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The total protein content, protein fractions and proteases activities of drone prepupae of *Apis mellifera* due to varrosis

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ABSTRACT. The proteins level and activities of acid and alkaline proteases in whole body extracts of drone prepupae of *Apis mellifera* naturaly infested with *Varroa destructor* were studied. The infested and a non-infested group did not differ significantly in their total protein content. However, some differences in protein profiles were found. A lack of three protein fractions of moderate and lower molecular weight in infested prepupae was noted. Moreover, some differences in the quantity of protein in most of the fractions were observed. The activity of acid proteases from infested prepupae was lower ($p < 0.05$) compared with the activity of these proteases from the non-infested one group. The infested drone had higher activity of alkaline proteases than non-infested but this difference was not statisticaly significant.

Key words: *Apis mellifera*, drone prepupa, honey bees, proteases, proteins, *Varroa destructor*.

Introduction

The lower effectiveness of forager bees due to *Varroa* infestation can arise from different reasons [1]. The most important is serious blood depletion, usually through septic injuries of the skin of capped brood and imago bees caused by *Varroa* mites [1, 2]. This depletion in mature worker honey bees is connected with haemocytopaenia, lower antibacterial rates of hematocytes and changes in their morphotic profile [3, 4, 5].

The harmful effects of parasites lead to significant losses in body weight or even malformation of the mature bee [1, 6, 7]. Septic, mostly viral and bacterial infections, exert such a potent negative impact on bees that they are able to shorten their life spans even by 50% [1]. Finally, it was found that oxidative stress is one of the basic pathways of varoosis [8].

The significant reduction of total protein content was observed in haemolymph of *Varroa* infested mature honey bee and drone larvae, especially in low molecular weight fractions. It seems to be connected with the proteolytic activity of digestive enzymes injected into the haemocell of the honey bee by the feeding *Varroa* mite [9, 10].

We hypothesized that similar phenomena concerning the protein fractions of total body protein content should also appear in infested drone prepupae because they suffer serious injuries and blood depletion [2]. To verify this hypothesis, we investigated the protein and protease activities in tissues of the *Varroa* infested drone prepupae, both in their body weight and total protein content.

Material and methods

276 prepupae (*A. mellifera*) taken in the middle of May 2004 from a naturally infested colony were used. Their body weights and infestation rates were noted. They were divided into infested and non-infested groups. From each of them 20 individuals were picked out randomly for the study. Each of the prepupa was separately homogenized in a glass Potter homogenizer with 2 ml 0.9% NaCl. The homogenates were centrifuged at 800 x g for 15 min at 4°C and the supernatant for enzyme determinations was carefully collected without destroying the upper lipid layer. The measurement of total protein content in the supernatant was performed according

to Bradford [11]. Proteins were separated through denatured electophoresis (SDS-PAGE) according to Laemmli [12], 56 μg of protein were applied to a 12% polyacrylamide gel. The Wide Range SigmaMarker™ was used to determine the molecular weight of protein fractions. Gels were stained with Coomassie Brilliant Blue R-250 (Sigma). Electrophoregrams were analyzed by the Gel Scan v. 01 program (Kucharczyk Ltd. Poland).

The activities of acid and alkaline proteases were estimated by Anson's method [13].

Results

The prevalence was 42.75%, the intensity of the infestation range was from 1 up to 3 *Varroa* per drone cell. On average, it was 1.74% per cell. The infested prepupae had a significantly lower body weight ($p < 0.05$) than the non-infested group. The total protein content in both groups did not differ significantly. Moreover, the infested prepupae contained slightly more protein (Table 1). PAGE-SDS has revealed 20 protein fractions in the tissues of non-infested prepupae, whereas in tissues of the infested ones only 17 protein strips were found (Fig. 1). We observed a lack of fractions number 12, 14 and 19 present in the non-infested larvae. The differences also occurred in the amount of proteins in the fractions which were comparable in molecular weight. Fractions no. 1, 3, 4 and 8 originating from infested prepupae contained less proteins, whereas fractions number 7, 11, 13, 16, 18 and 20 had more proteins than comparable molecular weight fractions from non-infested prepupae (Table 2).

The activity of alkaline proteases from infested prepupae was higher than those originating from not-infested groups. However, both groups did not differ significantly in these values because of the high level of standard deviations. Furthermore, the activity of acidic proteases in tissues of infested prepupae were half of those from the non-infested group. The difference between average values for

Table 1. The proteases activity, protein level and body weigth of prepupa drone of *Apis mellifera* infested with *Varroa destructor*

Factors	Non-infested[a]	Infested[a]
Alkaline proteases (mmol/mg protein)	1.21 ± 0.48	1.84 ± 0.98
Acid proteases (mmol/mg protein)	4.20 ± 0.20	2.03 ± 0.34*
Protein (mg/g body)	107.38 ± 16.21	110.69 ± 12.77
Body weigth (g)	0.412 ± 0.056	0.337 ± 0.061*

[a]Mean ± SD (n = 20), *$p < 0.05$

Table 2. The protein fractions in the extracts from non-infested and infested drone prepupae

Non-infested			Infested		
Fraction No	Molecular weight (kDa)	Relative percent	Fraction No	Molecular weight (kDa)	Relative percent
1	86.26	3.60	1	86.46	2.63
2	82.11	4.18	2	82.61	4.38
3	67.87	15.01	3	68.21	10.86
4	64.17	3.83	4	64.32	2.01
5	56.65	16.60	5	57.11	17.06
6	54.24	1.74	6	54.87	2.46
7	51.03	5.63	7	51.23	6.85
8	47.74	6.32	8	48.30	5.32
9	43.76	2.85	9	43.14	3.11
10	42.39	2.18	10	42.01	2.34
11	40.02	8.56	11	40.23	10.71
12	36.77	1.90	12	—	—
13	35.62	3.99	13	35.62	6.62
14	32.37	1.55	14	—	—
15	30.28	2.97	15	30.62	3.50
16	27.60	2.97	16	27.68	4.57
17	24.36	7.55	17	24.72	7.89
18	22.92	3.50	18	23.09	7.53
19	21.82	4.11	19	—	—
20	17.50	0.93	20	17.50	2.14

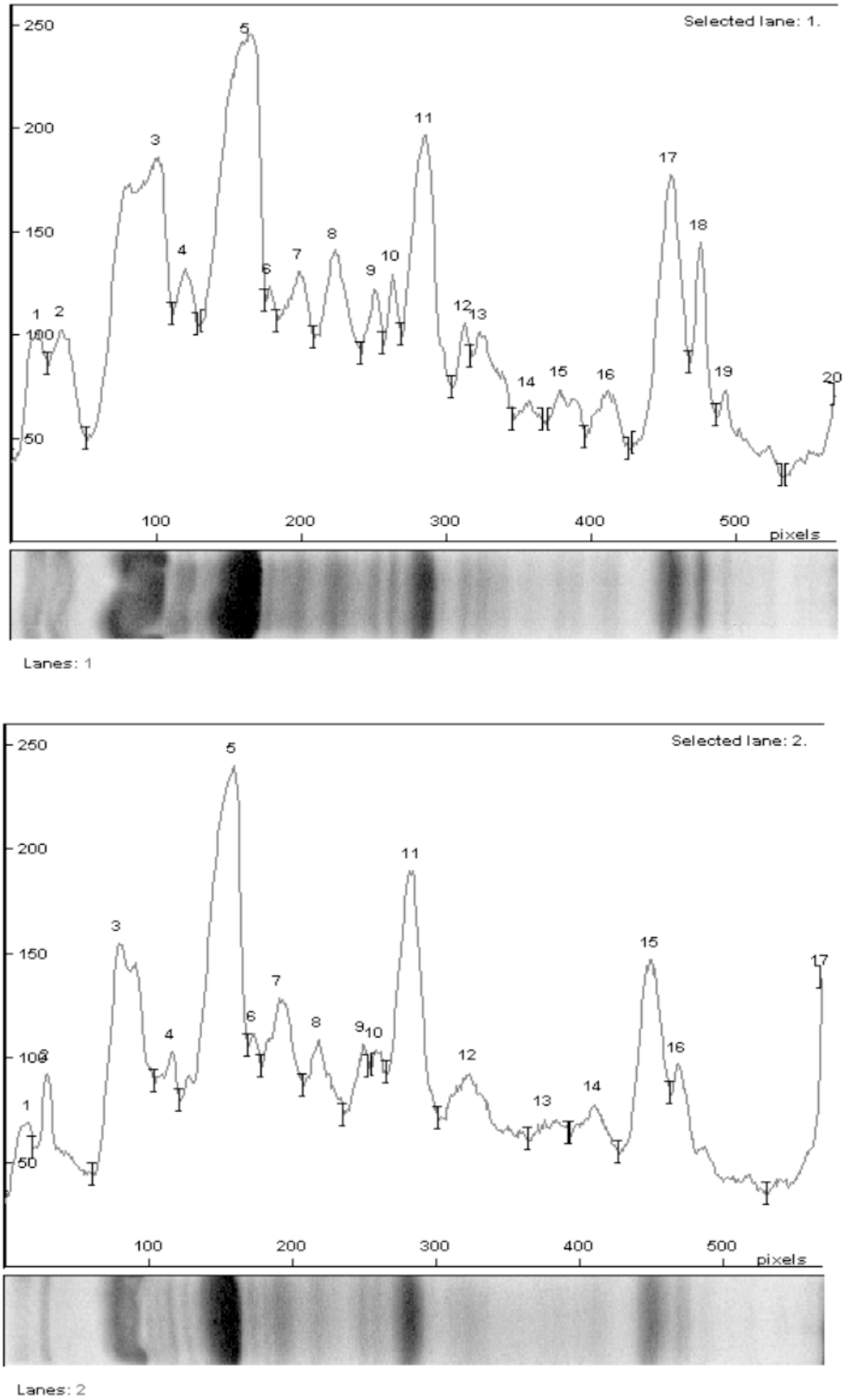

Fig. 1. Typical electrophoregrams and densitometer scans of proteins from non-infested (line 1) and infested (line 2) drone prepupae

both groups was statistically significant (Table 1).

Discussion

Drone larvae are especially sensitive to *Varroa* mite infestation [14]. Particularly, blood protein depletion and oxidative stress, cause smaller and malformed drones which are less effective in reproduction than larger drones [8, 9, 15]. These diminished reproductive abilities are seen in decreased effectiveness of mating flights, reduction of the number of spermatozoons and even changes in glycoprotein receptors on the spermatozoan surface [16, 17].

Our finding that *Varroa*-infestation diminishes the body weight of prepupae (Table 1) indicates that the process of dwarfing of drones begins shortly after capping the drone brood. This phenomenon is linked with the fact that we separated only 17 water soluble protein fractions in the tissues of infested prepupae, compared with 20 separated from the non-infested group. These observations agree with the results of Gliński and Jarosz [9] who noticed a reduction in lower molecular weights proteins in the haemolymph of *Varroa* infested drone honeybee larvae. However, the increase in the level of moderate and low molecular weight proteins in infested prepupae – ranging from 40 kDa to 17.5 kDa (Table 2) – suggests an immune origin [18]. The decrease in the number of protein fractions is not connected with the diminishing of total protein content in the whole body extracts from infested prepupae (Table 1). Contrary results were obtained by Gliński and Jarosz [9] and Sokół [10] for haemolymph *Varooa* infested drone prepupae and adult worker bees. The reduction in total protein content in this body fluid from infested insects was *ca* 30%.

Another negative aspect of *Varroa* infestation is a decrease in the activity of acid proteases. Their lower activity may result from the diminishing synthesis of these enzymes or from the inhibitory effects of the secretory products of *Varroa* on their activities. The inhibition of the activity of the host's proteolytic enzymes by the parasite often occurs during endoparasitosis [19, 20, 21]. We also can not exclude enhanced hydrolysis of the host proteins, including enzymes, by proteases released from the digestive tract of *Varroa* to drone prepupea coeloma during the sucking process [2]. This possibility was suggested by Gliński and Jarosz [9].

To conclude, the significant impact of *V. destructor* on the body weight and metabolism of proteins in the drone prepupa of *A. mellifera* indicates that the process of nutrition depletion and subsequent weakening and malformation of bees, especially in view of immunological reactions connected with oxidative stress, starts in the early stages of a capped brood. As this complicated phenomenon is still far from elucidation, it should be the subject of more detailed studies.

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