Arch. Biol. Sci., Belgrade, 56 (1-2), 9–13, 2004.

THE INFLUENCE OF DIETARY PROTEIN QUALITY ON MIDGUT AND BRAIN PROTEINS IN *MORIMUS FUNEREUS* LARVAE

LARISA ILIJIN, MILENA JANKOVIĆ–TOMANIĆ, MARIJA MRDAKOVIĆ, MILENA VLAHOVIĆ, VESNA PERIĆ–MATARUGA, JELICA LAZAREVIĆ and VERA NENADOVIĆ

"Siniša Stanković" Institute for Biological Research, 29. Novembra 142, 11060 Belgrade, Serbia and Montenegro

Abstract — The response of starved *Morimus funereus* larvae refed with an artificial diet (Galford, 1967) was examined in the present paper. Larvae were offered diets varying in protein quality (soya protein, casein, and gelatin). Refeeding with the Galford diet (G I) and two modifications of it in which soya protein was supplemented with casein (G II) or gelatin (G III) led to an increase of body mass. Different protein quality in the nutritive substrate influenced both the quantity and quality of midgut and brain proteins.

UDC 595.768 : 591.53.063.084

INTRODUCTION

The influence of food quality and quantity can be detected at different levels of biological organization in insects. Protein quality in insect nutrition affects proteolytic activity (Brodway and Duffey, 1988), energy metabolism (Karowe and Martin, 1989) and fitness components (Cooper and Schal, 1992).

Sapwood represents both the external medium and the nutritive substrate for *Morimus funereus* larvae. Wood mass is characterized by a high content of carbohydrates (starch, cellulose) and lignin, while the percentage of nitrogen is quite low (Mattson, 1980). Protein concentration in insect food also shows seasonal variability (Slansky and Scriber, 1985). Amino acid content of food proteins varies depending on the plant species and even between parts of the same plant. Free amino acids constitute up to 5% of plant nitrogen available for nutrition. They are important for phloem feeders and change in response to changes in host plant physiology (Bernays and Chapman, 1994). *Morimus funereus* larval development lasts from 3 to 4 years in nature, while it is much shorter in laboratory populations and lasts only 6.5 months (Ivanović *et al.*, 1991). Among other factors, intensified protein and lipid metabolism, increase in proteolytic activity, and activation of protocerebral neurosecretory neurons could explain the decrease of development time on an artificial diet (Nenadović *et al.*, 1989, Ivanović *et al.*, 1992).

Examing the effects of food and its compounds on survival, growth, development, and reproduction have become a very important part of insect dietetics (Reese, 1979).

Our goal was to determine the grbwth response of *M. funereus* larvae reared under constant laboratory conditions to starvation and refeeding as two physiological states. We also analyzed the effects of artificial diet varying in protein quality on the quantity and quality of midgut and brain proteins.

MATERIAL AND METHODS

Experimental groups and rearing conditions — *Morimus funereus* larvae were reared from egg hatching to molting into the fifth instar on an artificial diet (AD) (Roberts, 1986) under controlled laboratory conditions (constant temperature of 23°C, relative humidity of 70%, and in the dark). Larvae were exposed to 7–day total starvation from the 5th to 12th day of the fifth instar — (TS group) and after that refed for 7 days with the following diets:

Galford diet with 1.5% soya protein — (G I group);
Galford diet with 1.5% casein — (G II group);
Galford diet with 1.5% gelatine — (G III group).
Each group consisted of eight larvae.

Relative change in larval body mass was expressed in percentage of mass change during treatment (Mt – Mo) in relation to initial larval mass measured at the beginning treatment (Mo). Mass gain was calculated according to the formula:

$$((Mt - Mo)/Mo) \times 100\%$$

Biochemical methods — After decapitation, midguts and brains were dissected out on ice and weighed. Midguts and brains were homogenized in RIPA buffer, 1:5 wet wt/vol. (50mM TRIS pH7.4, 150mM NaCl, 1% Nonidet P–40, 0.5% Triton X–100, 0.1% SDS) and centrifuged at 20 000g for 5 min. Therefore SDS–PAGE electrophoresis was performed according to Laemmli (1970), on 13.5% and 16% gels. The gels were stained for proteins with 0.1% $AgNO_3$ and destained in a 0.025% formaldehyde–3% Na_2CO_3 solution. Molecular weights of proteins in SDS–PAGE were estimated using commercial standards with Mr range of 2.500–17.000 Daltons (Sigma) and 14.000–70.000 Daltons (Sigma).

RESULTS AND DISCUSSION

An important part of insect dietetics is the use of artificial diets, which gives insight into the biological significance of particular classes of molecules during the insect life cycle.

Constant food availability is necessary for growth, development, and maintaining of homeostasis in developing larvae (Reese, 1979). After a period of nutritional deficit, compensatory growth can occur if conditions improve, but it is usually regulated at optimal rather than maximal rates (Metcalfe and Monoghan, 2001).

Changes in *M. funereus* larval mass upon starvation and refeeding with the Galford diet (G I) and two modifications of it in which soya protein was supplemented with casein (G II) or gelatin (G III) are presented in Fig.1. Student's t–test for dependent samples was used for estimation of mass change significance. Statistical significance of the differences between experimental groups was evaluated by the LSD test. Significant changes in body mass were noticed in the TS and G I groups. In the TS group, mass change was significantly different from other experimental groups. Significant increase in larval body mass was detected in the group refed with the Galford diet.

Soya contains about 39% protein and the dominant amino acids are glutamic acid, lysine, and arginine. Casein is a protein that contains all essential amino acids, phosphorus, sialic acid, and galactose. The dominant amino acids are leucine, lysine, valine, and aspartic acid. Gelatin is a protein consisting of only nine amino acids with domination of glycine, praline, and hydroxyproline.

Comparing the larval mass gain, we observed that the amino acid composition of soya protein and that of casein have a stimulatory effect on *M. funereus* larval growth after total starvation.

The electrophoretic patterns of midgut proteins from all experimental groups are presented in Fig. 2. Differences in midgut protein patterns in the region of high molecular masses (14.2–66 kD) are shown in Fig. 2A. The protein profile of starved larvae is characterized by a protein band with Mr of about 66 kD, which is absent in profiles of those reared on the Galford diet and modifications of it. This region (48–68 kD) is known as the region of α–amylase molecular masses in insects (Buonocore *et al.*, 1976). A band of about 20 kD was detected only in the TS and G II groups. In G I group, two bands with Mr of about 24 kD were detected, while at the same position in the G II group, only one barely visible band was detected. In the protein profile of the G III group, a clearly visible band appeared in that region. The range of 20–35 kD is known as the Mr of trypsin and a

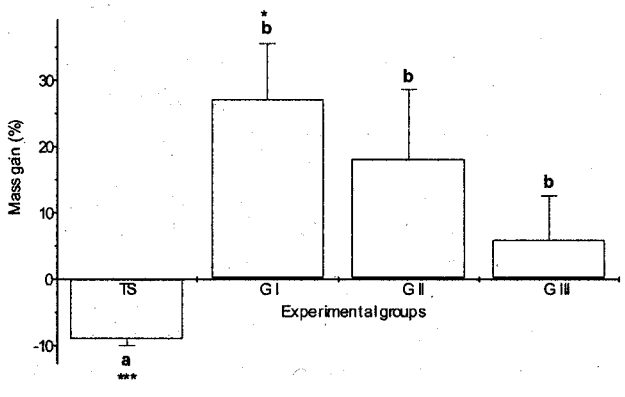

Fig. 1. Changes in body mass of Morimus funereus larvae after 7–day total starvation (TS) and 7–day refeeding with Galford diet supplemented with soya protein (G I), casein (G II) and gelatine (G III).

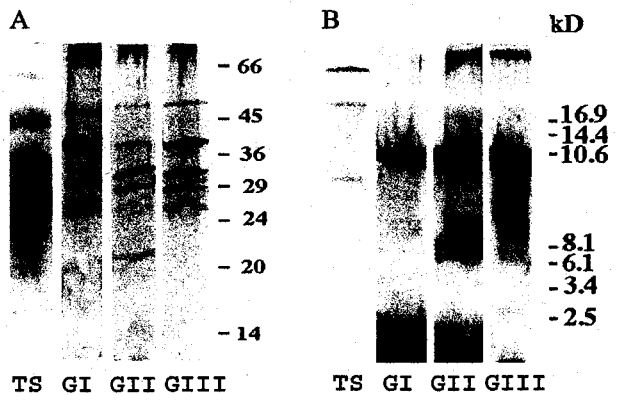

Fig. 2. Electrophoretic patterns of crude midght homogenates of Morimus funereus larvae obtained by 13.5% (A) and 16% (B) SDS–PAGE. Abbreviations are explained in the legend for Fig. 1.

chymotrypsin–like enzyme in insects (Christeller *et al.*, 1989; Applebaum, 1985). All experimental groups were characterized by intensive bands with molecular masses of about 50, 40, and 36 kD. These molecular masses are known as the Mr of three trypsin–like forms in *M. funereus* larvae (Đurđević, 1997).

Changes in protein patterns in the region of low molecular masses, i.e., 17–2.5 kD, are presented in Fig. 2B. The protein profile of the TS group is quite different from the others. Protein bands of about 17 and 10.6 kD were detected only in this group. All three experimental groups of larvae refed with the Galford diet and modifications of it were characterized by a most intensive band with molecular mass of about 10.6 kD. In the protein profiles of the G II and G III groups, several intensive bands appeared in the region of molecular masses from 6.1–10.6 kD. Those bands could not be detected in the TS and GI groups. Also, a band with Mr of about 2.5 kD was present in the G I and G II protein profiles, while it was barely visible in the G III group and not detected at all in the TS group. These protein bands in low Mr regions in *M. funereus* midgut protein patterns could represent glycosylated proteins from the peritrophic matrix, the subunits of some enzymes, products of protein digestion, or neuropeptides from midgut endocrine cells (East *et al.*, 1993; Nishiitsutsuji–uwo *et al.*, 1981; Sehnal and Žitnan, 1996).

The insect neuroendocrine system regulates biochemical and physiological processes, but also plays a role in adaptive responses to environmental changes. Thomsen and Möller (1963) hypothesized that medial neurosecretory cells and the *corpus allatum* are involved in regulation of midgut proteases. Experiments with injection of the crude cerebral complex to *M. funereus* larvae have confirmed the role of neurohormones in regulation of midgut proteases (Ivanović et al.,1998).

Changes in brain electrophoretic patterns are presented in Figs. 3A (Mr region of 66–14.2 kD) and 3B (Mr region of 17–2.5 kD). It can be seen from Fig. 3A that there are more protein bands with Mr lower than 20 kD in all experimental groups. The most intensive bands were with Mr of about 20 kD; there were two very close bands in that region in the TS and G III groups. In the G I and G II groups, those bands are more intensive.

The neurohormone allatotropin is synthesized in brain medial neurosecretory cells and released in *corpora allata* (allatotropins stimulate the synthesis of juvenile hormone). Kataoka *et al.*, (1989) have described a 13–amino–acid structure of this hormone, whose Mr is 20 kD in *Manduca sexta*.

Many close bands appeared in the region of molecular masses from 14.2 to 24 kD in all experimental groups.

Figure 3B represents the brain protein patterns in the region of low–molecular masses. All protein bands in the G I group are barely visible. In all experimental groups, intensive protein bands were detected in the Mr region of about 14.4 kD, and two bands were detected in the region of 9 kD. Patterns of the G I and G III groups are characterized by two scarcely visible bands in the region of 8 kD. Two bands with Mr of about 6 kD can also be detected in the G II and G III groups, together with one band with Mr of 3.4 kD in the G II group and two bands (one of lower and the other of higher Mr) in the G III group.

The detected changes in brain protein patterns are present in the Mr regions of bursicon and prothoracicotropic hormones (Ishizaki and Suzuki, 1988). The highest total brain protein concentration was detected in the G II group, i.e., Galford modification with casein (Al Arid, 2001). Casein is very rich in amino acids, especially aromatic ones, so there is a possibility of induced elevation of trypsin and chymotrypsin–like enzymes. A response of the neurosecretory system to nutritional stress could be sought at the level of allatoptropins.

It is obvious that dietary protein quality influenced both the quality and the quantity of midgut and brain proteins. Dietary protein quality can induce a switch in digestive enzyme isoforms. Changes in brain protein patterns reflect responses of the neurosecretory system to environmental changes.

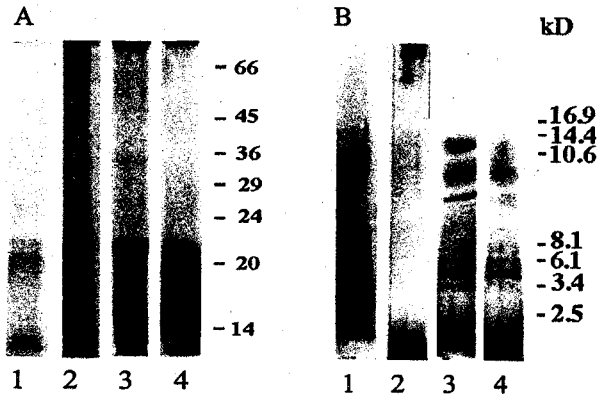

Fig. 3. Electrophoretic patterns of crude brain homogenates of Morimus funereus larvae obtained by 13.5% (A) and 16% (B) SDS–PAGE. Abbreviations are explained in the legend for Fig. 1.

Acknowledgments — This work was supported by the Ministry of Science, Technology, and Development of Serbia (grant No.1615).

REFERENCES

Al Arid, L. (2001). Effect of food quality on midgut and brain proteins in *Morimus funereus* larvae (Coleoptera; Cerambycidae). *M.S. Thesis*, University of Belgrade, Belgrade.

Applebaum, S. W. (1985). *Biochemistry of digestion. In: Comprehensive Physiology, Biochemistry and Pharmacology* (Eds. G.A. Kerkut and Gilbert L.I.), 279–311. Pergamon Press, New York.

Bernays, E. A., Chapman, R. F. (1994). *Host–Plant* Selection by Phytophagous Insects. Chapman and Hall, London.

Brodway, R. M., Duffey, S. (1988). Insect digestive physiology and the toxicity of plant proteinase inhibitors. *J. Insect Physiol.* **34**, 1111–1117.

Buonocore, V., Poerio, E., Silano, V., Tomasi, M. (1976). Physical and catalytic properties of α–amylase from *Tenebrio molitor* larvae. *Biochem. J.* **153**, 621–625.

Christeller, J. T., Shaw, D. B., Gardiner, S. E., Dymok, J. (1989). Partial purification and characterization of the major midgut proteases of grub larvae (*Costelytra zealandica*) Coleoptera, Scarabeidae. *Insect Biochem.* **19**, 221–231.

Cooper, R. A., Schal, C. (1992). Effects of protein type and concentration on development and reproduction of the German cockroach, *Blattela germanica*. *Entomol. Experim. Appl.* **63**, 123–134.

Đurđević, A. (1997). Analysis of midgut proteolytic activity in xylophagous *Morimus funereus* larvae. *M.S. Thesis*. Facutly of Chemistry; University of Belgrade.

East, I. J., Fitzgerald, C. J., Pearson, R. D., Donaldson, R. A., Vuocolo, T., Cadogan, L. C., Tellam, R. L., Eisemann, C. H. (1993). *Lucilia cuprina*: inhibition of larval growth induced by immunization of host sheep with extracts of larval peritrophic membrane. *Int. J. Parasitol.* **23**, 221–229.

Galford, J. R. (1967). Artificial rearing of 10 species of wood–boring insects. U.S. Departement of Agriculture Forest Service Research notes NE–102. *N East Forest Experimental Station*, pp. 6.

Ishizaki, H., Suzuki, A. (1988). An insect brain peptide as a member of the insulin family. *Horm. Metab. Res.* **20**, 426–429.

Ivanović, J., Stanić, V., Rauschenbach, I., Janković–Hladni, M., Nenadović, V., Budker, V. (1991). Influence of nutrition substrate on neuroendocrine system in ontogenesis of a cerambycid, *Morimus funereus* (Insecta). *J. Gen. Biol.* **52**, 205–213.

Ivanović J., Janković–Hladni, M., Đorđević, S., Stamenović, S., Lazarević, J. (1992). The effect of high temperature on metabolism of *Morimus funereus* larvae during an intermolt period. *J. Insect Physiol.* **38**, 877–883.

Ivanović, J., Lazarević, J., Đorđević–Stajić, S., Leković, S., Nenadović, V. (1998). Influence of diet composition and neurohormones on digestive enzyme activities in *Morimus funereus* larvae. *Acta Entomol. Serbica.* **3**, 43–53.

Karowe, D. N., Martin, M. M. (1989). The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilisation, nitrogen budget, and metabolic rate of the fifth instar of *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). *J. Insect Physiol.* **35**, 699–708.

Kataoka, H., Troetschler, R. G., Li, J. P., Kramer, S. J., Carney, R. L., Schooley, D. A. (1989). Identification of an allatotropin from adult *Manduca sexta*. *Science*. **243**, 1481–1483.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227**, 680–685.

Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Ann. Rev. Ecol. Syst.* **11**, 119–191.

Metcalfe, N. B., Monoghan, P. (2001). Compensation for a bad start: grow now, pay later. *Trends in Ecology and Evolution*. **16**, 254–260.

Nenadović, V., Janković–Hladni, M., Ivanović, J. (1989). The effect of food substrate on the neuroendocrine system and metabolism of *Morimus funereus* (Insecta, Coleoptera). *Gen. Comp. Endocrinol.* **74**, 211–212.

Nishiitsutsui–uwo, J., Endo, Y. (1981). Gut endocrine cells in insects: the ultrastructure of he endocrine cells in the cockroach midgut. *Biomed. Res.* **2**, 30–44.

Reese, J. C. (1979). Interactions of allelochemics with nutritients in herbivore food. In: *Herbivores — Their Interactions With Secondary Plant Metabolites* (Eds. G.A. Rosenthal and Janzen D.H.), 309–330. Academic Press, London.

Roberts, D.B. (1986). *Drosophila: A Practical Approach*, 15–19. JRL Press, Oxford.

Sehnal, F., Žitnan, D. (1996). *Midgut endocrine cells.* In: *Biology of Insect Midgut.* (Eds. Lehane M. J. and Billingsley P. F.), 55–86. Chapman & Hall, London.

Slansky, F. Jr. and Scriber, J. M. (1985). Food consumption and utilisation. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, **4**, (Eds. G.A. Kerkut and Gilbert, L.I.), 87–163. Pergamon Press, Oxford.

Thomsen, E., Möller, I. (1963). Influence of neurosecretory cells and *corpus allatum* on intestinal activity in the adult *Calliphora erytrocephala* Meig. *J. Exp. Biol.* **40**, 301–321.

УТИЦАЈ ДИЈЕТА РАЗЛИЧИТОГ ПРОТЕИНСКОГ САСТАВА НА ПРОТЕИНЕ СРЕДЊЕГ ЦРЕВА И МОЗГА ЛАРВИ *MORIMUS FUNEREUS*

ЛАРИСА ИЛИЈИН, МИЛЕНА ЈАНКОВИЋ–ТОМАНИЋ, МАРИЈА МРДАКОВИЋ, МИЛЕНА ВЛАХОВИЋ, ВЕСНА ПЕРИЋ–МАТАРУГА, ЈЕЛИЦА ЛАЗАРЕВИЋ и ВЕРА НЕНАДОВИЋ

Институт за биолошка истраживања "Синиша Станковић",11060 Београд, Србија и Црна Гора

У овом раду је испитиван утицај вештачке дијете по Галфорду (Galford, 1967) и њене две модификације различитог протеинског састава (протеин соје, казеин, желатин) на ларве *Morimus funereus* након тоталног гладовања. Телесна маса ларви се повећала након исхране дијетом по Галфорду (G I), као и њеним модификацијама у којима је протеин соје замењен казеином (G II) односно желатином (G III). Присуство протеина различитог квалитета у хранљивом субстрату утиче и на квантитет и на квалитет протеина средњег црева и мозга ларви ове врсте.