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## FEASIBILITY OF USING PROBIOTICS FOR BEES

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*The main mechanism of action of probiotics is to normalize the composition of the biological microflora of the gastrointestinal tract, that is, its colonization by competitive strains of bacteria-probionts, which carry out non-specific control over the number of conditionally pathogenic microflora by displacing it from the intestinal biocenosis, strengthening the barrier functions of the intestinal mucosa in bees, and also activates the synthesis of hemolymph cells, while stimulating digestion and strengthening the immune status of the body.*

*The article presents the results of studies of the antimicrobial properties of Bilact and Enteronormin preparations against putrefactive pathogens (*Penibacillus larvae*, *Melisococcus pluton*) in the laboratory. Their influence on the factors of non-specific resistance of bees is determined. Experimental studies to verify antimicrobial properties were performed by Agar diffusion. The criterion for evaluating effectiveness was the size of the growth retardation zone (mm). To determine the effect of the drugs "Bilact" and "Enteronormin" on the body of adult bees, studies were conducted in bee colonies. Families of the I-th experimental group were added to the feed of the drug "Bilact", the II-th group – the drug "Enteronormin", control – pure sugar syrup (1:2). Hemolymph was taken from bees before the experiment. During the experiment, hemolymph samples were taken after 7, 14, and 21 days and the activity of lysozyme, phagocytosis, and hemolymph bactericide were determined.*

*It was found that the experimental strains *Penibacillus larvae* and *Melisococcus pluton* were sensitive to both microbiological preparations: the growth retardation zone for crops even at the lowest concentration of  $1.0 \times 10^3$  Kou/cm<sup>3</sup> exceeded 15 mm. It was found that the preparations "Bilact" and "Enterohormin" showed approximately the same effectiveness in relation to pathogens of bee brood rot. Both drugs at a maximum concentration of  $1 \times 10^9$  Kou/cm<sup>3</sup> caused growth retardation of both putrefactive pathogens in  $24.9 \pm 0.71$  mm and  $24.5 \pm 0.51$  mm (Bilact) and  $23.8 \pm 0.8$  mm and  $24.2 \pm 0.84$  mm (Enteronormin).*

*It was found that the activity of lysozyme in the hemolymph of bees of Group I was 1.4 times higher 7 days after feeding a mixture of sugar syrup with Bilact 21 days later – 1.7 times higher than in the control. The activity of lysozyme in the hemolymph of bees of Group II was 1.5 times higher 7 days after feeding the drug "Enteronormin", after 21 days – 1.7 times more than in the control. Lysozyme activity in the hemolymph of bees of groups I and II 7 days after the end of top dressing was increased by 43.8% and 45.7%, respectively, compared to the control.*

*Differences in the indicators of bactericidal activity of hemolymph indicators before and after the use of probiotics were revealed. The results obtained indicate that feeding the preparations "Bilact" and "Enteronormin" contributes to an increase in the factors of non – specific resistance of the Bee body (lysozyme activity by 1.4, 1.7 times, phagocytosis by 1.1 times, bactericidal factor of hemolymph-by 2.0 times).*

**Keywords:** bees, pathogens of bacterial diseases, probiotics, hemolymph, humoral and cellular immune factors.



## ДОЦІЛЬНІСТЬ ЗАСТОСУВАННЯ ПРОБІОТИКІВ ДЛЯ БДЖІЛ

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*Основний механізм дії пробіотиків полягає в нормалізації складу біологічної мікрофлори шлунково-кишкового тракту, тобто його заселенні конкурентоспроможними штамами бактерій-пробіонтів, які здійснюють неспецифічний контроль над чисельністю умовно-патогенної мікрофлори шляхом витіснення її з кишкового біоценозу, посиленні бар'єрних функцій слизової оболонки кишечника у бджіл, а також активізує процеси синтезу клітин гемолімфи, при цьому стимулюється травлення та посилюється імунний статус організму.*

*У статті викладено результати досліджень антимікробних властивостей препаратів «Білакт» та «Ентеронормін» щодо збудників гнильців (*Penibacillus larvae*, *Melisococcus pluton*) у лабораторних умовах. Визначено вплив їх на фактори неспецифічної резистентності бджіл. Експериментальні дослідження щодо перевірки антимікробних властивостей проводили методом дифузії в агар. Критерієм оцінки ефективності була величина зони затримки росту (мм). Для визначення впливу препаратів «Білакт» та «Ентеронормін» на організм імаго бджіл дослідження проводили у бджолиних сім'ях. Сім'ям I-ї дослідної групи додавали в корм препарат «Білакт», II-ї групи – препарат «Ентеронормін», контрольним – чистий цукровий сироп (1:2). У бджіл перед дослідом відбирали гемолімфу. В процесі досліду зразки гемолімфи відбирали через 7, 14, 21 добу та визначали активність лізоциму, фагоцитозу та бактерицидності гемолімфи.*

*Встановлено, що дослідні штами *Penibacillus larvae* та *Melisococcus pluton* виявились чутливими до обох мікробіологічних препаратів: зона затримки росту для культур навіть у найменшій концентрації  $1,0 \times 10^3$  КОУ/см<sup>3</sup> перевищила 15 мм. Встановлено, що препарати «Білакт» та «Ентеронормін» проявили приблизно однакову ефективність по відношенню до збудників гнильців розплоду бджіл. Обидва препарати у максимальній концентрації  $1 \times 10^9$  КОУ/см<sup>3</sup> викликали затримку росту обох збудників гнильців у  $24,9 \pm 0,71$  мм і  $24,5 \pm 0,51$  мм (Білакт) та  $23,8 \pm 0,8$  мм і  $24,2 \pm 0,84$  мм (Ентеронормін).*

*Встановлено, що активність лізоциму у гемолімфі бджіл I групи була більше в 1,4 рази через 7 діб після згодовування суміші цукрового сиропу з препаратом «Білакт» через 21 добу – в 1,7 рази більше ніж у контролі. Активність лізоциму у гемолімфі бджіл II групи була більше в 1,5 рази через 7 діб після згодовування препарату «Ентеронормін», через 21 добу – в 1,7 рази більше ніж у контролі. Активність лізоциму у гемолімфі бджіл I та II групи через 7 діб після закінчення підгодівлі була більше на 43,8 % та 45,7 % відповідно в порівнянні з контролем.*

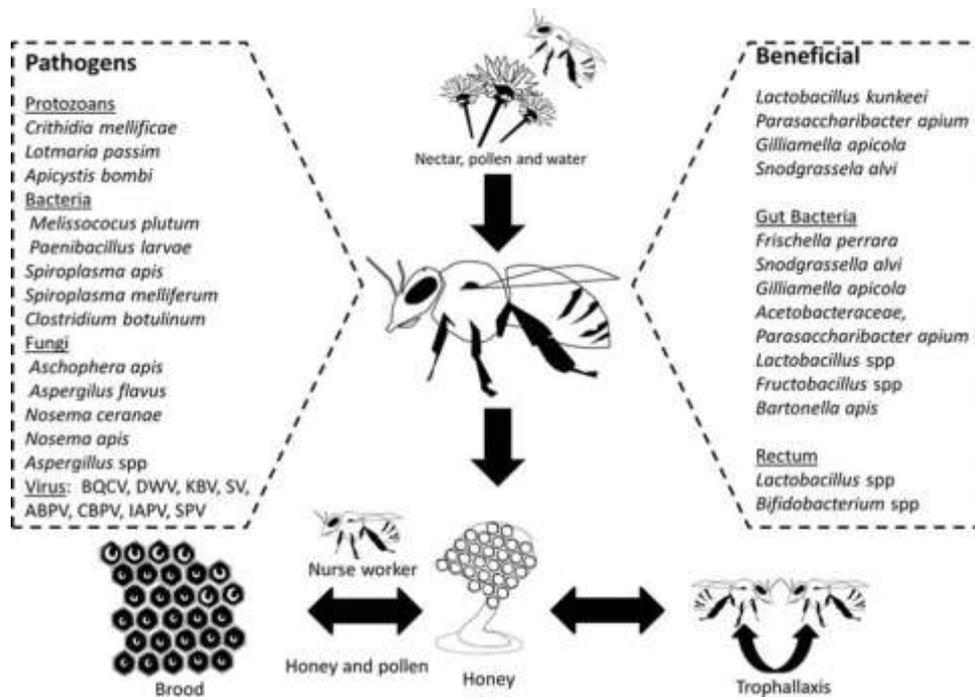
*Виявлено відмінності показників бактерицидної активності показників гемолімфи до і після застосування пробіотиків. Отримані результати вказують на те, що згодовування препаратів «Білакт» та «Ентеронормін» сприяє підвищенню факторів неспецифічної резистентності організму бджіл (активності лізоциму в 1,4, 1,7 рази, фагоцитозу в 1,1 рази, бактерицидного фактору гемолімфи – в 2,0 рази).*

**Ключові слова:** бджоли, збудники бактеріальних хвороб, пробіотики, гемолімфа, гуморальні та клітинні фактори імунітету.



The environment, honeybees and their microflora are the only ecological system that responds to any changes. In the normal physiological state of bees, the relationship between the body and microflora is generally symbiotic, which was formed and fixed in the process of evolutionary development. Nectar, pollen, and water enter the hive from the environment (DeGruttola, A. K. et al., 2016; Glenny, W. et al., 2017; Vagner de Alencar Arnaut de Toledo et al., 2020). However, it is important to know the diversity of microorganisms in the environment – hive biocenosis, especially due to the spread of pathogens that pollute bee products and the bee nest in the hive (fig. 1).

The figure schematically shows how parasites (varroa mites, Acarapis), protozoa (Nosema spp. Malpighamoeba mellifica), pathogenic viruses (black queen bee (BQCV), deformed wings (DWV), Kashmir Bee (KBV), sac-like brood (SBV), acute and chronic paralysis (ABPV, CBPV), Israel acute paralysis (IAPV) slow paralysis (SPV)) enter the middle of the nest and are distributed throughout it.



**Fig. 1. Pathogens and beneficial microorganisms in honeybees: one of the ways – bee food infection occurs through nectar, pollen (on flowers) and water collected by worker bees in the environment; the second – food is stored in the Hive and can be transmitted by trophalaxis between workers and brood; the third – is the consumption of infected honey and/or pollen from other hives by honeybees (based on materials: Honey Analysis. New Advances and Challenges. Edited by Vagner de Alencar Arnaut de Toledo, Emerson Dechechi Chambo (2020).**

There is also a niche of beneficial microbiota in the digestive tract (anterior, middle, and posterior intestines). Arrows indicate the transfer of microorganisms internally by hive bees by food route between individuals( brood and Imago), honey reserves, and parchment in the hive (Vagner De Alencar Arnaut de Toledo, 2020; Daisley, B., et al.2020).

Beneficial microorganisms can secretly remain in them, waiting for the moment when the environment becomes suitable for their development. Among the beneficial



bacteria found in bee products, we can mention some bacteria that act as probiotics when ingested (Paytuví-Gallart, A. Et al., 2020; Tushak, S., 2018).

However, in modern conditions, insects are exposed to a whole range of adverse factors that affect the normal functioning of the main vital systems: on the one hand, the deterioration of the environmental situation, an increase in the number of stressful situations due to insufficient quantity and variety of pollen and honeybees, and on the other - the mass uncontrolled use of chemotherapeutic drugs, both for plants and bees. A significant level of microbial contamination of feed and environmental objects leads to advanced colonization of the insect gut by pathogenic microorganisms, which slows down and even prevents the formation of normal intestinal microflora (Piccini C. et al., 2004; Kotsiumbas I. Ya. et al., 2013, Kalinichenko S. V et al., 2013, Ogrodowczyk, A. M. et al., 2020).

The increase in output and growth rates of beekeeping products may be a natural consequence of the introduction of new technologies in the fields of microbiology and biochemistry, in particular in the correction of biocenoses of the gastrointestinal tract of bees-the artificial introduction of representatives of beneficial microflora into the hive. They are called probiotics, the main purpose of which is the formation of a metabolically active population of probiotic bacteria in the digestive tract, which contributes to a qualitative change in the composition of intestinal microflora and the displacement of pathogenic microorganisms. In addition, the use of probiotics promotes the synthesis of enzymes and other biologically active substances, in particular vitamins, bacteriocins, etc. (Kalinichenko S. V. et al, 2013).

Probiotics are living microorganisms that have a positive effect on bee health through anti-infective defense mechanisms, immunomodulatory effects, increased barrier functions, metabolic effects and positive effects on intestinal motility and function.

According to the form of release, probiotics are divided into two groups - liquid and dry. Dry probiotics are freeze-dried microorganisms that can be found in powder, capsules, or tablets. The shelf life of dry preparations is longer than that of liquid ones, in addition, they are less dependent on environmental conditions and, thus, do not require strict compliance with storage criteria, and are also much more convenient to transport. The disadvantage of dry probiotics is that during lyophilization, bacteria lose some of their useful properties, and after using the Drug, time is needed for the transition of bacteria from suspended animation to the active form and the onset of action (Kalinichenko S. V. et al, 2013).

Liquid probiotics are bacteria "with an active life position", that is, they retain all their valuable properties and begin to act immediately after entering the body. However, firstly, this requires strict compliance with the shelf life conditions, and secondly, the shelf life of these drugs is shorter than lyophilized analogues-no more than three months. Liquid probiotics can consist not only of bacteria that are in a physiologically active state, but also of a special nutrient medium that serves as a source of nutrition for them; additionally introduced ingredients that enhance the effectiveness of the drug (water - soluble vitamins, micro-and macronutrients, amino acids, etc.); metabolites (bacterial waste products).

The most dangerous infectious diseases of bee brood are: American (malignant) rot, the causative agent of which is the spore-forming Rod *Penibacillus larvae* larvae, European (benign) rot, the main causative agent of which is *Melisococcus pluton* and others. Parasitic diseases (varooz and acarapidosis), diseases that cause protozoa (*Nosema* spp., *Malpighamoeba mellificae*), as well as pathogenic mold fungi and yeast weaken the insect body. Rot pathogens do not have the same pathogenicity and virulence, which largely depends on their concentration: the higher it is, the more often outbreaks of the



disease occur and the more affected families (Glinski, Z et al., 2001; Piccini C. et al., 2004, Kutsan O. T. et al. 2013; Daisley, B., et al.2020;).

Factors of non-specific resistance of bees include cells of the hemolymph, body fat (cellular immunity), as well as antimicrobial fluids (humoral immunity) of the body. Hemolymph performs protective functions due to the presence of such cells and fluids in it. It is characteristic that the activity of cellular defense factors depends on humoral ones. The process of phagocytosis is largely activated by lysozyme, microcins and opsonine. They increase the rate of phagocytosis. Lysozyme, which is adsorbed on the mucopetid cell wall of the microorganism, breaks it down. As a result, the osmotic balance is disturbed and hydrolysis of the microbial cell begins (Glinski, Z et al., 2001).

The cellular basis of the general mechanisms of non – specific resistance of insects is the antimicrobial activity of the hemolymph, which is associated with the functions of its shaped elements-hemocytes, including phagocytes, which create prerequisites for activating the synthesis of antibacterial proteins. Morphological heterogeneity of hemocytes determines the variety of associated protective processes in the insect body. Changes in the number, ratio of shaped elements and biochemical parameters of bee hemolymph are noted both depending on the season, functional and age groups, and under the influence of pathogens of various diseases and the means of their elimination used (Szymaś B. et al., 2003; Larsen, A. et al., 2019; Danihlík, J. et al.,2018).

Purpose of research. To study the antimicrobial properties of probiotic drugs: "Bilaktu", which includes *Lactobacillus* spp. and *Bifidobacterium*; dry - "Enteronormin", containing *Enterococcus* spp in its composition., *Lactobacillus* spp., *Bacillus* spp. on pathogens of rot (*Penibacillus* larvae, *Melisococcus pluton*) in the laboratory. To study the effect of these drugs on humoral and cellular factors of non-specific resistance of bees: bactericidal and biochemical parameters of the hemolymph in general, lysozyme activity in particular, as well as the cellular composition of the hemolymph.

**Materials and methods.** Epizootic strains of pathogens of American rot – *Penibacillus* larvae, European rot – *Melisococcus pluton* were used in experiments, in the form of a suspension of 3-day cultures of vegetative cells in the amount of  $1.0 \times 10^6$  PFU/cm<sup>3</sup>, the sensitivity of pathogens of rot to drugs was determined: "Bilact" is a liquid composition of bacteria of the genus *Lactobacillus* spp. and *Bifidobacterium*; "Enteronormin" is a dry-form compound with *Enterococcus* spp., *Lactobacillus* spp., *Bacillus* spp.

The preparations were used in concentrations:  $1.0 \times 10^3$ ,  $1.0 \times 10^6$ ,  $1.0 \times 10^9$  PFU/cm<sup>3</sup>. Saline solution of 0.9% was used as a control. The study was performed by Agar diffusion (Labynskaya, A. S., 1978). Two petri dishes were used in parallel for each drug.

At the first stage, the lower layer of Agar was poured into sterile petri dishes (for the causative agent of American rot – Willis–HoBZ medium, for European rot – skull) in the amount of 10 cm<sup>3</sup>, left for 30 minutes. for solidification. Three cylinders (10 mm high and 8 mm in diameter) were placed on the surface of the lower layer at a distance of 4 and 2.5 mm from each other and from the edge of the Cup, respectively. Then the top layer of selective Agar (for each pathogen) was poured in an amount of 10 cm<sup>3</sup> into Petri cups, evenly distributed over the surface and allowed to harden. At the second stage, 1 cm<sup>3</sup> of suspensions of three–day *penibacillus* larvae culture was applied to the surface of Willis – HoBZ Agar, and *Melisococcus pluton* culture was applied to the surface of Skull Agar and evenly distributed over the agar surface. After 30 minutes. excess suspensions were removed by sucking out a Pasteur pipette, and the cylinders were removed with tweezers. Each well was numbered clockwise on the reverse side of the cup and 0.5 cm<sup>3</sup> of the test drug was added at the above concentrations (well volume). Petri dishes were



kept in a thermostat at a temperature of  $37 (\pm 1) ^\circ\text{C}$  for (48-120) hours. (depending on the type of pathogen). The growth retardation zone of the microorganism was determined in mm by measuring through the center of the well diameter. The experiment was repeated 3 times, the results were processed statistically.

The criterion for evaluating the effectiveness of a probiotic (quantitative indicator) was the size of the growth retardation zone: – up to 10 mm – the strain was considered insensitive; – from 11 mm to 15 mm – insensitive; – from 15 mm to 25 mm – sensitive; – more than 25 mm – highly sensitive.

To determine the effect of the drugs "Bilact" and "Enteronormin" on the body of adult bees, studies were conducted in bee colonies. For this purpose, two experimental and one control groups of bees were formed, ten families each. Families of the I-th experimental group were added to sugar syrup the drug "Bilakt", the II-th group – the drug "Enteronormin", the control group was fed pure sugar syrup (1:2). Hemolymph was taken from bees before the experiment. During the experiment, hemolymph samples were taken after 7, 14, 21 days and lysozyme activity, bactericidal and biochemical parameters of hemolymph, as well as the species and quantitative composition of hemolymph cells were determined.

The bactericidal activity of hemolymph was studied by diffusion into Agar. As test crops, pathogenic microorganisms for bees were used-pathogens of rot: American, European, and non – pathogenic – *Escherichia coli*. On the surface of agar, a culture of the microorganism at a concentration of 1 billion tons was introduced into a petri dish. cells /  $\text{cm}^3$ . The cups were kept in a thermostat at  $37 ^\circ\text{C}$  for 2 hours. With a marker, holes with a diameter of (3-4) mm were made, into which hemolymph samples were introduced. The reaction results were recorded after 3, 6, 12, and 24 hours. (Labynskaya, A. S., 1978). Determination of lysozyme activity in the hemolymph was performed by turbidimetric method. *Micrococcus lysodeikticus* culture was prepared on phosphate buffer, and combined hemolymph samples were diluted 10 times with saline. Experimental and control samples were examined simultaneously. The amount of lysozyme in the hemolymph sample was calculated using the calibration curve in mcg/ml (Labynskaya, A. S., 1978).

Laboratory methods of research in biology were used to determine the biochemical parameters of protein metabolism in the hemolymph of bees (Vlizlo et al., 2012).

Smears were prepared from the hemolymph, fixed with methyl alcohol and stained with Azur-eosin. After washing and drying, the smears were microscopized at magnification (X900) using a Biolam microscope. 100 cells were counted in a single smear, assessing the morphological composition of bee hemolymph (Errapcaliu et al., 2009; Barakat et al., 2016).

**Research results and discussion.** It is known from the literature that probiotics are multifactorial therapeutic agents by their mechanism of action. They exhibit antagonistic activity against a wide range of pathogenic and opportunistic microorganisms, have a corrective effect on the biocenosis and stimulate reparative processes in the intestine, activate the body's defenses, improve metabolism, and affect non-specific factors of bee immunity (Brumfitt W. et al, 2002; Kalinichenko S. V. et al, 2013).

Analyzing our results, it should be noted that the experimental strains *Penibacillus* larvae and *Melisococcus pluton* were sensitive to both microbiological preparations: the growth retardation zone for crops even at the lowest concentration of  $1.0 \times 10^3 \text{ PFU /cm}^3$  exceeded 15 mm (table. 1).



Table 1

**Sensitivity of probiotic rot pathogens (Agar diffusion method)**

Drug name concentration,	Concentration, PFU / cm <sup>3</sup>	growth retardation zone, mm	
		<i>P. larvae</i>	<i>M. pluton</i>
«Bilakt»	1,0 × 10 <sup>3</sup>	16,9±0,62	17,1±0,7
	1,0 × 10 <sup>6</sup>	20,8±0,4	20,5±0,52
	1,0 × 10 <sup>9</sup>	24,9±0,71	24,5±0,51
«Enteronormin»	1,0 × 10 <sup>3</sup>	20,2±58	20,9±0,47
	1,0 × 10 <sup>6</sup>	21,4±0,56	21,8±0,46
	1,0 × 10 <sup>9</sup>	23,8±0,8	24,2±0,84
Control (physical solution)	0,9	–	–

Note: “ – ” - no growth retardation zone

Thus, when comparing the antimicrobial effect of the drugs "Bilakt" and "Enterohormin", it was found that they showed approximately the same effectiveness in relation to pathogens of bee brood rot. Both drugs at a maximum concentration of 1 x 10<sup>9</sup> PFU / cm<sup>3</sup> caused growth retardation of both putrefactive pathogens in 24.9±0.71 mm and 24.5±0.51 mm (Bilakt) and 23.8±0.8 mm and 24.2±0.84 mm (Enteronormin), respectively.

Beneficial microorganisms increase the bactericidal effect of bee hemolymph due to the accumulation of lactic acid or the formation of a significant number of specific metabolic products (peptides, carbonyl compounds, hydrogen peroxide, etc.), which have antibacterial properties. Bactericidal and biochemical parameters of bee hemolymph were determined in samples taken before the start of the experiment and 21 days after the end of top dressing. The research results are shown in Table 2.

Table 2

**Bactericidal and biochemical parameters of bee hemolymph**

Bee groups / Day (n=10)		hemolymph parameters			
		Bactericidal factor, h.	Total Protein, g / l	Total nitrogen, mg %	residual nitrogen, mg%
Before feeding with probiotics		6	48,4±1,3	575,0±10,2	412,0±35,5
Top Dressing "Bilakt"	21	12	63,6±4,2	787,0±50,8	289,0±12,2
Top Dressing "Enteronormin"	21	12	65,0±3,1	808,0±56,3	268,9±12,2
Control (sugar syrup)	21	6	52,4±3,1	568,4±65,0	345,0±34,2

The study of the bactericidal factor of bee hemolymph showed that it did not differ in individuals who received top dressing with probiotics. Thus, it was found that hemolymph delayed the growth of cultures of pathogens of American rot for 12 hours and exceeded this indicator twice compared to the control, where sugar syrup was fed without any impurities and the initial indicator (before the experiment). The study of the bactericidal factor of bee hemolymph showed that it did not differ in individuals who received top dressing with probiotics.



Thus, it was found that hemolymph delayed the growth of cultures of pathogens of American rot for 12 hours and exceeded this indicator twice compared to the control, where sugar syrup was fed without any impurities and the initial indicator (before the experiment). Indicators of protein metabolism also indicate a positive effect of probiotics on the body of bees. A significant increase in the content of total protein and nitrogen in the hemolymph of bees fed probiotic preparations was established against the background of a decrease in the amount of residual nitrogen. On Day 21, the total protein index increased by 31.4% when feeding "Bilaktu" and 34.3 % – "Enteronormin", total nitrogen increased by 36.9%, respectively; and 40.5%, residual nitrogen decreased by 29.9% and 34.9%, respectively.

In the process of interaction of representatives of the beneficial flora, which was a component of probiotic agents and pathogenic microbes, which was located in the Bee cattery, the death and destruction of pathogen cells and parts of beneficial microorganisms occurred. At the same time, lysozyme was released from the bacterial wall, which enhanced the effect of its own from the hemolymph of bees.

When studying the effect of the drugs "Bilact" and "Enteronormin" on the body of bees, an increase in the activity of hemolymph lysozyme was found (table. 3).

Table 3

**Activity of bee hemolymph lysozyme**

Selection period, day	Group of bees. Lysozyme activity, mcg / ml(n=10)		
	I experienced «Bilakt»	II experimental "Enteronormin"	Control (sugar syrup)
Before the start of the experiment	36,4 ± 1,5		
7	51,9 ± 3,8 <sup>1)</sup>	52,6±0,3 <sup>1)</sup>	36,1 ± 1,4
14	54,2 ± 4,1 <sup>1)</sup>	57,7 ± 0,8 <sup>1)</sup>	36,4 ± 1,5
21	60,7 ± 4,1 <sup>1)</sup>	62,7±1,2 <sup>1)</sup>	36,2 ± 1,5

Note: 1) – the difference in results is likely compared to the results before the start of top dressing and the control group,  $p < 0.05$ .

From the data in Table 3, it can be seen that the activity of lysozyme in the hemolymph of bees of groups I and II 7 days after the end of top dressing was higher by 43.8% and 45.7%, respectively, compared to the control.

After 21 days, this difference was 66.7% for the first group, 73.2% for the second group compared to the control group of bee families. Lysozyme activity in bees from Control families did not increase during the experiment.

The activity of certain types of hemocytes forms an immune response to the entry of a significant number of microorganisms into the insect body (Gábor et al., 2020). To interpret the ability of certain agents to have any effect on non-hemolymph of bees, it is important to fix the morphological (species) and quantitative composition of the latter's cells. The effect of probiotics "Bilakt" and "Enteronormin" in a field experiment on ten bee colonies was evaluated by manufacturing and microscopy of hemolymph smears: before the experiment; when using probiotics for feeding bees with sugar syrup after 7, 14, 21 days. Differentiation of hemocytes was based on their morphological and quantitative characteristics (Barakat et al., 2016; Jazlovitskaya et al., 2014; Richardson et al., 2018).



The results obtained indicate that feeding Bilact and Enteronormin preparations increases the factors of non – specific resistance of the Bee body (lysozyme activity by 1.4, 1.7 times, hemolymph bactericidal factor-by 2.0 times).

It is known that feeding bees with sugar syrup is a natural technological technique used during the period when there are no bribes in the environment and bees do not have enough food for the existence and development of families. In addition, it is sugar syrup that is most often the carrier of drugs that are used for preventive and curative purposes (Ptaszyńska et al., 2016; Saranchuk et al., 2021; Frizzera et al., 2020). The results of studying the quantitative and morphological characteristics of bee hemolymph cells on the Slave are shown in Table 4.

Table 4

**Quantitative and morphological composition of hemocytes in bee hemolymph**

Bee groups / day of experience (n=10)	Hemocyte classes					
	Proleuko-cytes	Phagocytes		Spherulo-cytes	Enocytoids	
		neutrophilic	eosinophilic			
Before top dressing	14,46±1,33	30,62±0,29	22,0±0,79	25,93±1,2	3,0±0,37	
Top Dressing "Bilakt"	7	15,00±2,69	33,0±5,26	24,2±1,08	25,8±4,38	3,0±0,73
	14	15,40±0,45	32,4±0,45	24,12±1,32	25,0±0,93	3,0±1,0
	21	15,62±0,82	31,25±1,45	23,2±4,18	26,00±1,38	3,0±0,35
Top Dressing "Enteronormin"	7	15,83±0,88	35,62±1,01	26,37±1,28	26,83±0,73	2,66±0,68
	14	16,20±1,88	35,0±2,89	25,60±3,05	27,60±2,14	3,0±0,94
	21	16,0±0,65	34,50±4,48	24,0±3,79	27,87±0,66	2,87±0,38
Control (sugar syrup)	7	14,50±2,49	31,20±1,71	22,33±0,85	24,0±3,68	3±0,65
	14	14,00±1,27	32,33±0,94	22,62±0,65	23,60±2,56	1,80±0,65
	21	13,62±0,98	33,53±1,45	23,06±2,22	22,75±0,76	1,62±0,29

When microscopy smears of individual hemolymph samples at the beginning of the experiment (before feeding the drugs), the quantitative indicators of hemolymph were: prodrug cells – 14.46%, phagocytes -56.59%, of which neutrophils – 33.53%, eosinophilic – 23.06%, spherulocytes – 25.93%, enocytoids – 3.0 %.

Comparison of the indicators of hemolymph smears that were examined before the start of feeding and after 21 days during feeding with sugar syrup indicates a decrease in proleukocytes by 0.84%, secretory cells (spherulocytes – 3.18, enocytoids – 1.38 %); an increase in phagocytic cells – neutrophils 2.91 %; which indicates the aging of bees and an increase in bacteria in their body, which, in turn, stimulate the growth of phagocytic function to maintain the body's homeostasis, eosinophils 1.06%, respectively.

Comparison of the results of microscopy of hemolymph smears of the control group on days 7, 14 and 21 with the groups receiving probiotics "Bilact" and "Enteronormin" showed a decrease in neutrophilic and eosinophilic phagocytes and an increase in proleukocytes and spherulocytes. This may indicate a positive trend in the hemolymph for the use of drugs and serve as confirmation of the immunostimulating and activating effect.

The dynamics of a decrease in the number of neutrophilic and eosinophilic phagocytes was also observed, especially in comparison with the control. A more pronounced decrease in phagocytic cells was observed in the group in which



Enteronormin was used. Such results indicate a high activity of probiotic drugs, the destruction of foreign substances in the hemolymph and the stabilization of cellular immunity. Analyzing the data, it should be noted that the drugs in sugar syrup have a more active effect on inactivating foreign inclusions and stabilizing immune processes.

Internal protection is provided by cellular and humoral factors. In (most) higher animals, two fluids circulate in the body: blood, which performs a respiratory function, and lymph, which performs mainly the function of delivering nutrients. The blood of insects differs significantly from the blood of higher organisms and therefore received a special name – hemolymph. It is the only tissue fluid in the body of insects. Like vertebrate blood, it consists of a liquid intercellular fluid – plasma and the cells that are in it – hemocytes. Unlike vertebrate blood, hemolymph does not contain cells that contain hemoglobin or other respiratory pigment. As a result, the hemolymph does not perform a respiratory function. All tissues and cells take the nutrients and other substances they need from the hemolymph and release metabolic products into it. Hemolymph transports digestive products from the intestinal walls to all organs, and waste products – to the excretory organs.

The composition of the hemolymph as the internal environment of the insect body is one of the criteria that characterizes the physiological or pathological state of the honey bee. The cellular basis of the general mechanisms of non – specific resistance of insects is the antimicrobial activity of the hemolymph, which is associated with the functions of its shaped elements-phagocytes, which create prerequisites for activating the synthesis of antibacterial proteins (lysozyme, Agglutinins, apidacins, abecins) in the cells of the fat body of bees and their entry into the plasma of the hemolymph. Proleukocytes are known to be precursors of all other classes of hemocytes. Neutrophilic and eosinophilic phagocytes appear in those that perform a microphagocytic function. Encytoids have a secretory function. Spherulocytes perform protective and secretory functions.

Each type is an independent group of hemocytes that are not related to each other by origin and do not have morphological transitions. Due to transformations, hemolymph cells located in different morphological positions can perform different functions. Usually, each type of Hemocyte accumulates in the maximum amount during a certain period of life. The number of hemocytes in the hemolymph decreases especially sharply from the 10th day of life of bees.

With age, the number of young forms of hemocytes decreases, and mature ones increase. Any stage of bee development, its age and physiological state is characterized by a specific hemogram that reflects the percentage of different types of hemocytes. According to the hemogram, it is easy to determine physiological changes in the insect's body. It can be used to determine the “fatness” of an insect and diagnose diseases in the early stages of the disease, parasite infestation, and insecticide poisoning.

The insect's hemolymph washes all internal organs and is the environment in which all cells of the Bee's body live and function. It performs a number of functions, including protective. This function involves plasma proteins, hemocytes that have the ability to phagocytosis, and cells that form Hemocyte capsules around multicellular parasites. Hemocytes also have the ability to accumulate in places where damage to the body has occurred, forming a kind of plug that closes the wound. In this case, hemocytes multiply and Dead Cells phagocytosis occurs.

Maintaining the immunity of bees is an important link for the Prevention of infectious diseases. In particular, the cellular mechanisms of the immune defense of *Apis mellifera* are responsible for a number of protective barriers that promote the destruction of foreign agents and ensure the ability of phagocytic cells to respond by lysis and phagocytosis reactions to the penetration of bacterial pathogens or cause their uptake for



neutralization (Larsen et al., 2019). However, it should be noted that the activity of phagocytic neutrophils of the hemolymph in laboratory conditions is slightly lower than in the conditions of natural existence. Moreover, in bee colonies living in hives, there is a phenomenon of so-called social immunity, in which the bees of a particular colony are able to orally transfer immunological compounds between the members of the hive (Harwood et al., 2021). This type of immune metabolism is probably activated in the event of increased resistance of each individual Bee from the insect colony (synthesis of certain antibodies). Our results indicate that it is advisable to use pro biotic preparations for feeding bees, either with sugar syrup or in the form of Kandy as a stimulant to increase the resistance of bee colonies and maintain protective properties at the proper level.

### Conclusion

1. Based on the above, we can conclude that among the scientific and practical directions, the development of modern methods for studying the composition and activity of insect microbiocenoses is relevant; detailing the molecular, biochemical and other mechanisms of action of probiotics for their effective use in the prevention and treatment of various diseases, an in-depth assessment of the safety of drugs containing probiotic strains.

2. Thus, there is no doubt that the scientifically based use of probiotics is an important method for preserving and restoring bee health.

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