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THE EFFECT OF PROBIOTIC MICROORGANISMS ON CATALASE ACTIVITY, FRACTIONAL COMPOSITION OF SOLUBLE PROTEINS, AND INTESTINAL MICROBIOTA OF HONEY BEES

Recently, there has been a trend toward the use of new effective natural preparations to fight diseases and improve the health of honey bees. It is also known that a well-balanced structure of the intestinal microbiota of honey bees is the basis for their growth, development, strengthening of the immune response, and resistance to infections. It has been established that some strains of lactic acid bacteria that have antibacterial, anti-inflammatory, and immunomodulatory properties, are promising for the development of broad-spectrum probiotic preparations based on them. Therefore, the ***aim*** *of the work was to determine the effect of probiotic strains Lactobacillus* casei *IMV B-7280 and L. plantarum IMV B-7679 on catalase activity, protein content and protein profile of hemolymph, as well as microbiota spectrum of different parts of the intestines of Apis mellifera honey bees.* ***Methods****. To conduct the research, a control and two experimental groups of 60-90 bees each were formed. The bees of the control group were fed 60% sugar syrup + 1 mL of distilled H_2O for 28 days. The experimental group of bees D1 received 1 mL of 60% sugar syrup + 1 mL of aqueous suspension containing cells of the L. casei IMV B-7280 strain at a concentration of $1 \cdot 10^6$ CFU/mL every day; experimental group of bees D2, in addition to 1 mL of 60% sugar syrup, received 1 mL of aqueous suspension containing cells of L. plantarum IMV*

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B-7976 strain at a concentration of $1 \cdot 10^4$ CFU/mL. Catalase activity of the whole organism tissues was determined using the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts on a spectrophotometer at a wavelength of 410 nm against water. The amount of protein in the whole organism tissues was determined by the Lowry method. The content of total protein in the body of bees was carried out according to the Kjeldahl method. Determination of the content of individual fractions of soluble proteins of the hemolymph was carried out by the method of vertical electrophoresis in a 7.5% polyacrylamide gel. The relative content of protein fractions was determined using the TotalLab TL120 program and expressed as a percentage of the total pool. To determine the qualitative and quantitative spectrum of the gut microbiota of bees, the hindgut and midgut were sampled (separately) from bees of control and experimental groups. The obtained samples were plated on eight selective solid media for cultivation of different groups of microorganisms. ***Results***. *A tendency to increase the catalase activity of bee tissues after 28 days of L. casei IMV B-7280 strain use and a consistently higher activity of this enzyme throughout the experimental period under the action of L. plantarum IMV B-7679 strain was established. In the control group of 28th days, the content of bees and catalase activity remained at a constant level. It was shown that on the 14th day and total protein in the body of bees that received L. casei IMV B-7280 strain increased significantly. Water-soluble fractions of hemolymph proteins were found in bees of both groups: γ-globulins, β-globulins, α2-globulins, and α1-globulins. It should be noted that the albumin fraction was not detected. It has been shown that the hindgut contains a much larger number of microorganisms than the midgut. The use of L. casei IMV B-7280 strain led to an increase in the number of lactic acid bacteria and bifidobacteria in both parts of the gut, as well as to a decrease in the number of staphylococci, streptococci, and microscopic fungi. The use of L. plantarum IMV B-7679 strain had a similar effect, but the changes in the composition of gut microbiome were less pronounced.* ***Conclusions***. *The use of probiotic strains L. casei IMV B-7280 and L. plantarum IMV B-7679 for feeding bees under the conditions of a laboratory thermostat led to quantitative changes in the composition of the intestinal microbiota of bees, namely an increase in the number of lactic acid bacteria and bifidobacteria, as well as a decrease in the number of some other groups of microorganisms in the gut. Probiotic strains stimulated catalase activity of bee's body tissues, increased the level of total protein, and did not significantly affect the ratio of hemolymph protein fractions.*

Keywords: *Apis mellifera bees, lactobacilli, catalase, protein fractions, hemolymph, intestinal microbiome spectrum.*

The honey bee (*Apis mellifera L.*) plays an important role in biodiversity conservation, ecosystem stability, and agricultural production through pollination, which contributes to increased yields. The bees are important producers of safe and ecological products — wax, pollen, royal jelly, propolis, etc.

Modern beekeeping is not limited to the production and profit from the sale of honey and other products. Large losses of the population of honey bees *A. mellifera* over the past decades threaten catastrophic consequences for both the ecosystem and food security of various countries. Particular attention is paid to identifying factors that worsen the morphofunctional state of bees.

It has been shown that stress factors of abiotic, biotic, and anthropogenic origin (parasites, viruses, pesticides, insecticides, heavy metals, climate change, etc.) disrupt physiological processes in the body of *A. mellifera*, suppressing their immunity, which leads to the death of entire colonies [1—3].

It becomes clear that the immune system of bees becomes very vulnerable under such conditions, therefore the search for mechanisms of activation of the defense systems of honey bees is extremely relevant. Defense responses of the honey bee include both cellular and humoral responses, combining many interrelated systems such as antibacterial peptides, hemagglutinins, phenoloxidase, and the antioxidant system (AOS).

One of the factors that negatively affects the health of bees and the development of colonies and causes their death is the deterioration of the forage base. A slight violation of the component composition or a lack of food can weaken the immune system of bees and make them more vulnerable to the use of chemicals and diseases

of various origins. This situation promotes the excessive generation of reactive oxygen species (ROS), which in turn leads to the development of oxidative stress.

Oxidative stress is an important process that can cause serious negative consequences in eukaryotic organisms. ROS are formed during normal metabolic processes and are responsible for oxidative stress. To prevent or reduce oxidative stress caused by ROS, insects use various enzymatic mechanisms that cause oxidative inactivation (superoxide dismutase, catalase, and peroxidase) or removal of ROS at the intracellular level with the help of glutathione peroxidase and glutathione reductase enzymes [4, 5]. One of the main enzymes that neutralize hydrogen peroxide is catalase, which is sensitive to external stimuli, which makes it possible to consider this enzyme as an indicator of the general state of the antioxidant system [5, 6].

Recently, various fungicides, antibiotics, heterocyclic organic compounds (indoles), and bacteriophages [7] have been used to combat honey bee diseases, which is promising for controlling the growth of pathogens both *in vitro* and *in vivo*. In addition, in many EU countries, the use of antibiotics in beekeeping is prohibited by law [8] due to the risks of the spread of antimicrobial genes [9, 10].

Therefore, there is a tendency to use new effective means of the natural origin for combating diseases and improving the health of honey bees, which helps to avoid many side effects, since their mechanisms of action differ from synthetic ones due to the activation of the body's protective reactions at the physiological level [11, 12].

It is known that a well-balanced structure of the intestinal bacterial microbiota of honey bees is the basis for their growth, development, strengthening of the immune response, and resistance to pathogens [13, 14].

It has been established that the lactic acid bacteria strain *Lactobacillus casei* IMV B-7280, which has antibacterial, anti-inflammatory, and immunomodulatory properties, is promising for the development of probiotic preparations [15]. It is recommended to make dietary supplements and medicines based on this strain for the prevention and treatment of infectious, inflammatory, and other diseases.

The *L. casei* IMV B-7280 strain is characterized by effective therapeutic action associated with the normalization of intestinal bacterial microbiota and participation in the modulation of inflammatory reactions. Its selective positive effect on factors of innate immunity, cellular immunity, and cytokine profile was also noted [16].

After entering the gastrointestinal tract, microorganisms with probiotic properties exert both a direct effect on pathogenic and opportunistic microorganisms and an indirect effect by activating specific and non-specific protective systems of the organism.

So, the **aim** of this study was to determine the influence of probiotic strains *L. casei* IMV B-7280 and *L. plantarum* IMV B-7280 on the catalase activity, protein content, protein profile of hemolymph, and spectrum of the intestinal microbiome of bees.

Materials and methods. The research was conducted on honey bees of the Carpathian breed from the laboratory apiary-vivarium of the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine. Lyophilized probiotic strains *L. casei* IMV B-7280 and *L. plantarum* IMV B-7976 were used in the research. These strains were previously isolated in the Department of Problems of Interferon and Immunomodulators of the Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences (ZIMV NAS) of Ukraine from the associated culture of biological material and deposited in the Ukrainian collection of microorganisms of the ZIMV NAS of Ukraine. Before each experiment, the viability of lyophilized strains was checked by monitoring their growth on Man-Rogosa-Sharpe agar (MRSA) medium at 37 °C for 24—48 h.

To conduct the research, a control and two experimental groups, D1 and D2, of 60-90 bees each, similar in weight and age, were formed. In the summer-autumn period, Bees of the control group (C) were fed 60% sugar syrup (SS) + 1 mL of distilled H_2O in the amount of 1 mL/group/day. Group D1 received 1 mL of 60% SS + 1 mL of *L. casei* IMV B-7280 cell suspension in distilled water at a concentration of $1 \cdot 10^6$ colony-forming units (CFU)/mL every day, and Group D2, in addition to 1 mL of 60% SS, received a cell suspension of probiotic strain *L. plantarum* IMV B-7976 at a concentration of $1 \cdot 10^4$ CFU/mL. Bees of the control and experimental groups were kept in cages with a volume of 4 dm^3 in similar conditions of the TS-80M-3 laboratory thermostat with microventilation at a temperature of 30 °C and humidity of 74—76% for 28 days of the study.

After the end of the experiment, 25 bees were taken from each group. To prepare the homogenate of the whole organism of honey bees, they were crushed and three parallel samples were formed. A group of bees weighing 0.5 g was homogenized with NaCl in a ratio of 1:10 using a homogenizer (Homogenizer Type 302, Poland) on ice. The samples were centrifuged at 3000 g for 5 min. The supernatant was used for further enzymatic measurement. The activity of catalase (1.11.1.6) was determined using the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts on a spectrophotometer (Unico, USA) at a wavelength of 410 nm against water [17]. The amount of protein in the extract was determined according to the Lowry method [18] using a set of reagents (IE Danysh, Ukraine). Catalase activity was calculated in μgMol/min·mg of protein. The content of total protein in the body of bees was determined according to the Kjeldahl method [19].

For the selection of hemolymph, honey bees were gradually cooled in a refrigerating chamber at –1 °C. 10 bees were selected from each group and mechanically fixed in a Petri dish. Hemolymph was collected using insulin syringes, puncturing the body of the bee between the third and fourth tergite from the dorsal surface. The selected hemolymph was diluted with electrode buffer (pH 8.3) in a ratio of 1:2. The content of individual fractions of soluble hemolymph proteins was determined by the method of vertical electrophoresis in a 7.5% polyacrylamide gel TL120 program (Nonlinear Dynamics Limited, Great Britain) and expressed as a percentage of the total pool.

To determine the qualitative and quantitative spectrum of the gut microbiota of bees, before the start of the experiment and on the 14th and 28th days, the midgut and hindgut were sampled (separately) from the bees of each experimental and control group. The obtained samples were homogenized in a sterile 0.15 M NaCl, and serial 10-fold dilutions from 10^{-1} to 10^{-7} were made for each sample. Then 100 μL of appropriate dilution was plated on a selective agar medium for cultivation of each of the groups of microorganisms:

- Meat-peptone agar (MPA) for aerobic and facultative anaerobic bacteria,
- BAIRD-PARKER-Agar (Merck, Germany) for *Staphylococcus* spp.,
- KF-Streptococcus agar (Merck, Germany) for *Streptococcus* spp.,
- MRSA (HiMedia, India) for lactic acid bacteria,
- Bifidobacterium agar (BA, HiMedia, India) for *Bifidobacterium* spp.,
- Endo (HiMedia, India) for coliform bacteria,
- Sabouraud agar (HiMedia, India) for microscopic fungi,
- Pseudomonas agar (HiMedia, India) for *Pseudomonas* spp.

Plates were incubated at the appropriate conditions, and colonies of typical morphology for each microorganism group were counted. The data were expressed as Lg of CFU in 1 mg of a tested sample [21, 22].

The research was conducted in accordance with the «General Ethical Principles of Ani-

mal Experiments» (VII National Congress of Bioethics, Kyiv, 2019) and the European Convention on the Protection of Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

All obtained digital data were processed using the computer program STATISTICA and the method of variational statistics, as well as the Excel program from Microsoft Office-2007 and 2010. Numerical data are presented as the arithmetic mean (M) and standard error (± m). Differences between groups were considered statistically significant at $p < 0.05$.

Results. The comparative study results indicate the stimulating effect of both probiotic strains, *L. casei* IMV B-7280 and *L. plantarum* IMV B-7976, on the catalase activity of the entire body tissues of bees (Fig. 1). In particular, on the 14th day of the use of probiotics, the catalase activity of body tissues was 125.6% ($P < 0.05$) in Group D1 and 133.4% in Group D2 compared to the control ($P < 0.01$).

Further use of probiotic strains with sugar syrup in the feeding of bees maintained a higher level of catalase activity of their tissues, which on the 28th day amounted to 129.9% ($P < 0.05$) in Group D1 and 134.0% in Group D2 compared to the control ($P < 0.01$).

Analysis of the results presented in Fig. 2 confirms the changes during the experimental period in the content of total protein in the tissues of the whole organism of bees that were added with *L. casei* IMV B-7280 or *L. plantarum* IMV B-7679 to sugar syrup.

Thus, the content of total protein in bees of Group D1 increased on the 14th and 28th days by 11.1% ($P < 0.05$) and 16.4% ($P < 0.01$), as compared to the control group.

In the bees of Group D2, the content of total protein also increased, but these differences were unreliable, which may indicate the absence of a significant effect of *L. plantarum* IMV B-7679 on the concentration of proteins in bee tissues. It is known that proteins are a labile system that reflects the state of the body as well as the changes that occur in it under the influence of internal and external factors.

The results of analysis of the effects of probiotic strains *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 on the body of bees showed detected fractions of proteins that correspond to the literature data regarding their electrophoretic mobility in a polyacrylamide gel of blood serum proteins of mammals: γ-globulins, β-globulins, α2-globulins, and α1-globulins (Table 1).

The highest level was established for β-globulins (71.29—72.76%), followed by γ-globulins (10.30—11.74%), α2 (8.93—9.92%), and α1-globulins (6.70—8.04%), respectively. The detected changes in the ratio of the indicated protein fractions in the control and experimental groups are not reliable. Therefore, it can be assumed that the addition of *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 to SS for the experimental groups of bees did not significantly affect the ratio of protein fractions of bee hemolymph.

Microbiological studies of the midgut of bees showed that on the 0th day of the study (preparatory period) in all groups and throughout the entire period of the experiment in the control group, a wide range of microorganisms of different groups was represented in the intestines, in particular, staphylococci, streptococci, microscopic fungi, and coliform bacteria (Table 2). Pseudomonads were detected only in two samples of the midgut of bees of the control and experimental groups in the preparatory period (0th day) in small quantities.

The use of both probiotic strains led to a decrease in the number of aerobic and facultative anaerobic microorganisms in the midgut on the 14th and 28th days in bees receiving probiotic strain *L. casei* IMV B-7280 (Group D1) and on the 28th day in bees receiving *L. plantarum* IMV B-7679 (Group D2). The number of staphylococci and streptococci in the midgut decreased only under the influence of *L. casei* IMV B-7280

strain (Group D1) on the 14th—28th days and on the 28th day, respectively.

It should be noted that the use of both probiotic strains did not reduce the number of coliform bacteria, which are representatives of the normal microbiota. On the contrary, the use of *L. casei* IMV B-7280 strain increased their number in the midgut on the 28th day of the study.

The number of microscopic fungi decreased on the 14th and 28th days after the use of both probiotic strains; on the 28th day in Group D2, microscopic fungi were not detected at all. Pseudomonads were not identified in the midgut on the 14th and 28th days after the use of both *Lactobacillus* strains.

The number of lactic acid bacteria in the bee midgut was significantly higher than that for the control in both groups receiving probiotic strains, both on the 14th and 28th days (Fig. 3). The number of bifidobacteria increased in the group of bees receiving *L. casei* IMV B-7280 during the entire observation period, and in the group receiving *L. plantarum* IMV B-7679 only on the 28th day.

It was shown that in the hindgut of bees of the control group, the number of microorganisms was greater than in the midgut (Table 3).

The use of both probiotic strains led to a decrease in the number of aerobic and facultative anaerobic microorganisms in the hindgut on the 14th and 28th days in bees receiving *L. casei* IMV B-7280 probiotic strain (Group D1) and on the 28th day in bees receiving *L plantarum* IMV B-7679 (Group D2) strain. The number of staphylococci and streptococci in the hindgut decreased under the influence of *L. casei* IMV B-7280 strain (group D1) on the 14th—28th days and on the 28th day, respectively. Under the influence of *L. plantarum* IMV B-7679 strain, only the number of staphylococci decreased on the 14th and 28th days, respectively.

The use of both probiotic strains had no effect on the number of coliform bacteria in the hindgut of bees.

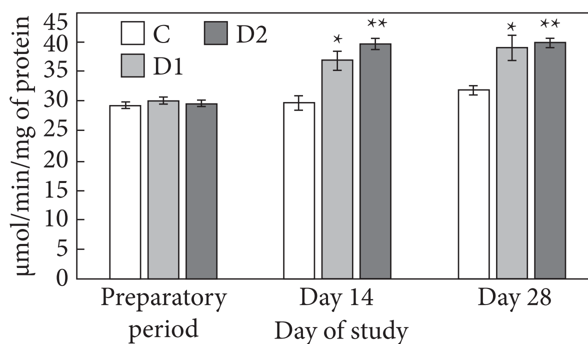

Fig. 1. Catalase activity of body tissues of bees, μmol/min/mg, of protein (M ± m, n = 3), where C is the control group, which received sugar syrup (SS), Group D1 received SS + *L. casei* IMV B-7280), and D2 (received SS + *L. p lantarum* IMV B-7679). *P < 0.05, **P < 0.01 compared to the control group on the same day of the study.

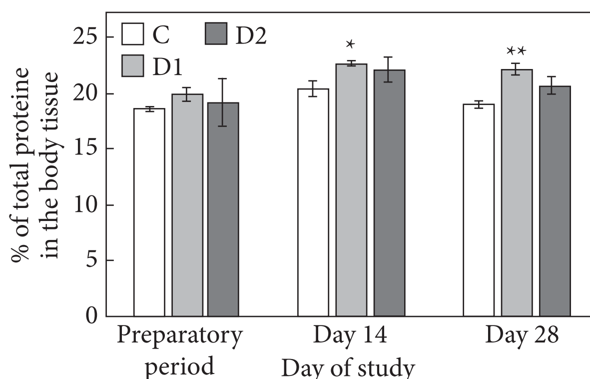

Fig. 2. The content of total protein in the body tissues of bees (M ± m, n = 3), where C is the control group (received SS), D1 (received SS + *L. casei* IMV B-7280), D2 — second experimental group (received SS + *L. plantarum* IMV B-7679). *P < 0.05, **P < 0.01 compared to the control group on the same day of the study

Table 1. **Fractional composition of bee hemolymph proteins, (M ± m, n=3)**

Protein fractions, %	Control group	Group D1 (SS + *L. casei* IMV B-7280)	Group D2 (SS + *L. plantarum* IMV B-7679)
γ-globulins	10.99 ± 0.96	10.30 ± 0.14	11.74 ± 1.67
β-globulins	72.76 ± 1.15	71.91 ± 0.67	71.29 ± 1.69
α2-globulins	9.55 ± 1.21	9.92 ± 0.52	8.93 ± 0.49
α1-globulins	6.70 ± 0.97	7.87 ± 0.36	8.04 ± 0.30
Albumins	Not found	Not found	Not found

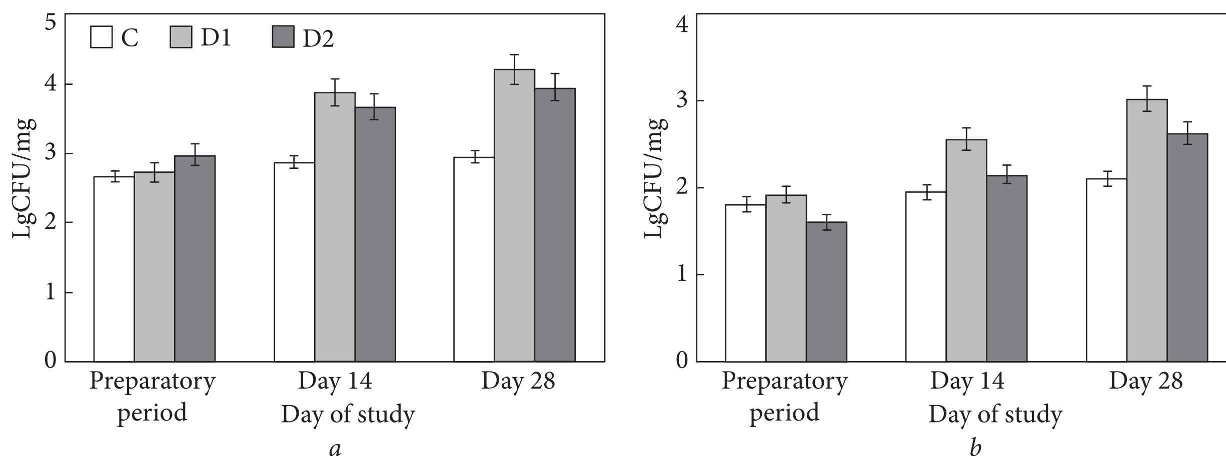

Fig. 3. The number of (*a*) lactic acid bacteria and (*b*) bifidobacteria in the midgut of bees (Lg CFU/mg), where C is the control group (received SS), D1 — experimental Group 1 (received SS + *L. casei* IMV B-7280), D2 — experimental Group 2 (received SS + *L. plantarum* IMV B-7679). *P < 0.05, **P < 0.01 compared to the control group on the same day of the study

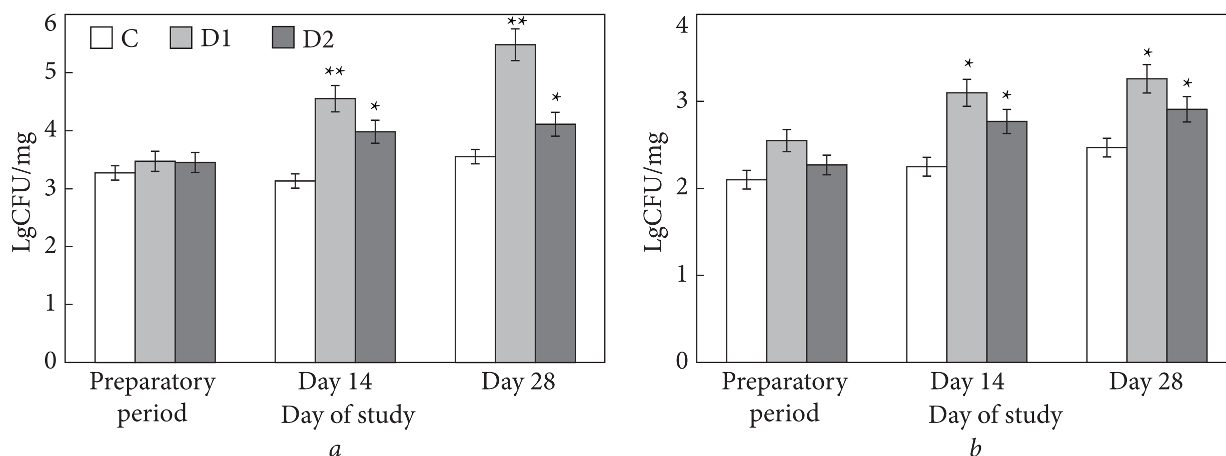

Fig. 4. The number of (*a*) lactic acid bacteria and (*b*) bifidobacteria in the hindgut of bees (Lg CFU/mg), where C is the control group (obtained SS), D1 — experimental Group 1 (received SS + *L. casei* IMV B-7280), D2 — experimental Group 2 (received SS + *L. plantarum* IMV B-7679). *P < 0.05, **P < 0.01 compared to the control group on the same day of the study

The number of microscopic fungi decreased on the 14th and 28th days after the use of both probiotic strains, and on the 28th day in Group D2, microscopic fungi were not detected at all. The number of pseudomonads decreased on the 14th and 28th days after the use of both strains, but complete elimination occurred only on the 28th day after the use of *L. casei* strain IMV B-7280.

The number of lactic acid bacteria and bifidobacteria in the hindgut of bees was higher than in the control on the 14th and 28th days in both experimental groups (Fig. 4), but these changes were more pronounced after the use of *L. casei* IMV B-7280 strain (Group D1).

Discussion. The obtained data indicate a tendency to increase the catalase activity of bee tis-

sues with longer use of *L. casei* IMV B-7280 and a consistently higher activity of this enzyme during the entire experimental period under the action of *L. plantarum* IMV B-7679. In the control group of bees that received SS throughout the experiment, catalase activity remained at a constant level. It is known that one of the main indicators of the general state of the antioxidant system is catalase, which is involved in the protection of the body against excessive action of ROS, which leads to the development of oxidative stress. Thus, probiotics can have a stimulating effect on the resistance and viability of the body of honey bees [23, 24]. Under the conditions of studying their influence, it is advisable to determine the dosage of probiotic strains and the schemes of the use of them.

Table 2. **Spectrum of the midgut microbiota of bees fed *L. casei* IMV B-7280 (D1) and *L. plantarum* IMV B-7679 (D2) strains**

Group of study	Day of study	The number of microorganisms on selective nutrient media (Lg CFU/mg)					
		MPA	BAIRD-PARKER-Agar	KF-Streptococcus agar	Endo	Sabouraud agar	Pseudomonas agar
Control	0	4.80 ± 0.12	3.77 ± 0.06	2.97 ± 0.11	3.47 ± 0.05	2.97 ± 0.07	<1
	14	4.55 ± 0.04	3.42 ± 0.12	3.02 ± 0.06	3.58 ± 0.08	3.11 ± 0.12	<1
	28	4.19 ± 0.07	3.14 ± 0.09	3.13 ± 0.08	3.17 ± 0.04	2.72 ± 0.09	1.25 ± 0.25
D1	0	4.92 ± 0.04	3.82 ± 0.06	3.12 ± 0.11	3.51 ± 0.05	2.85 ± 0.06	<1
	14	3.99 ± 0.05*	2.77 ± 0.08*	2.87 ± 0.09	3.78 ± 0.07	1.75 ± 0.10**	<1
	28	3.28 ± 0.06**	2.45 ± 0.05*	2.45 ± 0.05*	3.91 ± 0.05*	<1**	<1
D2	0	4.77 ± 0.11	3.91 ± 0.11	3.27 ± 0.05	3.22 ± 0.09	2.91 ± 0.04	1.60 ± 0.20
	14	4.17 ± 0.14	3.15 ± 0.09	3.12 ± 0.09	3.40 ± 0.05	1.75 ± 0.10**	<1
	28	3.62 ± 0.04*	3.27 ± 0.04	3.45 ± 0.20	3.65 ± 0.08	1.25 ± 0.15**	<1

*P < 0.05, **P < 0.01 compared to the control group on the same day of the study.

Table 3. **Spectrum of the hindgut microbiota of bees fed *L. casei* IMV B-7280 (D1) and *L. plantarum* IMV B-7679 (D2) strains**

Group of study	Day of study	The number of microorganisms on selective nutrient media (Lg CFU/mg)					
		MPA	BAIRD-PARKER-Agar	KF-Streptococcus agar	Endo	Sabouraud agar	Pseudomonas agar
Control	0	5.20 ± 0.06	4.48 ± 0.06	4.52 ± 0.14	5.80 ± 0.11	3.28 ± 0.11	2.25 ± 0.08
	14	5.47 ± 0.08	4.02 ± 0.11	4.68 ± 0.22	5.82± 0.09	3.66 ± 0.06	2.48 ± 0.10
	28	5.28 ± 0.11	4.45 ± 0.04	4.80 ± 0.12	5.14± 0.14	3.89 ± 0.12	3.41 ± 0.05
D1	0	5.17 ± 0.05	4.04 ± 0.04	4.57 ± 0.07	5.44± 0.07	3.65 ± 0.08	3.47 ± 0.03
	14	4.62 ± 0.06**	3.55 ± 0.08*	4.02 ± 0.04	5.68± 0.03	1.15 ± 0.10**	1.50 ± 0.08*
	28	4.11 ± 0.09**	3.01 ± 0.04*	3.18 ± 0.11*	5.92± 0.18	<1**	<1**
D2	0	5.44 ± 0.11	4.78 ± 0.11	4.65 ± 0.05	5.81± 0.05	3.15 ± 0.08	2.80 ± 0.03
	14	5.11 ± 0.06	3.86 ± 0.05*	4.10 ± 0.10	5.21± 0.11	2.30 ± 0.11*	2.01 ± 0.07*
	28	4.88 ± 0.03*	3.40 ± 0.11*	4.28 ± 0.06	5.16± 0.08	2.10 ± 0.05*	1.10 ± 0.10**

*P < 0.05, **P < 0.01 compared to the control group on the same day of the study.

It should be noted the absence of albumin fractions, which is also noted in the results of studies by other researchers [25]. Protein water-soluble fractions in the blood plasma of vertebrates consist mainly of albumins and globulins. In clinical settings, measuring the percentage of albumins and globulins can provide information about clinical conditions, such as hydration, oncotic pressure, body condition, or inflammatory processes. In our study, the method used failed to detect this protein in the hemolymph samples, which may indicate its absence or small (trace) amounts. Some authors suggest that insects do not need this protein to maintain oncotic pressure that liposoluble hormones can be transported by hemocyanin, and the nutritional function is performed by a network of intestinal diverticula that reach all parts of the body [25, 26].

The *Lactobacillus* genus is widely used for fermentation of plant materials and dairy products. These species are typically found in highly specialized environments, with the bee gut serving as one of the niche locations where *Lactobacillus* was detected [27]. The bee-associated bacterial communities did not differ in hive constructions and varied slightly over the honey production season. Samples of the gut were usually dominated by taxa belonging to the *Lactobacillus*, *Bifidobacterium*, *Bartonella*, *Snodgrassella*, *Gilliamella*, and *Frischella* genera, as observed in previous studies [28].

While many recent studies support the idea of a core microbiota in the guts of younger in-hive bees, it is unknown whether this core is present in forager bees or they carry the pollen back to the hive. Additionally, several studies hypothesize that the foregut (crop), a key interface between the pollination environment and hive food stores, contains a set of 13 lactic acid bacteria, which inoculate collected pollen and act in synergy to preserve pollen stores [29].

Nowadays, honey bees are stressed by a number of biotic and abiotic factors that may compromise to some extent the pollination service and the hive productivity. The EU's ban on antibiotics as therapeutic agents against bee pathogens has stimulated the search for natural alternatives. The increasing knowledge of the composition and functions of the bee gut microbiota and the link between balanced gut microbiota and health status has encouraged research on the use of gut microorganisms, especially lactobacilli, to improve bee health [30].

Fanciotti et al. determined the impact of *L. salivarius* A3iob, a honey bee gut-associated strain (GenBank code access KX198010), on honey yield. *L. salivarius* A3iob cells prove to be a natural alternative that will positively impact the beekeepers' economy by providing a higher honey yield: all treated bees produced between 2.3 to 6.5 times more honey than the controls [31].

Due to their social behavior, honey bees can be infected by a wide range of pathogens including the microsporidia *Nosema ceranae* and the bacteria *Paenibacillus larvae*. The use of probiotics as food additives for the control or prevention of infectious diseases is a widely used approach to improve human and animal health. Another study has shown that a mixture of four *L. kunkeei* strains isolated from the gut microbial community of bees has a potential beneficial effect on larvae and adult bees [32].

Also, A-Tai Truong et al. showed that *L. apis* HSY8_B25, *L. panisapium* PKH2_L3, and *L. melliventris* HSY3_B5 can be potential probiotic candidates, and they were selected for probiotic development to prevent diseases caused by *Paenibacillus larvae* [33].

Therefore, it can be considered appropriate to continue research on probiotic strains of lactobacilli to create a complex drug capable of increasing the life expectancy and honey production of bees, as well as to support the homeostasis of their microbiome, which will provide natural protection against widespread diseases of different origin.

Conclusions. The use of the *L. casei* IMV B-7280 and *L. plantarum* IMV B-7679 probi-

otic strains for feeding bees under the conditions of a laboratory thermostat stimulated the catalase activity of their body tissues in both experimental groups ($P < 0.05$, $P < 0.01$) both on the 14th and 28th days of the study compared to the control group. The content of total protein in the body tissues of bees that received *L. casei* IMV B-7280 increased by 13.9% and 11.4% on the 14th and 28th days of the experimental period compared to the control group. The use of both probiotic strains led to quantitative changes in the composition of the intestinal microbiota of bees, in particular, to an increase in the number of lactic acid bacteria and bifidobacteria and a decrease in the number of some other groups of microorganisms.

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ВПЛИВ ПРОБІОТИЧНИХ МІКРООРГАНІЗМІВ НА АКТИВНІСТЬ КАТАЛАЗИ, ФРАКЦІЙНИЙ СКЛАД РОЗЧИННИХ БІЛКІВ ГЕМОЛІМФИ ТА СПЕКТР КИШКОВОЇ МІКРОБІОТИ МЕДОНОСНИХ БДЖІЛ

Останнім часом спостерігається тенденція до використання нових ефективних засобів натурального походження для боротьби з хворобами та покращення здоров'я медоносних бджіл. Також відомо, що добре збалансована структура кишкової мікробіоти медоносних бджіл є основою для їх росту, розвитку, підсилення імунної відповіді та стійкості до інфекцій. Встановлено, що деякі штами молочнокислих бактерій, що мають антибактеріальні, протизапальні та імуномодулювальні властивості, є перспективними для розробки на їх основі пробіотичних препаратів широкого спектру дії. Отже, метою роботи було визначення впливу пробіотичних штамів *Lactobacillus* casei *IMB B-7280* і *L. plantarum IMB B-7679* на активність каталази, вміст білка та білковий профіль гемолімфи, а також спектр мікробіоти різних відділів кишечника медоносних бджіл *Apis mellifera*. **Методи.** Для проведення дослідження було сформовано контрольну та дві дослідні групи по 60—90 бджіл у кожній. Бджоли контрольної групи протягом 28 діб отримували підгодівлю з 60 % цукрового сиропу + 1 мл дистильованої H_2O. Дослідна група бджіл Д 1 щодня отримувала 1 мл 60 % цукрового сиропу + 1 мл водної суспензії клітин штаму *L. casei* IMB B-7280 в концентрації $1 \cdot 10^6$ КУО/мл; дослідна група бджіл Д 2 додатково до 1 мл 60 % цукрового сиропу отримувала 1 мл водної суспензії клітин штаму *L. plantarum* IMB B-7976 в концентрації $1 \cdot 10^4$ КУО/мл. Активність каталази в тканинах організму бджіл визначали за допомогою здатності гідрогенпероксиду утворювати із солями молібдену стійкий кольоровий комплекс на спектрофотометрі при довжині хвилі 410 нм проти води. Кількість білка в тканинах організму бджіл визначали за методом Лоурі. Вміст загального білка в організмі бджіл проводили за методом Кендаля. Визначення вмісту окремих фракцій розчинних білків гемолімфи проводили методом вертикального електрофорезу в 7,5 % поліакриламідному гелі. Відносний вміст білкових фракцій визначали за допомогою програми TotalLab TL120 і виражали у відсотках від загального пулу. Для визначення якісного та кількісного спектру кишкової мікробіоти бджіл проводили забір середнього та заднього кишківника (окремо) від бджіл контрольної та дослідних груп. Отримані зразки висівали на вісім селективних поживних агаризованих середовищ для культивування окремих груп мікроорганізмів. **Результати.** Встановлено тенденцію до підвищення каталазної активності тканин бджіл за тривалішого (28 діб) застосування штаму *L. casei* IMB B-7280 і стабільно вищої активності цього ензиму впродовж всього дослідного періоду за дії штаму *L. plantarum IMB B*-7679. У контрольній групі бджіл активність каталази залишалася на сталому рівні. Показано, що на 14 та 28 добу достовірно збільшувався вміст загального протеїну в організмі бджіл, які отримували штам *L. casei* IMB B-7280. У бджіл обох груп виявлено водорозчинні фракції білків гемолімфи: γ-глобуліни, β-глобуліни, α2-глобуліни, α1-глобуліни. Варто зазначити, що фракція альбумінів не виявлена. Показано, що задня кишка містить значно більшу загальну кількість мікроорганізмів, ніж середня. Застосування штаму *L. casei* IMB B-7280 призводило до збільшення кількості молочнокислих бактерій та біфідобактерій в обох відділах кишківника, а також до зниження кількості стафілококів, стрептококів та мікроскопічних грибів. Застосування штаму *L. plantarum* IMB B-7679 мало схожий ефект, але зміни у складі мікробіому кишківника були менш виражені. **Висновки.** Застосування пробіотичних штамів *L. casei* IMB B-7280 і *L. plantarum* IMB B-7679 для підгодівлі бджіл за умов лабораторного термостату приводило до кількісних змін у складі кишкової мікробіоти бджіл, зокрема до збільшення кількості молочнокислих бактерій та біфідобактерій, а також зменшення кількості деяких інших груп мікроорганізмів в кишківнику. Пробіотичні штами стимулювали каталазну активність тканин організму бджіл, підвищували рівень загального білка, але суттєво не впливали на співвідношення білкових фракцій гемолімфи.

Ключові слова: *бджоли Apis mellifera, лактобактерії, каталаза, фракції протеїнів, гемолімфа, спектр мікробіому кишківника.*