THE CONCENTRATION OF CARBOHYDRATES IN THE DEVELOPMENTAL STAGES OF THE *Apis mellifera carnica* DRONE BROOD

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Summary

The concentrations of sugars were investigated in the *Apis mellifera carnica* drone brood in successive development stages. These stages are from the newly hatched larvae to the freshly emerged imago**.** Brood of all stages contained glycogen, trehalose and glucose. The glycogen concentration was higher in the fresh matter of all stages than trehalose and glucose. It was high in 1-2 day-old larvae (92.1 mg/g tissue), then decreased by half in 3-4 day-old larvae, and then reached 117.0 mg/g just before sealing. Sealed larvae contained almost 50% less glycogen than that close to cell capping. A subsequent increase occurred in the prepupae and pupae. The highest concentration (127.4 mg/g) was observed in pupae with pink eyes. Freshly emerged drones had the lowest level (6.1 mg/g) of glycogen.

The lowest concentration of trehalose was observed in four-day-old larvae (1.5 mg/g). A significant increase occurred before sealing; but the highest concentration was found in larvae spinning the cocoon (12.9 mg/g). After that it continuously decreased. Freshly emerged drones had little trehalose (3.2 mg/g). Differences between all described means were statistically significant.

The glucose concentration was low in brood of all developmental stages and ranged from 1.3 – 3.0 mg/g of fresh matter. Only the concentration in prepupae was significantly lower than in other stages.

Keywords: *Apis mellifera*, brood development, carbohydrates, glycogen, trehalose.

INTRODUCTION

It is generally agreed that sugars are the most important energy sources for honeybees (Hepburn et al. 1979, Let et al. 1996, Panzenböck and Crailsheim 1997). More sugars are needed for drone brood breeding than for worker brood rearing (Hrassningg and Crailsheim 2005). This is manifested not only by their higher body weight but also by carbohydrate content, i.e. the percentage of glycogen (Hrassningg and Crailsheim 2005).

At this point it must be noted, that the worker honey bee larva at hatching time weighs about 0.08 mg. By the time the cell is sealed and feeding ceases, its weight increases up to 144 - 162 mg. After this, the use of stored materials to reorganize the body, reduces the weight of the emerging imago to about 112 mg. Similarly, drone larvae reach a maximum fresh weight at the time of cell sealing (262 - 419 mg). After that their weight decreases. The weight of freshly emerged drones of European honeybees was found to be 277 - 290 mg (reviewed by Hrassningg and Crailsheim 2005).

In general, honeybees consume carbohydrates in the form of nectar or honey. The enzymes such as invertase and α-glycosidases break the nectar sugars into

glucose and fructose. These sugars are used directly or converted into fat body and glycogen, which is the main sugar reserve (Morse and Hooper 1985, Caron 1999).

Glucose and fructose are very important in bee haemolymph and are present in large quantities compared to other insects (Morse and Hooper 1985, Fell 1990). The other reserve sugar circulating in the haemolymph of insects is disaccharide – trehalose. Trehalose and glycogen are synthesised in the body fat from glucose (Morse and Hooper 1985, Blatt and Roces 2001). Fructose is transformed into glucose, which is metabolised in the tissue cells and it is also transformed into trehalose and glycogen in the fat body (Gmeinbauer and Crailsheim 1993, Candy et al. 1997, Pazenböck and Crailsheim 1997, Lorenz et al. 1999).

Adult honeybees store only limited amounts of glycogen in the flight muscle and fat body. They use negligible quantities of amino acids as fuel. For these reasons they are highly dependent on intestinal and haemolymph energy supplies for most activities (reviewed by Blatt and Roces 2001, Woodring et al. 2003). This is facilitated by the fact that the honey sac is large. It consists of up to 30% of the total body weight of a worker bee (Lorenz et al. 2001).

Surprisingly, there is only fragmentary data concerning the concentrations of the sugars in the developmental stages of the *Apis mellifera* (Bishop 1925, Hepburn et al. 1979, Panzenböck and Crailsheim 1997). Even more surprising, is the lack of data regarding the earlier stages of drone development; this was already noticed by Hrassningg and Crailsheim (2005).

Since carbohydrates are crucial for the physiology of the worker honeybees (Wheeler 1996, Hoover et al. 2006), as well as drones (Hrassningg and Crailsheim 2005) we decided to fill this gap in bee biology by studying the different concentrations of sugars during the development of the *A. m. carnica* drone.

MATERIAL AND METHODS

Honeybee combs, containing unsealed and sealed drone broods, were removed in May of 2005 from five colonies of the *A. m. carnica* in an apiary situated ca. 20 km west from the city of Olsztyn. These combs were selected randomly and wrapped in warm, moist towels to maintain their appropriate temperature and humidity during immediate transport to our laboratory (ca. 20 minutes). Upon arrival at the laboratory, the drone brood was immediately removed intact from the brood combs. Next, each bee was individually classified according to the stage of development. This was done mainly by the size and shape, according to Jay (1962, 1963). Next this material was weighed and immediately frozen at – 71°C for further analysis. The whole procedure took between 40-60 minutes.

Groups were formed as follows: (i) - nunsealed brood: one- or two- (L1-2), three- (L3), four- (L4) and six-day old larvae (L6), (ii) - sealed brood was divided into: seven-day old larvae (L7) spinning larvae (L8 and L9), prepupae (PP), pupae with white (P1), pale pink (P2), pink (P3), brown eyes and yellow thorax (P4), pupae with dark eyes and dark thorax (P5). The investigations were also carried out on freshly emerged drones (A).

For biochemical analysis, thirty drones were randomly selected from each age group. Then each selected individual was analyzed separately. However, in the case of the first three developmental stages (L1-2, L3 and L4), due to their very low weight, the analyzed sample consisted of n = 100, n = 50 and n = 30 larvae respectively. All the analyses were repeated three times.

Preparation of extracts from age-related developmental stages of drones:

Each drone was homogenised separately in a glass Potter homogenizer with 4 ml of 0.9 mg/g NaCl. The homogenate was then centrifuged for 15 minutes at 900 x g at 4°C. The supernatant was used to determine glycogen, trehalose and glucose content.

The glycogen content was determined by the Söling and Esmann method (1975). High pressure liquid chromatography (HPLC) was used to determine trehalose and glucose content. The separation was carried out on Rezex RMN Carbohydrate Na^+ column (30 x 0.78 cm) at the flow rate of deionized water of 0.4 ml per minute, in a Shimatzu chromatograph with a refractometric detector. The results were expressed in mg of sugar per g of fresh matter of tissue. ANOVA was applied and the Tukey test was used to determine significant differences between means, $p < 0.05$ was considered as significant.

RESULTS

The weight of the drone brood increased quickly until cell capping (Table 1). The drone brood had the heaviest average weight on the seventh day of development (L7). Later, significantly reduced body weight was continuously observed up to the pupae stage with pink eyes (P3). After the last moult, a significant reduction of body weight was noted. In effect, the body weight of freshly emerged drones (A) was 30% lower than the larvae just after sealing (L7).

Drone brood contained all three determined sugars in its tissues during the whole period of development (Fig. 1 and Fig. 2). The total concentration of sugars ranged as follows: glycogen, trehalose and glucose. It is worth noting that the glycogen concentration fluctuated during brood development and was always the highest (Fig. 1). It differed always by one order of magnitude compared to trehalose and glucose.

Glycogen concentration (Fig. 1) was quite high in the youngest larvae (92.1 ± 22.3 mg/g). It decreased significantly by a half in L3 and L4 larvae and then increased significantly to 117.0 ± 26.1mg/g in larvae (L6) just before cell capping. Capped larvae (L7 – L9) contained significantly almost 50% less glycogen than the L6 stage just before cell capping. In older stages the glycogen content continuously increased. The highest concentration (127.4 ± 18.4 mg/g) was noted in pupae with pink eyes (P3). Subsequently it declined significantly together with pupae development. The

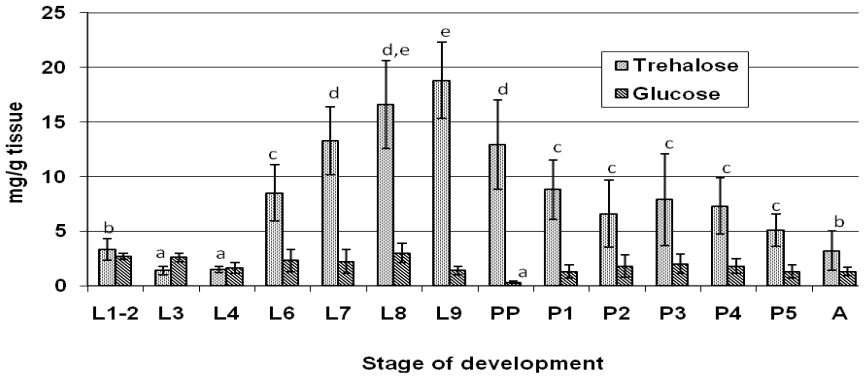

Fig.1. The changes of glycogen concentration during honeybee drone development. For explanations see Table 1. Error vectors present SD. Different letters at the top of bars indicate significant differences between means.

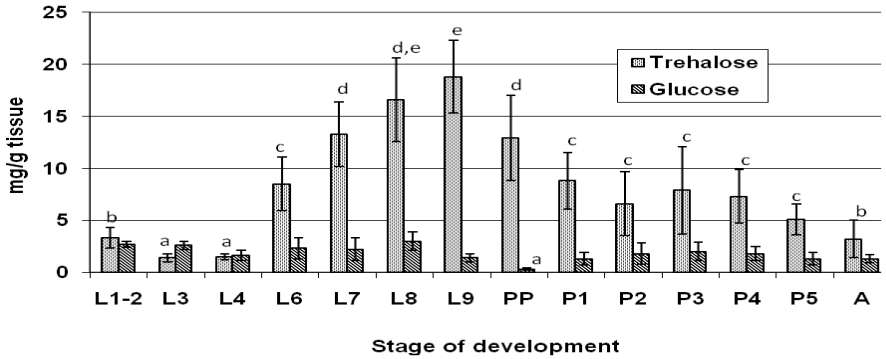

Fig. 2. The changes of trehalose and glucose concentrations during honeybee drone development. For explanations see Table 1. Error vectors present SD. Different letters at the top of bars indicate significant differences between means. In the PP stage glucose concentration was significantly lower than in the other stages.

freshly emerged drones (A) had the lowest (6.1 ± 3.9 mg/g) concentrations of glycogen (Fig.1).

The trehalose concentration was 3.3 ± 1.0 mg/g in L1-2 larvae (Fig. 2). Then, it decreased significantly that L4 larvae possessed the lowest amount of this sugar during the whole developmental period (1.5 ± 0.3 mg/g). It significantly increased before cell capping and reached

Table 1

Body weight of honeybee drones during successive developental stages.

Stage of development[1]	Mass (mg) (M ± SD)	Ranges of masses (mg)
L1-2 (n=398)	4.64 ± 1.32^{a}	2-11.2
L3 (n=176)	16.53 ± 5.33^{b}	8.3-31
L4 (n=112)	37.27 ± 9.12^{c}	28.3-41.5
L6 (n=80)	271 ± 31^{d}	186-296
L7 (n=88)	369 ± 42^{e}	310-412
L8 (n=60)	348 ± 58^{e}	280-420
L9 (n=60)	333 ± 23^{e}	256-374
PP (n=91)	330 ± 34^{e}	287-372
P1 (n=70)	323 ± 23^{e}	294-350
P2 (n=69)	281 ± 19^{d}	260-310
P3 (n=66)	275 ± 26^{d}	241-310
P4 (n=50)	$283 \pm 43^{d,e}$	252-330
P5 (n=45)	$284 \pm 42^{d,e}$	235-354
A (n=41)	253 ± 32^{d}	225-277

[1] one or two- (L1-2), three- (L3), four- (L4) and six- (L6), seven-day (L7) old larvae, spinning larvae (L8 and L9), prepupae (PP), pupae with white (P1), pale pink (P2), pink (P3), brown eyes and yellow thorax (P4), pupae with dark eyes and dark thorax (P5), freshly emerged drones (A). Different letters indicate significant differences between means.

maximum value in moulting larvae L9 (12.9 ± 4.1 mg/g). Its content then decreased significantly in pupae to 5.1 ± 1.5 mg/g (P5). Freshly emerged drones had significantly less trehalose (3.2 ± 1.8 mg/g) than pupae.

Glucose concentration (Fig. 2) was low during drone development and ranged from 1.3 to 3.0 mg/g of fresh matter. The lowest concentration (0.3 ± 0.1 mg/g) was found in prepupae (PP). This concentration was significantly lower than in the other stages. Interestingly, the glucose concentration was almost the same as the trehalose for up to six days after hatching.

DISCUSSION

We observed that the body weight of drone larvae increased 2-3 fold during the first days after hatching, and 10 fold between days 4 and 7 of their life (Table 1). This is congruent with observations by Schmolz et al. (2005) and Hrassningg and Crailsheim (2005).

We found that; (i) the average body weight of our freshly sealed larvae was lower, but also (ii) the speed of its decrease was lower during metamorphosis compared to findings by Jay (1963) and Schmolz et al. (2005). This seems to explain why our freshly emerged drones (Table 1) had a similar average body weight to those noted by these authors. This is despite obvious differences in genetic and environmental conditioning of this phenomenon (Jay 1963).

An exceptional increase in body weight took place between the fourth and seventh day of post hatching development (Table 1). Our result was in agreement with those of Schmolz et al. (2005) and Hrassningg and Crailsheim (2005). It seems to be associated with the period of intensive feeding the larvae with modified drone jelly (Winston 1987, Hrassningg and Crailsheim 2005, Hoover et al. 2006). The wide range of values of drone body weights observed by us (Table 1) seems to be the result of difficulties with precise assessment of the age of larvae. This is very important, because honey bees have an exceptionally fast growth rate compared to other animals and plants (Schmolz et al. 2005). This can cause high standard deviations of averages especially in the groups of younger larvae (Table 1).

We assume that larval glycogen reserves, decay faster during the first four days after hatching than it is rebuilt by synthesis. Probably this is connected with insufficient development of the fat body which is a place of synthesis and storage of glycogen (Bishop 1923, Woodring et al. 2003). The higher glycogen level in larvae older than 4 days than younger ones (Fig. 1) was in accordance with the data of Schmolz et al. (2005). It could be associated with the fact that older larvae may store glycogen for later development in the period when larval food contains more carbohydrates (Haydak 1957, 1970). Six day-old larvae had the maximal concentration of glycogen. The level of trehalose increased continuously reaching a maximum in the spinning larvae (L8, L9) This is despite the large amount of energy used for spinning movements (Jay 1963). In our opinion the conversion of glycogen into trehalose took place at this time, causing a decrease in the glycogen concentration. Afterwards the level of glycogen increased again in pupae. This is congruent with the results obtained by Strauss 1911 (after Hrassningg and Crailsheim 2005) and Bishop et al. (1925). Similarly, Schmolz et al. (2005) stated that decrease of carbohydrate content occurred just after larvae sealing, especially at the stage of spinning larvae and late pupae. These authors suggested that drones rebuild sugars into lipids at that time. Drones can collect more lipids than workers, although both are supplied by energy mainly from glycogen reserves (Schmolz et al. 2005).

The concentration of glucose was respectively low and stable, whereas there were some higher fluctuations of trehalose over the whole investigaton period (Fig. 2). This fact seems to confirm the hypothesis of Blatt and Roces (2001) which predicts that at higher metabolic rates trehalose synthesis is not fast enough to balance its consumption.

The last period of drone metamorphosis was connected with a significant decrease of the concentration of glycogen and trehalose (Fig. 1 and Fig. 2). This observation is consistent with results of our previous enzymatic studies. The changes in the content of sugars positively correlate with total activity of α-glycosidases during drone development (Żółtowska et al. 2007). Further work needs to be carried out to elucidate the activity of specific enzymes connected with decomposition of their main reserve of the sugars – glycogen and trehalose. This will be the subject of our following investigations.

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ZAWARTOŚĆ CUKRÓW W CIELE STADIÓW ROZWOJOWYCH CZERWIU TRUTOWEGO *Apis mellifera carnica*

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

Celem pracy było oznaczenie stężenia cukrów w świeżej masie czerwiu kolejnych stadiów rozwojowych trutni *Apis mellifera carnica*, poczynając od świeżo wylęgłej larwy, a na wygryzającym się trutniu kończąc. U wszystkich stadiów czerwiu stwierdzono obecność glikogenu, trehalozy oraz glukozy.

Stężenie glikogenu było zawsze wyższe niż trehalozy i glukozy. Było ono stosunkowo wysokie u larw 1-2 dniowych (92,1 mg/g), po czym obniżało się prawie o połowę u larw 3-4 dniowych, a następnie rosło do 117,0 mg/g u larw gotowych do zasklepienia. Zasklepione larwy zawierały prawie o połowę mniej glikogenu niż tuż przed zasklepieniem. Następnie stężenie glikogenu wzrastało u przedpoczwarek i poczwarek. Najwyższe (127,4 mg/g) było u poczwarek z różowymi oczami. Najmniej glikogenu (6,1 mg/g) stwierdzono u świeżo wylęgniętych trutni. Najniższe stężenie trehalozy (6,1 mg/g) zaobserwowano u larw 4 dniowych. Znacząco wzrastało ono tuż przed zasklepieniem, najwyższe (12,9 mg/g) było u larw przędzących. Następnie stale obniżało się i u świeżo wylęgniętych trutni wynosiło 3,2 mg/g. Różnice między powyższymi średnimi były istotne.

Stężenie glukozy u wszystkich stadiów rozwojowych trutni było niskie i wahało się w granicach 1,3 – 3,0 mg/g. Najniższe stężenie stwierdzono u przedpoczwarek.

Słowa kluczowe: *Apis mellifera*, rozwój czerwiu, cukry, glikogen, trehaloza.