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Quantitative changes in hemogram of bees using probiotic «Enteronormin»

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The article considers the main quantitative changes in hemolymph cells under the action of the drug «Enteronormin» when fed with 50% sugar syrup during 10 days compared with the changes in the control group that received only sugar syrup. On the 3rd day, the hemogram of the bees that received the drug did not differ from the bees that received the sugar syrup only. Positive dynamics in the quantitative composition of the hemolymph was observed on the days 7–10. The number of phagocytic cells was 1.38% less than in the control group, and 2.0% of neutrophils less and 0.63–1.13% of eosinophils. The number of secretory cells increased, in particular, gliwocytes by 1.17% and 1.62%, respectively. It is noted that the medication «Enteronormin» has been extended to the extent of 3 days not a positive effect on the immune system of bees. Therefore, the drug «Enteronormin» should be fed for at least 7 days, taking into account the quantitative changes in hemocytes. On the twenty-first day the effect of the drug was preserved. The most appropriate term for drug feeding with the aim of activating the cellular immunity is defined to be 7–10 days.

Key words: bees, probiotik, «Enteronormin», hemogram, hemocytes, hemolymph, eosinophils, progemocytes, spherulocytes, enocitoid.

Introduction

The state of the immune system is important for a living organism homeostasis formation. Immune status determines the effectiveness and consistency of all systems and elements of immunity (Luss et al., 2000; Genersh and Aubert, 2010). The immunity of honey bees ensures the resistance of the bee family to the pathogenic microorganisms and their waste products. The formation of immunity involves the entire organism of bees as an integral system in which all the protective mechanisms are interconnected (Artemenko and Skrypnik, 1984; Boman and Hultmark, 1987; Genersh and Aubert, 2010). The internal protective mechanisms include the form elements of hemolymph and fatty body, as well as the antimicrobial properties of plasma, fluids and tissues of the insects' body. The protective properties of form elements' cells are called cellular immunity, while the protective properties of liquids – humoral immunity (Boman and Hultmark, 1987; Glinski and Jarosz, 2000). In the normal cell the composition of hemolymph varies depending on the breed, season of the year, conditions of keeping. In general, numerous studies on the factors of immunity in bees have shown that the stimulation of their activities causes complex biochemical changes in their organism, which, according to their spec-

ificity and efficiency are less significant than the analogous changes in the organisms of warm-blooded animals. Thus, any stage of bee development, its age and physiological state are characterized by a specific hemogram. Hemograms are very sensitive to physiological changes in the organism of insects. The hemogram of bees' hemolymph is used for early diagnosis of diseases, as well as for determining the effectiveness of the use of a particular drug (Artemenko and Skrypnik, 1984; Boman and Hultmark, 1987; Glinski and Jarosz, 2000; Sapcaliu et al., 2010).

The use of stimulants to prevent infectious diseases requires a more thorough examination of their effectiveness. According to the theory stating that all hemocytes have one source of origin (progemocytes) and claiming the ability of different cell types to transform into amoeba-like state for phagocytosis, cytological changes due to the immune drugs feeding can be analyzed. Moreover it is important to mention the study by V.P. Tishchenko (1986) proving that the insect hemogram reflects changes in their organism according to the physiological norm; in terms of hemocytes it is possible to evaluate their «fattening», to carry out an early diagnosis of diseases, parasite infections and cases of insect intoxication (Tyshhenko, 1986; Glinski and Jarosz, 2000; Sapcaliu et al., 2010).

One of the factors that significantly influences the change in cellular composition is the use of various biologically active drugs, in particular, probiotics. The beekeepers of Ukraine began to use the drug «Enteronormin», a probiotic, or a symbiotic, containing viable lactic acid and sporogenic bacteria in a unique symbiosis (*Enterococcus* spp., *Lactobacillus* spp., *Bacillus subtilis* spp.) with the addition of prebiotic chitosan and peptone. *Lactic acid bacteria* in the drug Enteronormin are specially selected to have the maximum ability to extract lactic acid, useful enzymes, essential vitamins, stimulators and modulators of the immune system, and other biologically active substances.

Enteronormin actively forms normal microflora, participates in activating the protective forces of the bee's organism, stimulates the development and productivity of bee colonies, exhibits antagonistic (suppressing) activity against a wide variety of pathogenic bacteria and fungi ([Rekomendacii po primeneniju ...](#)).

Analysis of recent research. Veterinary doctor Rubel I.S. from the company TD «Geotek» carried out the study on the life expectancy of bees using the probiotic «Emprobio». The effect of the probiotic on the hemolymph composition was not investigated. Domanska T. studied the effect of probiotics Vetom-1.1 and Vetom-3 for the treatment of bees families suffering from ascosferosis ([Rubel' et al., 2013](#)).

The objective of the research. To study the effect of probiotic «Enteronormin» on the hemolymph of bees. To identify the positive effects of the drug on the hemogram of bees, depending on the duration of treatment.

Materials and methods

The main tasks were: 1) to define the optimal term of the drug Enteronormin feeding taking into account the changes in hemolymph; 2) to detect in laboratory conditions the major changes that may occur in the composition

of hemolymph when feeding the probiotic drug Enteronormin, depending on the term of the drug feeding; 3) to identify presence of the drug prolonged action.

The analysis of the effect of the drug «Enteronormin» on the hemolymph of bees was carried out in the laboratory of the Department of Microbiology, Pharmacology and Epizootology of the Zhytomyr National Agroecological University during April–June 2017. The bees were kept in a thermostat with a passive ventilation system, where the temperature of 26 °C and a relative humidity of 35–70% were maintained. 100 species treated by the drug in a ratio of 1:5 with 50% sugar syrup were kept in entomophilic containers. Prior to the treatment, for the activation of microorganisms the required amount of the drug was dissolved in an aqueous solution of «Jodis + Se» in a proportion of 1 to 5 and infused during 16 h. The drug was prescribed orally during 3–10 days.

The hemolymph analyses were carried out on the 1st, 3rd, 7th, 10th and 21st with the modified method «SNAU, Vet-NSC Prokopovicha» in order to detect the effect of the drug on hemolymph of bees. This effect will indicate the activation of immune processes in the body of bees. The data was processed using the statistics method and Excel.

Results and discussions

The microscope analysis of smears of individual samples of bees hemolymph, showed that at the beginning of the research (before using the drug), the quantitative indices of hemocytes constituted: proleucocytes 21.69%, neutrophil phagocytes 28.56%, eosinophilic phagocytes 23.56%, spherulocytes 23.38%, enocitoid 2.75%.

The results of treatment with «Enteronormin» are presented in Table 1. Analyzing the data from Table 1, it should be noted that the number of hemocytes in bees is unstable and varies with the time depending on the environmental factors.

Table 1
Quantitative changes in bees hemolymph indices

Groups of bees	Proleucocytes		Neutrophilic phagocytes		Eosinophilic phagocytes	
Before the experiment	21.69 ± 2.27		28.56 ± 2.78		23.56 ± 2.99	
On	control	drug	control	drug	control	drug
3-d day	19.20 ± 1.75	19.0 ± 2.27	29.10 ± 1.37	29.25 ± 0.76	24.30 ± 1.83	24.75 ± 2.71
7-th day	18.25 ± 1.67	18.75 ± 1.28	29.63 ± 1.68	28.25 ± 1.39	24.38 ± 2.26	23.75 ± 2.43
10-th day	18.0 ± 0.75	19.13 ± 0.99	30.0 ± 1.07	28.0 ± 0.76	24.88 ± 0.64	23.75 ± 1.04
21-st day	17.5 ± 1.07	18.38 ± 0.92	31.0 ± 0.93	29.13 ± 2.85	25.13 ± 1.13	24.13 ± 0.64
Groups of bees	Enocitoids		Spherulocytes			
Before the experiment	2.75 ± 1.77		23.38 ± 2.82			
On	control	drug	control	drug		
3-d day	2.5 ± 1.78	2.37 ± 1.68	25.3 ± 1.66	24.87 ± 2.23		
7-th day	2.75 ± 0.83	3.4 ± 1.68	24.63 ± 1.92	25.8 ± 1.64		
10-th day	2.75 ± 0.71	3.13 ± 0.83	24.375 ± 0.52	26.0 ± 0.76		
21-st day	2.13 ± 0.83	2.88 ± 1.13	24.25 ± 1.67	25.5 ± 1.19		

When comparing the hemolymph index, analysed on the 1st day before the beginning of the experiment with control group on the 3rd, 7th, 10th and 21st day of the experiment, an increase in phagocyte cells was observed – neutrophil by 0.54%, 1.06%, 1.44% and 2.44% respectively, eosinophils by 0.94%, 0.82%, 1.32% and 1.57%, indicating the activation of hemocytes phagocytic reactions and the reduction of new cells reproduction, probably due to the unnatural laboratory conditions and aging. In this case, the comparison of cellular hemolymph response during and without the use of stimulants demonstrates the emergence of adaptive mechanisms in experimental groups kept in unnatural laboratory conditions (Fig. 1). Taking into account the conditions of bees maintaining in the thermostat (approximating the

natural conditions) a rapid increase in indicators is a norm.

The comparison of the microscopy results of the hemolymph smears of control group and the bees receiving by the drug is presented in Figure 1 and Figure 2. It should be indicated that on the 3-d day the positive changes in the hemogram were not observed, in particular, the quantity of proleucocytes remained constant. The number of phagocytes increased by 0.15%, neutrophilic phagocytes and eosinophilic phagocytes by 0.25%. Moreover, the decrease in secretory cells encitoids and spherulocytes was noted by 0.13% and 1.57% respectively. The results mentioned above indicate that the use of the drug during 3 days does not allow implementing the active stimulation of bees' organism.

Figure 1. The dynamics of quantitative changes of phagocytes in the hemolymph of bees from control group and those treated by «Enteronormin»

Figure 2. The dynamics of quantitative composition of hemolymph secretory cells of bees treated by «Enteronormin» and control group.

It should also be noted that positive changes in the hemogram were observed on the 7th and 10th days of the experiment. Thus, compared with the control group, the quantity of proleucocytes was 0.5% and 1.13% higher respectively, indicating a positive dynamics of the im-

mune processes in the organism of bees, in particular the ability of the body to produce «fresh» cells. The number of phagocytic cells was decreased in comparison with the control, therefore it can be concluded that the immune system of bees does not perceive the drug as an alien

body, but rather as a stimulant. The number of spherulocytes increased by almost 2% compared with control, moreover there appeared young forms of spherulocytes with small vacuoles.

Taking into account the results of the research, it should be noted that the feeding of the drug within 7–10 days gives positive results. From the 10th till the 21st day only sugar syrup was fed to bees. At the end of the experiment (the 21st day), smears of hemolymph of bees, constantly receiving sugar syrup and those receiving the drug up to the 10th day, were examined. It can be stated that the quantitative indices of hemolymph cells on the 21st day taken from the bees fed only by sugar syrup indicate the aging of the bees, so the proleucocytes constituted 17.5%, phagocytes – neutrophils 31% and eosinophils 25.13%, spherulocytes – 24.25%. The reason for the faster aging of the bees' organism is the conditions of keeping, since they are as close as possible to the natural, but not equal to them.

Regarding the morphologic changes in hemolymph cells of bees, we observed significant changes in hemolymph of bees in the control group. Clearly defined mature neutrophil phagocytes, manifested by the formation of pseudopodia at the opposite poles of the cell and their extension, cells acquired spindle-like shape, the nucleus shifted from the center of the cell to the periphery. These cells are highly specialized, actively carrying out phagocytosis and encapsulation of foreign substances. These

cells were observed on days 7–10 of the experiment in bees receiving a sugar syrup only. The results were different for the bees receiving the drug (Fig. 3).

Fig. 3. Mature forms of phagocytes

In the hemolymph of bees receiving the syrup the mixed with the drug, vivid morphological changes in the spherulocytes were observed. Thus, in hemolymph, the young forms of cells characterized by clear contours and a dense centered nucleus, the cytoplasm filled with small vacuoles, in contrast to the old, poorly colored, losing clarity of outlines, with the nucleus shifted to the periphery and often loose were detected (Fig. 4–5).

Figure 4. Young spherulocytes

Figure 5. Old forms of spherulocytes

Quantitative indices of hemolymph of bees that were fed the drug up to the 10th day were at the level of days 5–7, therefore we can claim that the drug «Enteronorm» stimulates the immune processes in the body of bees even after completing the feeding of the drug – the aging of the body is slower, the immune system is more stable to unfavorable environmental factors, indicating the prolonged effect of the drug.

On the 21st day there appeared a tendency towards the stabilization of the quantity of proleukocytes (precursor cells) in the experiment with Enteronormin, which indicates a slow but prolonged effect of this drug on the body of bees and their hemolymph.

Quantitative and qualitative changes in hemocytes of spring bees generation during their stay in laboratory conditions and the application of working doses of enteronormicum, indicate the dynamism of hemocytic indices of honeybee bees hemolymph and confirm the importance of cytological analysis for rational choice of immune drugs.

Conclusions

Feeding «Enteronormin» during 3 days did not show an effective result in hemolymph rates, since no positive changes in hemolymph were detected.

Effectiveness of the drug «Enteronorm» is actively manifested in feeding it with sugar syrup during 7–10 days, characterized by positive changes in hemolymph and the activation of the cellular immunity of bees.

The effect of «Enteronormin» is preserved during 21 days, the immune system of bees becomes more resistant to unfavourable environmental factors, the average life expectancy of bees in containers increases by 2–3 days compared with the control group. That confirms prolonged action of the drug and the activation of immune processes in the organism of bees.

The perspective of future research. The next stage of the research will be aimed at confirmation of the drug «Enteronormin» efficiency if used in field conditions as well as defining the degree of immunity activation using the hemolymph analysis. Determination of the effect of

the drug on the hygienic behavior of bees. Improvement of the method of probiotic «Enteronorm» application depending on seasons of the year and epizootological situation at the place of use.

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