

HYGIENIC BEHAVIOUR OF BEE COLONIES AND TOTAL PROTEIN CONTENT IN THE HAEMOLYMPH OF WORKER BEES (*APIS MELLIFERA* L.)

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Abstract

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Total protein content in the haemolymph of worker bees from families with varying degrees of hygienic behaviour has been determined. A total of 26 samples of haemolymph have been analysed, obtained from 250-300 bees from colonies with different manifestation of hygienic behaviour - superhygienic – those that clean over 95% of the cells with dead brood in a 5x5 cm area by the 24th hour after puncture; hygienic - colonies that clean more than 95% of the cells in the marked square by the 48th hour after puncture; nonhygienic - those that leave uncleaned cells in the area after the 48th hour. The total protein content of the haemolymph has been determined by the Biuret method. A variation in the mean values for total protein content of 13.14 ± 3.94 g.l⁻¹ has been found for the group of superhygienic bee colonies, up to 24.96 ± 4.93 g.l⁻¹ for the group of hygienic colonies. The greatest is the min/max difference in nonhygienic bee colonies, from 4.16 g.l⁻¹ to 54.53 g.l⁻¹. The determined LS estimate against the average for the model is positive (+1.93) in colonies with low level of manifestation of hygienic behaviour (nonhygienic), which is indicative of a tendency to increase the amount of total protein in the haemolymph of worker bees. The stated results suggest that the failure of these bee colonies to quickly detect and clean the sick larvae (in this case the dead larvae) puts them in a stressful situation. Probably, as a response, activation of the natural defense factors of the bee organism is triggered, incl. change in the protein composition of the haemolymph. The results of the present study show that the degree of hygienic behavior in bee colonies affects the total protein content in bee haemolymph.

Key words: honey bees, hygienic behaviour, haemolymph, total proteins

Introduction

Hygienic behaviour is an example of the social behavior of honey bees and it is inherited in the offspring (Rothenbuhler, 1964; Lapidge et al., 2002). The cleaning instinct in bees is taken as a natural mechanism for control of disease causing agents in bees. In this regard it is necessary to clarify in detail the mechanisms of manifestation and the factors that influence it.

In honey bees there are no mechanisms to build an immune response, which is why the natural protective function of the haemolymph with lysozymes, proteins, haemocytes (blood cells) and antibiotics contained in it plays an important role. The haemolymph is the internal medium of the organism in insects - all organs, tissues and cells are in direct

contact with it. In bee larvae the haemolymph accounts for 25-30% of their liveweight, whereas in adult bees – 8-10%.

One of the most important functions of the insect haemolymph, incl. honey bees, is the protective one - prevention from infections and other agents of biological origin. The protective function of haemolymph includes cellular and humoral barriers (Tushtenko, 1986; Gillespie et al., 1997), expressed in phagocytosis, encapsulation, cytotoxicity, humoral encapsulation, etc.

In our country studies related to determining the level of manifestation of hygienic behaviour are not many (Petrov, 1997; Gurgulova et al., 2003; Zhelyazkova and Gurgulova; 2003, Darkazanli, 2008; Lazarov et al., 2016). Petrov (1997) while studying the possibilities for the selection of bees resistant to *Ascosphaera apis* (causing agent of chalk brood)

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applied a modified method for the detection of hygienic bee colonies. The study did not demonstrate a positive correlation between the level of manifestation of cleaning behaviour and the resistance of bees to ascospores. The same author found negative correlation between *Varroa destructor* bee infestation and hygienic behaviour of the bee colony, i.e. colonies that are highly infested with the mite have low level of manifestation of hygienic behaviour and he did not find a positive effect of the presence of natural feed or supplemental feeding of bees with sugar solution on the cleaning instinct of nonhygienic bee colonies. Gurgulova et al. (2003) track the changes in the amount of lysozyme and total protein in the haemolymph of worker bees as factors of the natural resistance of bees to diseases. The authors point out that the content of lysozyme and total protein is higher in bees in colonies with high level of manifestation of hygienic behaviour compared to the nonhygienic colonies. Darkazanli (2008) made a study on the relationship of hygienic behaviour to certain factorial diseases. Some of the results confirm the findings by Gurgulova et al. (2003), namely that total protein content and lysozyme activity in the haemolymph of worker bees have higher values in bees from hygienic colonies (100% cell cleaning up to the 48th hour).

Due to the established relationship between the level of manifestation of hygienic behavior and the resistance of bees to diseases, as well as the results of the studies carried out in our country on the bee cleaning instinct, there are possibilities for expanding research in this area.

The objective of the present study is to investigate the different level of manifestation of hygienic behaviour and the total protein content in the haemolymph of worker bees (*Apis mellifera* L.).

Material and methods

The study was conducted during the active beekeeping season in 2015 and 2016.

Testing bee colonies for hygienic level

During these periods 99 bee colonies were tested from 10 apiaries (from different regions in the country) for level of manifestation of hygienic behavior and the total protein content in the the haemolymph of worker bees was determined. The apiaries are grouped in 3 groups based on the business line: apiaries from research institutes and for queen bee production (apiaries I, II, V, VII and VIII); stock apiaries with less than 50 bee colonies (apiaries III and VI); stock apiaries with over 50 bee colonies (apiaries IV, IX and X).

Bee colonies from the local breed of *Apis mellifera* L. were used in the study.

Testing of bee colonies for hygienic behaviour was performed by using a modified method (Gurgulova et al., 2003), similar to the one applied by Petrov (1997). A square template of 5 x 5 cm was prepared to outline the testing area. The template is clamped on a certain stretch of the bee comb with a sealed worker brood, with 100 worker cells enclosed in it. The caps of cells with sealed brood are pierced with entomological needle No. 1 (without destroying them) to kill the pupae.

Depending on the period and level of cleaning bee colonies are divided into two main groups: hygienic - the families that clean more than 95% of the cells in the outlined square up to the 48th hour of piercing; nonhygienic – the ones that leave uncleaned cells in the area after the 48th hour. In the present study, within the group of hygienic bee families a group of superhygienic ones has been separated that clean over 95% of the cells in the outlined area up to the 24th hour of piercing.

Determining the total protein content in the haemolymph of worker bees

To determine the total protein content in the haemolymph, aggregate samples of 250-300 bees have been formed. The hemolymph is obtained by a thin Pasteur pipette between the second and third abdominal tergite. The collected haemolymph has been stored in a freezer until analyses were performed. A total of 26 haemolymph samples were analyzed by the Biuret method (Stambolova et al., 1978). The analyses were performed at the National Reference Laboratory “Bee Health” at the National Diagnostic Scientific Research Veterinary Medical Institute - Sofia.

The study data have been processed by statistical variation on computer - Statistica 6 software.

Results and discussion

Results of testing bee colonies for hygienic level

The data in Table 1 show that 59 of the 99 bee colonies tested (59.6%) are hygienic (over 95% of cells opened and cleaned at the 48th hour of brood killing) and 40 (40.4%) are nonhygienic (less than 95% of opened and cleaned cells at 48th hour of brood killing). Of the group of hygienic bee colonies 23 (38.98%) are superhygienic (over 95% of opened and cleaned cells at the 24th hour of brood killing). The relatively high percentage of nonhygienic bee colonies can be explained by the fact that some of the apiaries in which the test was carried out are non-isolated stock holdings.

At the 48th hour of reporting, high ($P \leq 0.001$) reliability in the differences between superhygienic/hygienic, superhygienic/nonhygienic and hygienic/nonhygienic bee colonies was determined - Table 1.

Table 1
Opened and cleaned cells in testing bee colonies for hygienic level

Opened and cleaned cells							
24 th hour (%)				48 th hour (%)			
mean	SE	min	max	mean	SE	min	max
Superhygienic bee colonies (n = 23)							
97.62	0.41	92.13	100	99.55***	0.18	96.94	100
Hygienic bee colonies (n = 36)							
82.5	2	44.44	94	98.10***	0.3	95	100
Nonhygienic bee colonies (n = 40)							
65.48	2.74	23.71	90.91	85.69***	1.51	55.67	94.9

*** P<0,001

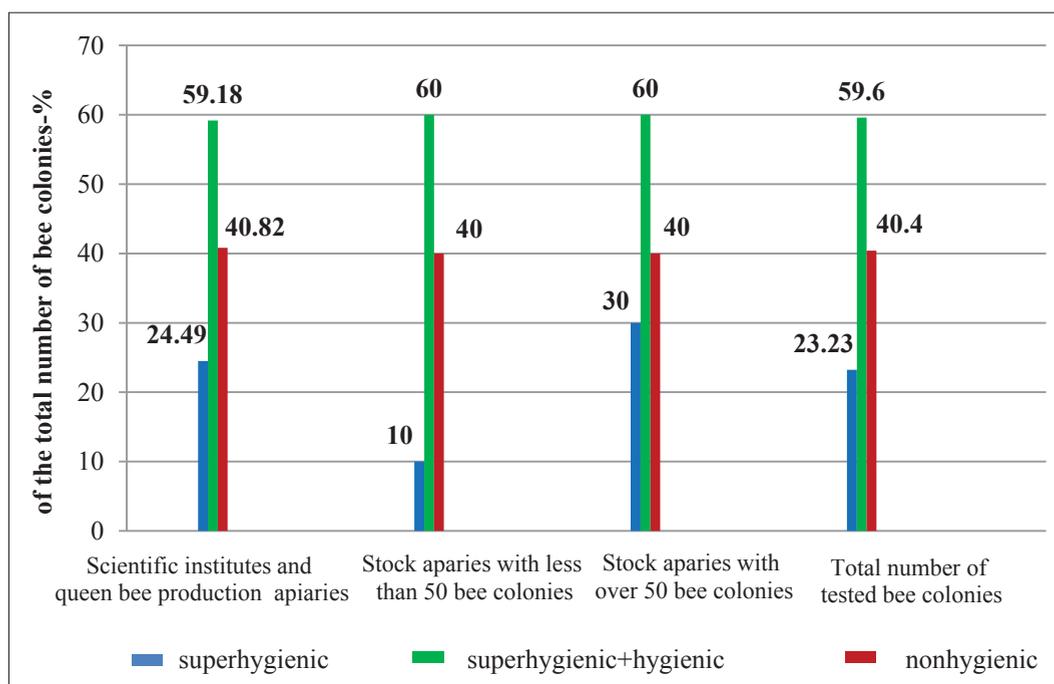


Fig. 1. Results from testing bee colonies for manifestation of hygienic behavior by apiaries – % of the total number

Fig. 1 presents the results from testing bee colonies for hygienic behaviour by apiaries. The apiaries are grouped in 3 groups, as follows: apiaries from scientific research institutes and apiaries for queen bee production (apiaries I, II, V, VII and VIII); stock apiaries with less than 50 bee colonies (apiaries III and VI); stock apiaries with over 50 bee colonies (apiaries IV, IX and X).

It is evident from the graph that for all groups of apiaries the number of hygienic bee colonies (superhygienic and

hygienic) ranged from 59.18 to 60% of the total number. The number of colonies with low level of manifestation of hygienic behaviour in the three groups ranged from 40 to 40.82%. The analysis of the results shows that for stock apiaries with over 50 bee colonies the percentage of colonies with high level of hygienic behaviour (superhygienic) is the highest (30%). A probable cause is that they often replace queen bees and queen bees purchased from queen bee production apiaries or own production from families with proven productivity and

hygiene are used. The intensive manner of rearing bee colonies in these apiaries is related to continuous control of the productive and other biological qualities of bees. The second by percentage of superhygienic bee colonies from the total number are the apiaries in the scientific research institutes and the queen bee production apiaries - 24.49%. The fewest superhygienic bee colonies were found in stock apiaries with less than 50 beehives - 10% of the total number of tested colonies. The comparative analysis shows that for the above group of apiaries the percentage of colonies with high level of hygiene is 2.5 times smaller than in the group of stock apiaries with over 50 beehives and 3 times smaller than the colonies in the group of scientific research institutes and queen bee production apiaries.

The results of the present study in aphids' apiaries with a different business line (stock, queen bee production, in experimental stations and scientific research institutes) show that the percentage of superhygienic and hygienic bee colonies is higher where control and selection of the local bee have been performed compared to apiaries in which this trait has not been tracked.

Results from determining total protein content in the haemolymph of worker bees

The present study includes determining total protein in the haemolymph of worker bees due to the fact that this trait is considered a factor of natural immunity and to great extent defines the protective functions of the bee organism.

The results obtained (Table 2) show average values for total protein content of 19.05 ± 3.45 g.l⁻¹ for the group of hygienic colonies up to 22.63 ± 5.18 g.l⁻¹ for the nonhygienic colonies. The highest is the variation in nonhygienic bee colonies with low hygienic level - from 4.16 g.l⁻¹ to 54.53 g.l⁻¹. The reported differences in the amount of total protein in the haemolymph of worker bees from colonies with different manifestation of hygienic behaviour are statistically unproven.

The data in Table 2 show that in the negative direction to the average of the model are the LS-estimates of bee colonies

with high level of manifestation of hygienic behaviour (superhygienic and hygienic) and in positive direction for bee colonies with low hygienic level (nonhygienic) – (-1.65) and (+1.93), respectively. The reported positive LS-estimate, as well as the established maximum value (54.53 g.l⁻¹) indicates that in bee colonies with low level of manifestation of hygienic behavior there is a tendency to increase the amount of total protein in the haemolymph of worker bees.

A similar trend has also been observed with regard to the level of lysozyme in the haemolymph (Lazarov et al., 2016). According to the authors, a probable cause for the results obtained is the inability of nonhygienic bee colonies to quickly detect and clean the sick larvae (in this case the dead larvae), which puts them in a stressful situation. As a response, activation of the natural defense factors of the bee organism is triggered, including change in the protein composition of the haemolymph.

Figures 2 and 3 graphically present the results of the total protein content in the haemolymph of bees from bee families with high and low levels of manifestation of hygienic behaviour by apiaries.

The average value of the studied trait for the group of hygienic bee colonies is 19.05 g.l⁻¹ - Table 2. In 40% of the apiaries values above the average for the group are observed, while for the remaining 60% they are lower - Fig. 2. In colonies with high hygienic manifestation, the amount of total protein in the bee blood has value above the average in apiaries I, V, IX and X, i.e. in bee colonies from the group of scientific research institutes and queen bee production and in the group of stock apiaries with over 50 colonies. In bee samples from apiary X (stock with over 50 bee colonies), the highest value for the studied trait was recorded - 1.7 times more than the average for the group.

In bee colonies with low level of manifestation of hygienic behavior (nonhygienic), the total protein content in the haemolymph of the studied bee samples is 22.63 g.l⁻¹ on average for the group - Table 2, Fig. 3. For 62.5% of the included bee colonies, the defined values of the studied trait are above the

Table 2
Amount of total protein in the haemolymph of worker bees from bee colonies with different level of manifestation of hygienic behavior

Hygienic level	Total protein g.l ⁻¹				LS-estimate
	mean	SE	min	max	
Superhygienic + hygienic bee colonies (n = 14)	19.05	3.45	1.21	43.76	-1.65
Nonhygienic bee colonies (n = 12)	22.63	5.18	4.16	54.53	1.93
Average for the model	20.7	2.98	-	-	

average for the group (apiaries II, V, VI, VII and IX) - from 1.03 to 2.41 times higher. The aforementioned apiaries are from the group of scientific research institutes and queen bee production and from the group of stock apiaries with over 50 colonies.

The study confirms the fact that haemolymph as an internal medium of the bee organism, responds quickly to a number of external factors - the way of rearing, nutrition, diseases, environmental state, etc. (Gliniski and Grzegorzczuk, 1995; Gliniski and Kauko, 1995; Gurgulova et al., 1997; Zhelyazkova and

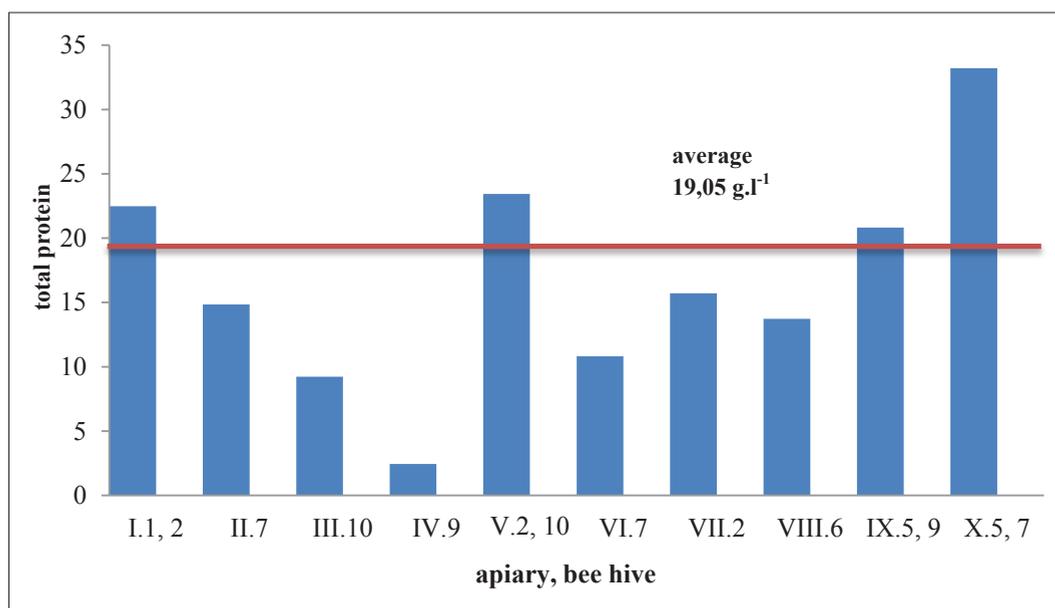


Fig. 2. Total protein content in the haemolymph of bees from bee colonies with high hygienic level (superhygienic and hygienic), g.l⁻¹

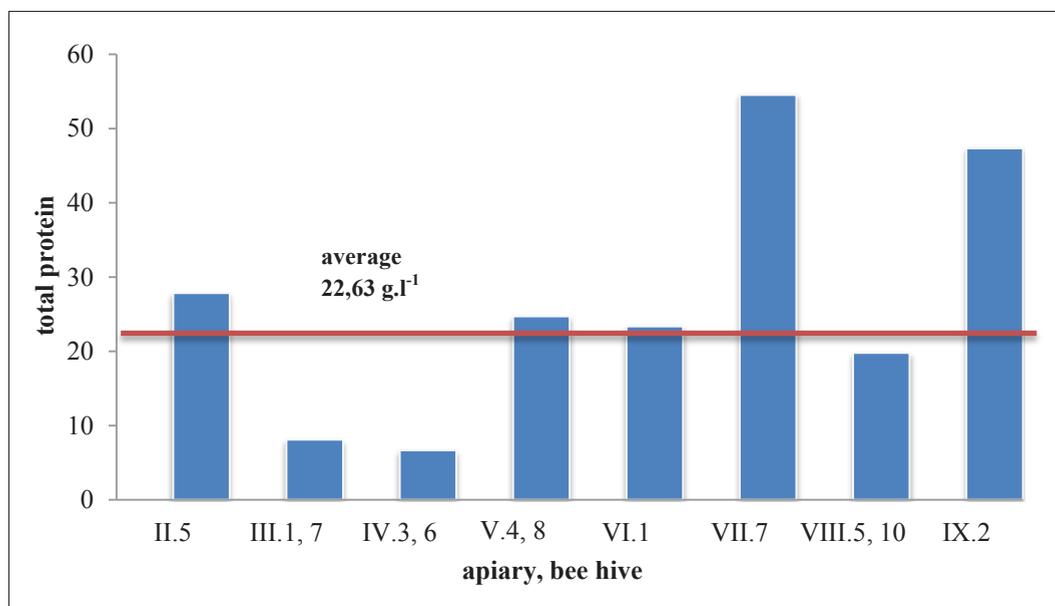


Fig. 3. Total protein content in the haemolymph of bees from bee colonies with low hygienic level, g.l⁻¹

Gurgulova, 1997, 2000; Kanchev et al., 1997; Gurgulova et al., 2001; Zhelyazkova et al., 2004; Shumkova, 2016).

On the other hand, the results obtained complement the above factors and show that the level of manifestation of hygienic behaviour in bee colonies also results in changes in the total protein content in the bee haemolymph.

Conclusions

Of the 99 bee colonies tested, 59.6% are hygienic (over 95% opened and cleaned cells at the 48th hour of brood killing) and 40.4% are nonhygienic (less than 95% opened and cleaned cells at the 48th hour of brood killing). From the group of hygienic bee colonies, 38.98% are superhygienic (over 95% opened and cleaned cells at the 24th hour of brood killing). Based on the results for apiaries of different business lines (stock, queen bee production, at experimental stations and research institutes) it is found that the percentage of superhygienic and hygienic bee colonies is higher where control and selection of the local bee were conducted compared to apiaries where this trait had not been traced (stock apiaries with less than 50 bee colonies).

In negative direction from the average for the model are the LS-estimates of the bee colonies with high level of manifestation of hygienic behavior (superhygienic and hygienic) and in positive direction for the bee colonies with low hygienic level (nonhygienic) – (-1.65) and (+1.93), respectively. The reported positive LS-estimate, as well as the established maximum value (54.53 g.l⁻¹), show that in bee colonies with low level of manifestation of hygienic behavior there is a tendency to increase the amount of total protein in the haemolymph of worker bees.

In bee colonies with high and low manifestation of hygienic behaviour, the amount of total protein in the bee blood has value higher than the average for the group in the apiaries of research institutes and queen bee production and stock apiaries with over 50 colonies.

In the present study, it was found that the level of manifestation of hygienic behavior in bee colonies resulted in changes in the total protein content of the bee haemolymph.

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