (1981). Abundance and distribution of the gut flora of the desert locust, *Schistocerca gregaria*. *J. Invertebr. Pathol.* 38, 378–385. doi: 10.1016/0022-2011(81)90105-1.

James D.E & Burkart M.R (1999). Agricultural Nitrogen Contributions to Hypoxia in the gulf of Mexico. Journal of Environmental Qualitative.850-859.

Lowry ,O.H., Rosebrough,N.J., Farr.,A.L., Randall,R.J., 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193,265-275.

Morihara, K., Tsuzuki, H., 1977. Production of protease and elastase by Pseudomonas aeruginosa strains isolated from patients. Infection and Immunity 15,679-685.

Rust MK, Reierson DA, Hansgen KH. (1991). Control of American cockroaches (Dictyoptera: Blattidae) in sewers. Journal of Medical Entomology 28: 210-213.

Rust, M.K., Reierson,D.A., Hansgen, K.H., 1991.Control of American cockroaches (Dictyoptera: Blattidae) in sewers.Journal of Medical Entomology 28,210-213.

Shihabi, Z.K., Bishop,C.,1971.Simplified turbidimetric assay for lipase activity.Clinical Chemistry 17, 7750-1153.

Somogyi,M.,1945. A new reagent for the determination of sugars. Journal of Biological Chemistry 160,61-68.

Vinokurov, K., Taranushenko, Y., Krishnan, N ., Sehnal, F., 2007 Proteinase, amylase,and proteinase-inhibitor activities in the gut of six cockroache species .Journal of Insect Physiology 53,794-802.