Acta entomologica serbica, 1998, 3 (1/2): 43-53 UDC 595.768:577.152.34

INFLUENCE OF DIET COMPOSITION AND NEUROHORMONES ON DIGESTIVE ENZYME ACTIVITIES IN *MORIMUS FUNEREUS* LARVAE

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The effects of diet differing in nutritional value, starvation and neurohormones contained in the extract of cerebral complex and *corpora cardiaca* (ECC) on the activity of digestive enzymes (mesenteric proteases and amylases) of cerambycid beetle *Morimus funereus* larvae have been studied. The results clearly demonstrated changes in the activity of mesenteric proteases dependent on nutritional value of the diet, as well as ECC effects on the one hand. On the other hand, feeding and application of ECC after starvation led to a decrease of amylolytic acitivity.

KEY WORDS: *Morimus funereus*, digestive enzymes, diet, neurohormones, starvation.

INTRODUCTION

In addition to temperature, humidity and oxygen supply, the diet as an environmental factor significantly affects processes of development and metamorphosis in phytophagous insect species. The diet itself, in a complex interaction with other dominant environmental factors influences dynamics of both the number and quality of a population (ROSSITER, 1992; LAZAREVIĆ, 1994; LAZAREVIĆ et al., 1994). It has been reported that nutrition can induce a change in diet preference in some butterfly species what is of a certain evolutionary significance (de BOER, 1992).

Postembryonic development of the cerambycid beetle *Morimus funereus* under natural conditions lasts for 3-4 years. Rearing of the larvae on a *Drosophila* sp. substrate (AD) containing increased protein concentrations and at 23 C, prominently reduces duration of postembryonic development (6.5 months). This reduction can be ascribed to the activation of all protocerebral neurosecretory neuron species and an

intensified metabolism of proteins and lipids accompanied by a conspicuous increase of proteolytic activity (six-fold) as shown previously (NENADOVIĆ et al., 1989; IVANOVIĆ et al., 1989; 1991).

However, the influence of diet composition on activity of digestive enzymes and its significance in the changes occuring during the life cycle of the species examined, remained still an open question. This prompted us to perform the present study and besides the diet composition, the effects of neurohormones contained in the cerebral complex extract (ECC) were investigated, as well.

MATERIAL AND METHODS

Experimental groups and rearing conditions

The experiments were performed using *Morimus funereus* larvae collected from oak trees at Fruška Gora Mt. during the period of intensive growth (spingsummer). To record the changes in the activity of digestive enzymes (proteases and amylases) during 48 h, two groups of larvae fed on natural substrate (minced subcortical oak layer) were formed. The first group of the larvae was kept under conditions simulating natural ones, while those of the second group were reared under controlled temperature conditions (16 h at 23 ^{o}C and 8 h at 8 ^{o}C). Samples consisting of 7-9 larvae were taken for analyses every 6 h.

In another series of experiments concentrated on the examinations of the influence of diet composition on the activity of digestive enzymes the larvae collected at the above locality were used. They were starving for 7 days and then sacrificed (total starvation group - TS). The second group was transferred for 24 h on agar after 7 days of starvation (TS+AG) and the third one also exposed to starvation for 7 days was offered an artificial diet (TS+AD). The larvae of the fourth group were injected with the extract of cerebral complex and *corpora cardiaca* (ECC). The larvae of all experimental groups were sacrificed 24 h upon the feeding, i.e. injection of ECC.

The group of larvae (D) serving for the preparation of ECC was fed AD substrate for 30 days. Each experimental group consisted of 7-9 larvae.

Biochemical methods

The digestive enzyme activities were determined in the aqueous midgut homogenates by applying spectrophotometric methods. The amylolytic activity was determined according to BERNFELD (1955) and proteolytic activity as described by KUNITZ (1947). Optimal conditions for digestive enzyme activities *in vitro* were described previously (IVANOVIĆ et al., 1975).

Total protein content was measured by the method of LOWRY et al. (1951). Specific amylolytic activity (SAA) was expressed as A_{550}/A_{750} nm/h and specific proteolytic activity (SPA) as A_{280}/A_{750} nm/h.

Statistical analysis

All data are presented as the meansS.E. Statistical significance of the differences between experimental groups was evaluated by ANOVA and Scheefe's multiple range test (SOKAL & ROHLF, 1981).

RESULTS

Changes of the examined enzyme activities of the cerambycid beetle *M. funereus* larvae during digestion

Changes of proteolytic activity of the larvae maintained for 48 h under simulated natural conditions and of those kept at controlled varying temperature (23 ^{o}C and 8 ^{o}C) during 48 h are depicted in Figs. 1, graphs A and B, respectively.

It can be seen that both in the larvae from simulated natural conditions (Fig. 1, Graph A) and in those reared at controlled varying temperature conditions (Fig. 1, Graph B) proteolytic activity increases and decreases regularly during digestion phases. An increase of SPA begins in the evening to reach a maximum value during the night at about 2 a.m. Minimum proteolytic activity was recorded during the day at about 2 h p.m. Similar changes in midgut proteolytic activity were observed in the *M. funereus* larvae kept at controlled varying temperatures.

Changes of amylase activity of all experimental groups of the *M. funereus* larvae examined throughout the present study are shown in Fig. 2. Irregulat fluctuations of mesenteric amylase activities in the larvae kept under simulated natural conditions can be seen (Fig. 2, graph A), while in the group maintained under controlled varying temperature conditions, these fluctuations were somewhat more regular (Fig. 2. Graph B).

Changes in digestive enzyme activities during starvation, accessible food of different nutritional value and upon the action of neurohormones contained in ECC

Changes of proteolytic enzyme activities of the larvae exposed to starvation (7 days), short-term action of the diet of different nutritional value (24 h, agar or AD) and neurohormones contained in ECC from laruge (group D) are graphically presented in Fig. 3. In larvae fed AD, the level of proteolytic activity was the highest. In the group starved for 7 days, a mild increase of proteolytic activity was observed,

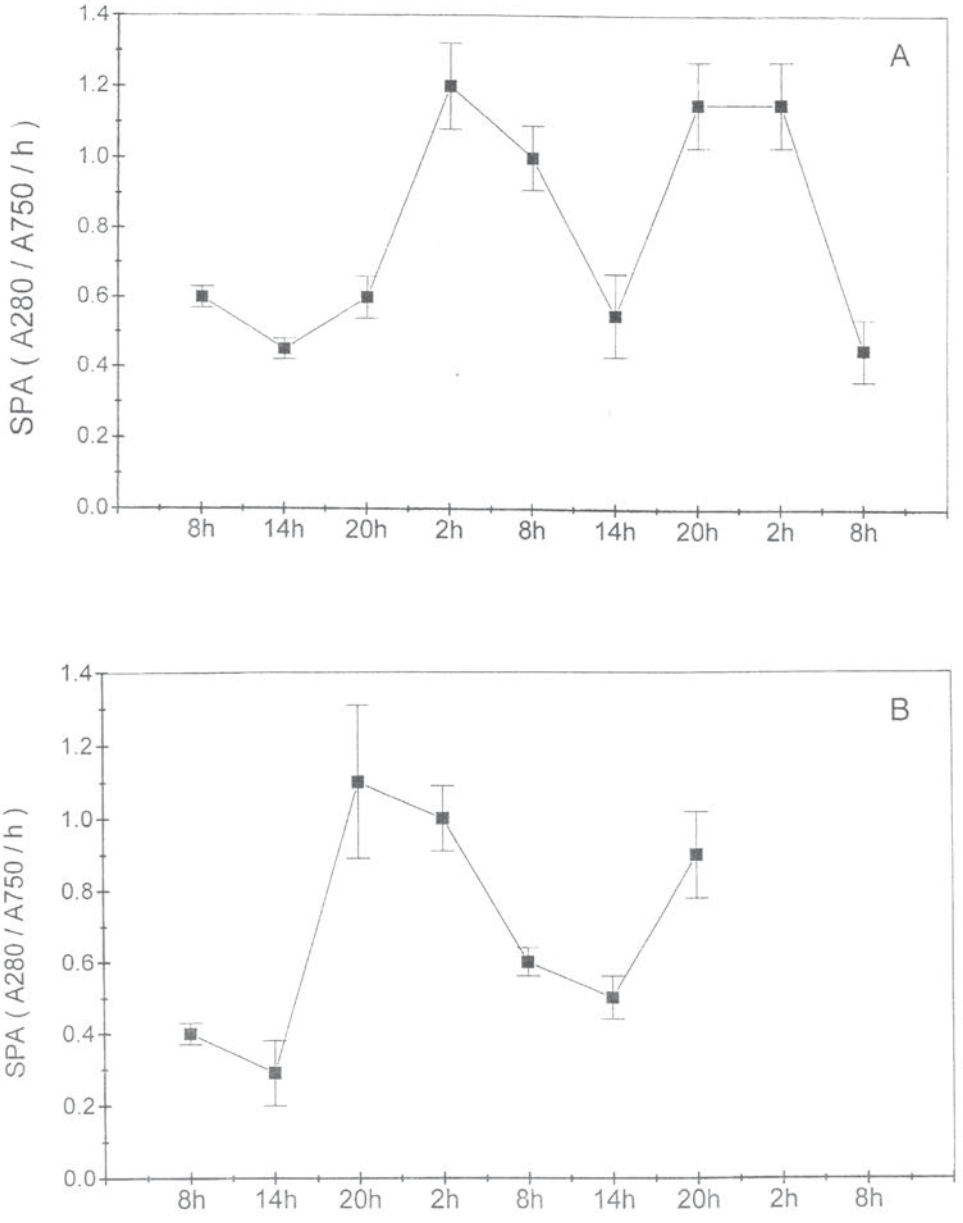

Fig. 1 (A) Changes of specific proteolytic activity (SPA) in *Morimus funereus* larvae maintained for 48 h under simulated natural conditions and **(B)** those kept at controlled varying temperature (23°C and 8°C).

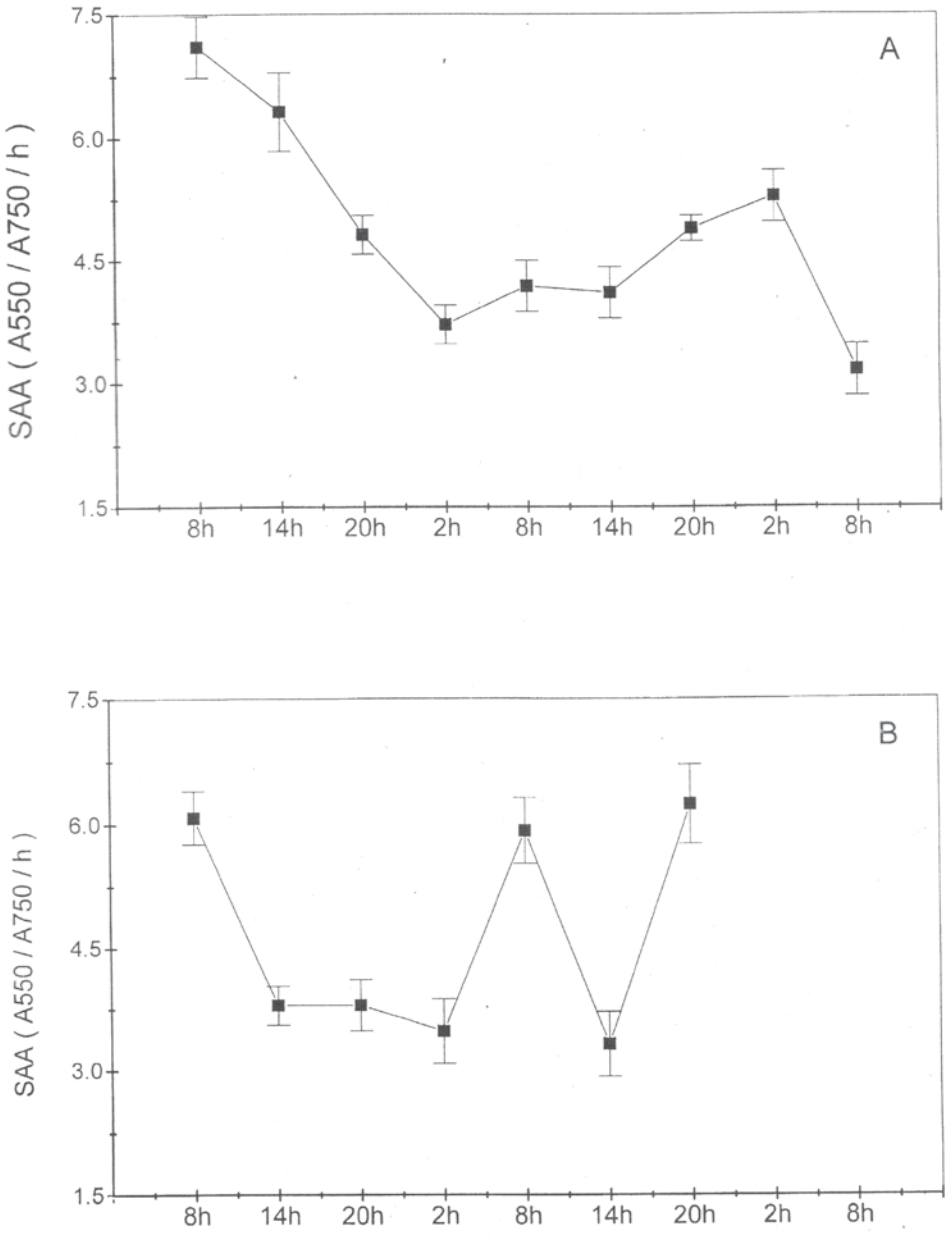

Fig. 2 (A, B) Changes of specific amylolitic activity (SAA) in *Morimus funereus* larvae maintained under the same experimental conditions as described in Fig. 1.

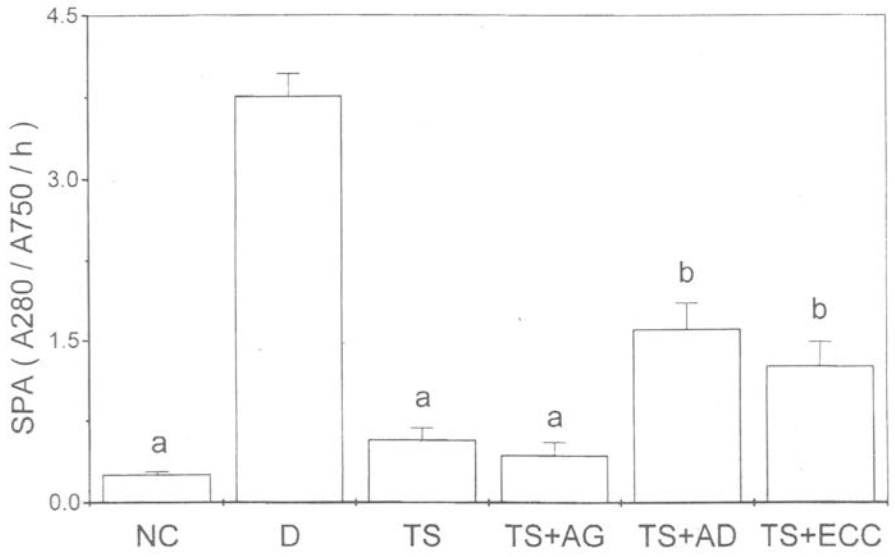

Fig. 3. Changes of SPA in *Morimus funereus* larvae exposed to starvation (TS), short-term (24 h) action of the diet of different nutritional value (agar-AG and artificial diet-AD) and neurohormones from ECC of donors (D) kept at AD for 30 days. Bars indicated by different letters (a, b) differ significantly (the

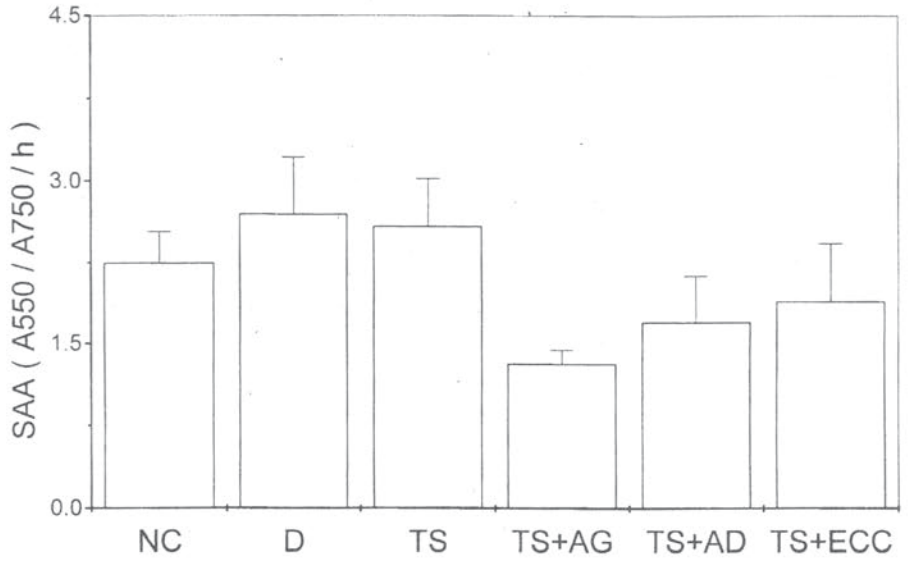

Fig 4. Changes of SAA in *Morimus funereus* larvae depending on starvation, diet composition and neurohormones. All abbreviations are the same as in Fig. 3. Statisticaly significant difference among experimental groups was not revealed by ANOVA and multiple range test.

although at the moment it is impossible to offer a logical explanation for this phenomenon. The data presented in Fig. 3 clearly demonstrate that the intensity of proteolytic activity changes strongly depends on the nutritional value of the diet. In the larvae offered agar known for its negligible nutritional value, a trend for a decline of proteolytic activity was recorded. In the group offered AD a significant increase of SPA was observed already after 24 h in comparison with both the NC and TS groups. The level of proteolytic activity was significantly increased in the group of larvae exposed to starvation for 7 days and injected with ECC.

Changes of amylolytic activity of the same groups of the larvae are shown in Fig. 4. As seen, no significant differences in the level of amylolytic activity were observed when the larvae from natural conditions (NC), those kept at AD and those exposed to starvation for 7 days (TS) were compared. Short-term action of the diet differing in nutritional value and application of ECC led to an insignificant decrease of amylolytic activity.

DISCUSSION

The influence of the diet and its nutritional value on individual performance of insect generalists that develop in the bark and wood mass of different deciduous and coniferous tree species differing not only with regard to content of tannins and other secondary metabolites, but also in the amounts of available proteins, amino acids and carbohydrates (BERNAYS & CHAPMAN, 1994) is insufficiently studied so far. In generally, in phytophagous and especially in xylophagous insect species the effect of the diet on digestive enzyme activities has been insufficiently examined due to the lack of knowledge on these enzymes and a high diversity of mesenteric peptidases (proteinases and exopeptidases) and other hydrolases (BIAN et al., 1996; ĐURĐEVIĆ et al., 1997), as well as because of the complexity of control mechanism of both secretion and synthesis of digestive enzymes in insects (BLAKEMORE et al., 1995).

For the cerambycid beetle *M. funereus,* the upper layer of the wood sap represents not only an external medium but also a nutrient substrate. Physiology of digestion of this insect species has not been sufficiently studied so far and as a consequence, it is not possible to claim that this species, i.e. its larvae belong to the group of insects that feed discontinuously. Food uptake and digestion of the 6th instar larvae reared on AD and constant temperature of 23 ^{o}C last some 24 h (unpublished results). However, among the larvae from natural conditions and those maintained under controlled conditions some of individuals with empty guts have been found, although this can't be connected to moulting. This suggests that hormones act as the regulators of both secretion and synthesis of digestive enzymes. Our results showing more expressed changes in proteolytic enzyme activities during digestion support

such a hypothesis (Figs. 1 and 2). Increased proteolytic activity of starved larvae injected with ECC (Fig. 3) or in ligated ones (IVANOVIĆ et al., 1978; IVANOVIĆ, 1991) may be taken as indirect proofs for hormonal regulation of activity of these enzymes. The data on this species reported so far suggest that digestive enzyme activities, especially of trypsin-lice enzymes are regulated by hormonal mechanisms. However, the possibility for the control of some exopeptidases and other hydrolases by secretagogues can not be excluded (ENGELMANN, 1969). It is also possible that the release and synthesis of digestive enzymes are subjected to a dual control (hormonal and through secretagogues) depending on physiological factors.

It is evident that the absence of food did not lead to a usual decrease of digestive enzyme activities in *M. funereus* larvae, although such an effect was observed in some other insect species (JANDA & MAIXNEROVA, 1972). Applying some immunological methods (incorporation of labelled cysteine into the neurosecretory protein) FRIEDEL & LOUGHTON (1980) observed a two fold decreased transport of neurosecretory material during starvation in comparison with the corresponding control. Short-term exposure to substrate of negligible nutritional value (agar) of *M. funereus* larvae that were previously subjected to starvation did not influence the level of proteolytic activity (Fig. 3) in comparison with the starved larvae. However, a substrate of a higher nutritional value (AD), as well as the injection of ECC resulted in a significant increase of proteolytic activity in the larvae examined throughout the present study.

Both the substrates used in the present work and ECC injection led to a decrease of amylolytic activity in relation to that found in the starved larvae. It should be pointed however, that there is the possibility that the results obtained upon the application of ECC to the larvae from natural populations could be the consequence of a high tissue concentration in the extract (2 CC/larva; to be published).

Previous studies performed on this model system demonstrated the influence of temperature not only on neuroendocrine system but also on all physiological processes which are under control of neurohormones, including digestive enzyme activities (IVANOVIĆ et al., 1975; 1988; 1989; 1991; ĐORĐEVIĆ et al., 1995), i.e. development and metamorphosis (STANIĆ et al., 1989; NENADOVIĆ et al., 1989; NENADOVIĆ, 1992).

Our results clearly demonstrate the influence of the diet on the life cycle of the cerambycid examined, the effects on the digestive enzyme activities being significant and by all probability controlled by neuroendocrine system.

Further studies along this line will be directed toward investigations on the role of individual diet components (especially of protein quality) on digestive enzyme activities, i.e. individual performance of cerambycid beetle.

ACKNOWLEDGEMENT

This work was supported by the Ministry for Science and Technology of Serbia, contract "03E13. The authors are thankful to dr Jelena Joksimović for useful suggestions.

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УТИЦАЈ ХРАНЕ И НЕУРОХОРМОНА НА АКТИВНОСТ ДИГЕСТИВНИХ ЕНЗИМА КОД ЛАРВИ *MORIMUS FUNEREUS*

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Извод

Изучавања утицаја хране као једног од доминантних фактора спољашње средине на метаболизам односно на активност дигестивних ензима код ксилофагних инсеката који се развијају у релативно сиромашном супстрату су релативно малобројна и имају спорадичан карактер.

У ранијим радовима нађено је да храна веће нутрицине вредности значајно скраћује дужину трајања ларвеног развића букове стрижибубе *M.funereus* што би могло бити проузроковано повећањем активности дигестивних ензима, нарочито мезентеричних протеаза (значајно повећање СПА код тзв. донора и при давању гладним ларвама АД) или повећањем ефикасности коришћења хране. Поред хране, њене нутрицине вредности на активност дигестивних ензима утичу и неурохормони о чему сведоче промене СПА током варења и код иницираних са ЕЦЦ гладних ларви, што потврђује раније добијен резултат на лигатурисаним ларвама ове стрижибубе.

Received December 25, 1998
Accepted March 10, 1999