

SUGAR CONTENT, TREHALASE ACTIVITY AND TREHALOSE LEVEL IN DRONE PREPUPAE OF *Apis mellifera carnica* PARASITIZED WITH VARROA

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S u m m a r y

A study performed on drone prepupae of honeybees *Apis mellifera carnica* naturally infested with *Varroa destructor* showed a significantly lower sugar content, $7.08 \pm 2.34\%$, as compared to $11.98 \pm 2.16\%$ in non-infested larvae. The trehalose level in infested larvae was also significantly higher than in their non-infested counterparts – $1.74 \pm 0.46\%$ and $2.23 \pm 0.39\%$ ($p < 0.05$), respectively. The latter difference may be the result of trehalase activity that was observed to be increased by one-half in infested prepupae. The results suggest that, as a result of Varroa infestation, the rate of saccharide consumption in drone larvae undergoes acceleration.

Keywords: drone prepupa, *Apis mellifera carnica*, *Varroa destructor*, varroatosis, sugars, trehalose, trehalase.

INTRODUCTION

Varroatosis is a honeybee (*Apis mellifera* L) disease (De Jong 1997). It is caused by the mite *Varroa destructor* (Anderson and Trueman 2000). Varroa infestation causes serious health disorders in honeybees. In order to control the adverse effects of the disease, such as weakening or even death of the capped brood and of the adult bees, it is necessary to study in depth the pathogenesis of varroatosis with particular emphasis on the changes in the physiology and biochemistry of the infested bee throughout its development.

Relatively much is known about the impact of Varroa on the protein metabolism in honeybees. The mite was found to lower the protein content of the hemolymph (Gliński and Jarosz 1984, Weinberg and Madel 1985, Sokół 1996, Bowen-Walker and Gunn 2001), especially the level of low molecular

weight proteins. It is linked to depressed immunological functions observed in parasitized bees (Gliński and Jarosz 1984, Żółtowska et al. 2005). It is also known that the abdomens of bees parasitized during their development by Varroa mites contain not only less proteins but also lower amounts of sugars (Bowen-Walker and Gunn 2001). Surprisingly, there is almost no information concerning the sugar metabolism in bees infested with Varroa mites. Apart from the paper by Bowen-Walker and Gunn (2001) no reference to that subject was found in the available literature. Sugars are known as an important energy source in insects, an especial role being ascribed to trehalose. Trehalose is one of the major saccharide occurring in honeybees (Morse and Hooper 1985). Apart from it, in the bodies of worker bees small amounts of glycogen, glucose, and fructose were found (Leta et al. 1996, Panzenböck and

Crailsheim 1997, Blatt and Roce 2001, 2002). It is also interesting that the hypotrehalosaemic hormone – Mas-AKH which in the majority of insects causes trehalose level to rise fails to produce that effect in honeybees due to the mobilisation of glycogen reserves. Indeed, in the northern races of *A. mellifera* - *A. m. carnica* (P) and *A. m. mellifera* (L) no traces of that hormone were detected (Woodring et al. 2003).

In this paper trehalose level as measured against the total content of saccharides and the activity of trehalase, the main enzyme involved in trehalose metabolism, were investigated in drone prepupae from naturally infested colonies that were parasitized with *V. destructor* and free of the mite.

Drone prepupae were chosen for the study, acting under the assumption that infestation-elicited changes in the level of saccharides should be greater in drones than in worker prepupae. Due to the longer duration of individual development and the preference of the mites for drone over worker larvae (De Jong 1997) drones are more vulnerable to massive infestation (Fuchs 1990, Boot et al. 1994, De Jong 1997).

MATERIAL AND METHODS

Drone prepupae of the honeybee *A. m. carnica* were used as the experiment material. They were sampled from an apiary situated in the commune of Jonkowo, ca. 20 km away from Olsztyn, and originated from colonies heavily infested with *V. destructor*. The combs from the apiary were transported to laboratory within ca. 20 min wrapped in slightly moistened towels to keep appropriate temperature and humidity.

Immediately after being transferred to the laboratory, the prepupae were isolated from the combs and divided into two groups: free of the parasite and parasitized.

The number of parasites feeding on the prepupae was counted. The individual prepupae were weighed and frozen at -71°C until analyzed. A total of 276 drone prepupae were collected of which 158 were non-infested individuals (controls) and 118 *Varroa*-parasitized individuals (infested group). From each group 30 individuals were picked at random to be analyzed biochemically.

Extracts were prepared by homogenizing individual prepupae separately in a glass Potter homogenizer with 4 ml 0.9% NaCl. Homogenate was centrifuged for 15 min. at $900 \times g$ and 4°C . The supernatant was used to make assays of total sugar content, trehalose, trehalase activity and protein.

The total sugars in prepupa extracts were assayed using the phenol-sulphuric acid method by Dubois et al. (after Keleti and Lederer 1974). Trehalose content was determined using high performance liquid chromatography (HPLC). The separation was done using a Shimadzu chromatograph with a refractometric detector on the Rezex RMN Carbohydrate Na^+ (30 x 0.78 cm) column at a flow rate of 0.4 ml per min. 20 μl of prepupa extract was applied on the column. Glucose content of the samples was measured enzymatically by using the analysis kit manufactured by Cormay, Lublin, Poland. The concentration of that sugar was read from a calibration curve plotted for the glucose standard (100 $\mu\text{g}/\text{ml}$) at a wavelength of 505 nm. Control samples to which prepupa extract was added only after incubation time were processed in the same way. Trehalase activity was expressed as μg of glucose released by the enzyme and converted to 1 mg of protein contained in the extract from prepupae.

Protein content was assayed using Bradford's method (1976). Ten μl aliquots of prepupa extract diluted 50-fold were used for the assays. The results were analysed using t-Student test.

Table 1

Selected physiological indicators in control vs. infected drone prepupae
of *Varroa destructor*

Indicator	non-infected prepupae	infected prepupae
Body weight (mg)	390.74±47.79	364.81±39.93 ^a
Protein (%)	9.41±2.01	9.08±1.59
Total sugars (%)	11.98±2.16	7.08±2.34 ^a
Trehalose (%)	2.23±0.39	1.74±0.46 ^a
Trehalase (µg glucose/mg protein)	150.17±43.81	231.83±54.22 ^a

^a significant difference between means of non-infected and infected group ($p < 0,05$).
Protein and sugar content were given as percentages of fresh prepupa body weight

RESULTS

The extent to which the prepupae were infested was high reaching 42.75% with average infestation rate of 1.74 mite per larva (ranging from 1 to 3 parasite per larva). The parasitized prepupae had a significantly ($p < 0.05$) higher body weight than did the control individuals (Table 1). Instead, the protein content of prepupa body extract in both groups did not differ significantly and was only slightly lower in parasitized drones. The total saccharide content of the bodies of non-infested prepupae was higher by 40% than that in parasitized drones. Likewise, trehalose level in non-infested prepupae was by 23% lower than that in infested prepupae. Mean group differences both for total sugar content and for trehalose concentration were statistically significant ($p < 0.05$) (Table 1). Trehalase activity in *Varroa* infested individuals was higher by more than by one-half of that in non-infested prepupae. The differences in the enzyme activity for both groups were statistically significant.

DISCUSSION

Drone larvae are particularly susceptible to infestation by *Varroa destructor* (De Jong 1997). It is also confirmed by high indicators of mite invasion recorded in this

study. Varroosis is known to be the cause of depressed body weight of parasitized insects (Bowen-Walker and Gunn 2001). It was borne out by the results of this study (Table 1). On the other hand we failed to find either in this or in previous studies (Zółtowska et al. 2005) any significant reduction of total protein level in the bodies of infested drone prepupae. It was only lower by 5% compared to that in the control group (Table 1). Those results partly contradict those reported by Gliński and Jarosz (1984) and by Weinberg and Madel (1985). It is probably due to the fact that we assayed the level of total protein in the bodies of drone pupae rather than that of the protein contained solely in the hemolymph as did the authors of the studies referred to above.

An important effect of the infestation by *Varroa* of drone prepupae was a significant ($p < 0.05$) reduction of the total carbohydrate concentration of their bodies (Table 1). A similar effect, though less pronounced, was observed by Bowen-Walker and Gunn (2001) in freshly emerged workers of *A. mellifera*.

Likewise, trehalose concentration in the bodies of parasitized drone prepupae was lower than that in the controls. The lower trehalose concentration in infested drone larvae can be accounted for by an elevated

activity of trehalase – trehalose - degrading enzyme. The activity of that enzyme in the infested prepupae was higher by as much as one-half of that in the non-infested ones (Table 1). The changes in trehalose level in hemolymph are still more interesting if one considers that the sugar, apart from being an energetic material in forager bees (Woodring et al. 2003), also performs the role of a signal compound that regulates the activity of the proventriculus controlling the inflow of essential energetic substrates (Roces and Blatt 1999, Blatt and Roces 2002).

It is surprising that the difference in trehalose level between the infested and non-infested group, though statistically significant, was much smaller than that for total sugar content (Table 1). The fact can be at least partly accounted for by the declining role of Mas-AKH to control sugar level of the hemolymph in the evolution of the European honeybee races (Woodring et al. 2003). As a result, the percentage of trehalose in total sugars of the control larvae was 18.61% being lower than that of the infested larvae (24.58%) (Table 1). The decomposition rate of that disaccharide in mite-parasitized larvae, resulting from an increased trehalase activity, is much higher than the trehalose breakdown rate in non-infested larvae. Being aware of those relationships among sugars in drone brood one can suggest that during the parasitizing by *Varroa* the following occurs: (i) either enhanced trehalose synthesis in infested prepupae or (ii) a simultaneous higher catabolic rate of other energetic reserves such as glycogen. It is not to be expected that other energetic substrates, such as lipids, should be involved since according to the report by Bowen-Walker and Gunn (2001) the level of those compounds in parasitized worker bees did not undergo any significant changes. The clarification of that issue will be the subject of our further studies.

In conclusion, the results obtained in this study warrant the statement that one of the effects of *Varroa* parasitizing drone larvae is an increase in sugar consumption by the prepupae. Supposedly, it is related to a higher demand for energy necessary, if not for other forms of insect activity, to enhance the defensive functions of honeybee.

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ZAWARTOŚĆ CUKRÓW, AKTYWNOŚĆ TREHALAZY ORAZ POZIOM TREHALOZY U PRZEDPOCZWAREK TRUTOWYCH *Apis mellifera carnica* DOTKNIĘTYCH WARROZĄ

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S t r e s z c z e n i e

Warroza jest chorobą pszczoł miodnych (*Apis mellifera* L.) wywołowaną przez odżywiający się hemolimfą roztoczą *Varroa destructor*.

Cukry są najważniejszym źródłem energii dla pszczoły miodnej, mimo to nie wiele wiadomo o wpływie tego pasożyta na metabolizm cukrowy żywiciela. Jedyne prace Bowen-Walker i Gunn (2001) dostarcza informacji o obniżeniu ogólnego poziomu cukrów w odwłokach wygryzających się robotnic *A. mellifera* dotkniętych warrozą. Nie badano tego zagadnienia u czerwiu zasklepionego, który może nawet dotkliwiej odczuwać ubytki sacharydów powodowane przez pasożyta niż osobniki dorosłe. Stało się to celem niniejszej pracy.

Materiałem badawczym były trutowe przedpoczwarki pszczoł miodnych *A. m. carnica*, naturalnie zarażone *V. destructor*. W ekstraktach z ich ciała badano ogólną zawartość cukrów, poziom trehalozy oraz aktywność trehalazy. Wykazano istotnie niższe stężenie ogółu cukrów $7,08 \pm 2,34\%$ u zarażonych przedpoczwerek w porównaniu do $11,98 \pm 2,16\%$ u larw niezarażonych. Również poziom trehalozy był u nich znacznie niższy niż u niezarażonych i wyniósł odpowiednio $1,74 \pm 0,46\%$ i $2,23 \pm 0,39\%$ ($p < 0,05$). Ta ostatnia różnica może być skutkiem wyższej o ok. 50% aktywności trehalazy, obserwowanej u zarażonych przedpoczwerek.

Uzyskane wyniki sugerują, że w następstwie warrozy wzrasta się tempo zużycia sacharydów u larw trutowych. Innym stwierdzonym następstwem parazytozy była istotnie niższa masa ciała zarażony przedpoczwerek oraz obniżony poziom ich białek rozpuszczalnych. W tym ostatnim przypadku różnice nie były statystycznie istotne.

Słowa kluczowe: przedpoczwarki trutowe, *Apis mellifera carnica*, *Varroa destructor*, warroza, cukry, trehaloza, trehalaza.