https://doi.org/10.15407/animbiol25.01.020
UDC 638.1; 577.115; 66.095
Received 03.10.2022 ▪ Revision 27.12.2022 ▪ Accepted 12.03.2023 ▪ Published online 31.03.2023

Lipid composition and peroxidation products in the body tissues in bees under the action of different doses of nanotechnological Ge citrate and the probiotic *Lactobacillus casei* B-7280

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IIK: Data curation; Formal analysis; Investigation; Methodology; Resources; Software.
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Declaration of Conflict of Interests:
None to declare.

Ethical approval:
Not applicable.

Acknowledgements:
None.

Modern methods of preserving honey bees focus on increasing their viability, feeding level and productivity development during different growth periods. There is a tendency to use new effective remedies of natural origin. Their action mechanisms differ from synthetic substances and drugs due to the activation of the body's protective reactions at the physiological level. The probiotic *Lactobacillus casei* IMV B-7280 has an effective stimulating effect on physiological processes in bees under various environmental and experimental conditions. The physiological effect of this probiotic is associated with normalizing of the intestinal bacterial microflora and participation in modulating body's protective reactions. The resistance of honey bees also depends on mineral nutrition, which affects metabolic processes at the tissue, organ and system levels and determines the vitality and resistance of the organism. Mineral elements are involved in protein, lipid, carbohydrate and energy metabolism, they activate enzyme systems. Therefore, the aim of the research was to determine the effect of the probiotic *L. casei* B-7280 in combination with different doses of Ge nanotechnological citrate (NTC) on the lipid composition and peroxidation products content in bees' tissues. Bees of the control group were fed 60% sugar syrup (SS) in the amount of 1 cm^3/group/day. The bees of the first experimental group (R1) were fed 1 cm^3 of SS, 0.1 μg of Ge NTC and a solution of probiotic *L. casei* B-7280 (10^6 cells/cm^3); the second experimental group (R2) received 0.2 μg of Ge citrate and probiotic *L. casei* B-7280 (10^6 cells/cm^3). The feeding duration was 34 days. It was established that feeding sugar syrup, *L. casei* and different doses of Ge citrate increased the content of phospholipids, mono- and diacylglycerols in the R2 group, cholesterol esterification in the R1 group, and decreased free cholesterol, non-esterified fatty acids compared to the K group and P period. The results indicate a dose-dependent effect of Ge NTC on lipid metabolism in body tissue homogenates. There was an increase in the triacylglycerols level in the lipids in all groups during the experimental period. The biological effect of nanotechnological Ge citrate and *L. casei* cause a decrease of lipid peroxidation processes in bees of the experimental groups.

Key words: bees, Ge citrate, probiotic, lipids, peroxidation products

Introduction

In addition to beekeeping products, the honeybee (*Apis mellifera*) plays an essential role in preserving biodiversity, ecosystem stability, and agricultural production by pollinating entomophilous plants, which increases its yield. In recent decades, the honeybee population's significant losses have threatened the ecosystem and the country's food security. Under these conditions, researchers and beekeepers pay special attention to factors that worsen the body's resistance, the functional state of its systems, and the productivity of bees. Therefore, the scientific support of modern beekeeping is aimed at obtaining safe ecological products, developing means and methods for stimulating reproduction, increasing the resistance of bees to various pathogens, and protecting them from adverse environmental conditions [21]. It has been shown that the influence of abiotic, biotic origin, and anthropogenic activity disrupts physiological processes in the body of *A. mellifera*, suppressing the antioxidant and immune systems, which leads to the death of entire colonies [1, 19, 29].

Therefore, today, an extremely urgent task is to find new means and BARs for activating the protective systems of the honeybee and to find out the mechanisms of their beneficial effects. The honeybee's defense responses include cellular and humoral responses that combine interconnected systems, including antibacterial peptides, hemagglutinins, phenoloxidase, and antioxidant systems (AOS). Under normal conditions, there is a balance between the generation of reactive oxygen species (ROS) and antioxidant processes. Oxidative stress occurs when the dynamic balance between the formation of ROS exceeds the ability of antioxidant protection to remove the toxic substances formed. Many studies have linked oxidative stress to stressors affecting honeybee health and colony productivity. Low temperature, high flight activity, pathogenic microorganisms in hives, and pesticides used to control pests on various crops disrupt the oxidative homeostasis of honeybees [25, 27].

Deterioration of the forage base or its sudden change is one factor that negatively affects the health of bees and the development of colonies and can cause their death [14, 33]. A deficiency of feed or a slight violation of the component composition can weaken the AOS, detoxification, and immune systems of bees, resulting in their body becoming more vulnerable to chemical plant protection drugs and diseases of various etiologies.

These factors contribute to the excessive generation of ROS in the body of bees, which leads to the development of oxidative stress [17]. Reactive oxygen species can react with polyunsaturated fatty acids of lipid membranes and induce lipid peroxidation (LPO), affecting cell membranes' physiological function. The final product of these reactions is malondialdehyde (MDA), a marker of LPO and, as a result, the oxidative stress. Therefore, there is a trend of active study of new effective means of natural origin to fight diseases and improve honey bees' health, which helps to avoid many side effects. It has been proven that the mechanisms of their action differ from synthetic substances and drugs due to the activation of the body's protective reactions at the physiological level [9, 11, 32].

Research on the physiological justification of the use of probiotics, the antibacterial and antifungal properties of which are due to high antagonistic activity against a wide range of pathogenic and conditionally pathogenic microorganisms, and the possibility of their synergistic combination with microelements, deserve special attention in the system of prevention of bee diseases [21, 24].

It is known that a well-balanced structure of the intestinal bacterial microflora of honey bees is the basis for their physiological growth, development, reproduction, strengthening of the immune response, and resistance to the action of pathogens [4, 21].

The probiotic *Lactobacillus casei* IMV B-7280 is characterized by an excellent therapeutic effect in various experimental infectious-inflammatory models [8, 23, 24]. The physiological effect of this probiotic is associated with the normalization of the intestinal bacterial microflora and participation in the modulation of inflammatory reactions. In the gastrointestinal tract, probiotics exert both a direct effect on pathogenic and conditionally pathogenic microorganisms and an indirect effect by activating specific and nonspecific protective systems of the body [8, 21].

It is known that the vital activity of the organism of honey bees also depends on mineral nutrition, which affects metabolic processes at the level of tissues, organs, and systems and affects the vitality and resistance of the organism [7, 22]. They participate in protein, lipid, carbohydrate, and mineral metabolism, activate enzyme systems, etc. Literary data indicate the possibility of using biotic trace elements produced by nanotechnology as highly active compounds in animal husbandry and veterinary medicine [3, 7, 20].

Adding some elements to bee feed, as metabolic stimulators of organic and inorganic origin, introduced in different doses, affects the correction of physiological and biochemical processes and increases their productivity and resistance [11, 20, 36]. Such mineral components include Co, Ge, Se, Cr, Ni, and others. The results of previous studies of the Institute of Animal Biology NAAS using citrates of certain microelements and probiotics [21, 31] provide a theoretical basis for developing new nano- and biotechnological means and drugs to increase the resistance and reproduction of bees. The effect of various amounts of mineral and organic compounds obtained based on nanotechnological citrates on the metabolic processes of the bees' bodies was clarified. Several works were published based on the research results [7, 9, 19, 20]. It has been established that adding nanocarboxylates of biotic elements is more effective than their mineral salts in bee feeding [21]. However, the biological effect of the newly synthesized nanotechnological mineral element Ge in combination with probiotic preparations of the *L. casei* B-7280 class has not been studied.

In connection with the purpose mentioned above of the research, we determined the effect of the probiotic drug *Lastobasillus casei* IMV B-7280 in combination with different doses of germanium citrate on the lipid composition and the content of lipid peroxidation products in the bees body.

Materials and Methods

Conducting the research

The research was conducted on the Carpathian breed honey bees, selected from the laboratory apiary of the Institute of Animal Biology NAAS. The research used the lyophilized probiotic strain *Lactobacillus casei* IMV B-7280, which was isolated in the department of problems of interferon and immunomodulators from the associated culture of biological material and deposited in the Ukrainian Collection of Microorganisms of the Zabolotny Institute of Microbiology and Virology NAS of Ukraine. The research was carried out under the conditions of a laboratory thermostat on three bee colonies, similar in weight, colony strength, and queen age. From this, 50–60 bees were selected and formed into three groups. Bees of the control and experimental groups were kept in cages-containers with a volume of 4 dm^3 in similar conditions of a TC-80M-3 laboratory thermostat with microventilation at a temperature of 30° C and humidity of 74–76% during the study.

Bees of the control (C) group were fed 60% sugar syrup (SS) in 1 cm^3/group/day. Experimental 1 group of bees (R1) in addition to 1 cm^3 of sugar syrup received 0.1 μg of Ge in the form of nanotechnological citrate (NTC) [18] and a solution of the probiotic *L. casei* B-7280 at a concentration of 10^6 cells/cm^3; experimental group 2 of bees (R2) additionally received 0.2 μg of Ge in the form of citrate and the probiotic *L. casei* B-7280 at a concentration of 10^6 cells/cm^3.

The duration of drinking SS, Ge citrate, and probiotic is 34 days. In the preparatory period (P), as well as on the 34th day of the experimental period, live bees were selected from the control and experimental groups for physiological and biochemical studies to determine the content of total lipids and the ratio of their classes and products of lipid peroxidation in tissue homogenates of the entire organism.

Obtaining total lipids

Homogenized tissue (1 g) was extracted with 20 cm^3 of a mixture of chloroform-methanol in a ratio of 2 : 1 (v/v) according to the Folch method [13]. A solution of 4 cm^3 of an aqueous solution of 0.74% KCl was added to each sample of lipid extract. After 24 h, the upper phase containing hydrophobic peptides was removed with a water pump, and the lower phase containing lipids was filtered (deashed filter, blue ribbon). Lipid extracts were evaporated to dryness, weighed on an analytical balance, and calculated in mg/g.

Separation of lipids into classes

Separation of lipids into classes was carried out by the method of thin-layer chromatography on silica gel (silica gel L 5/40μ, LSL 5/40μ, *Chemapol*, Czech Republic), the mobile phase was hexane-diethyl ether-glacial acetic acid in a ratio of 70 : 30 : 1 (v/v/v). Lipid classes were shown in crystalline iodine vapors. Rf values identified lipids. Quantitative analysis and calculation of the content of individual classes of lipids were performed by computer processing of phorograms using *TotalLab TL120* software (Nonlinear Dynamics Limited, UK) and expressed as a percentage of the total amount.

Determination of lipid peroxidation products

To prepare a homogenate of tissues of the entire body of honey bees of the control and experimental groups, they were ground and formed into three parallel samples. A group of bees weighing 0.5 g was homogenized with physiological saline in a ratio of 1 : 5 using a homogenizer (*Homogenizer Type 302*, Poland) on ice. The samples were centrifuged at 3000 g for 5 minutes. The supernatant was used to measure the content of lipid peroxidation products further [35] in bee tissues on the 34th day of SS, Ge citrate, and probiotic use.

Determination of TBA reactive substances (MDA)

The basis of the method is the reaction between MDA and thiobarbituric acid (TBA), which at high temperatures and in an acidic environment forms a trimethine complex containing one MDA molecule and two TBA molecules. 5 cm^3 of 20% phosphotungstic acid was added to 0.5 cm^3 of the prepared homogenate. The tubes were closed, mixed, and left in the cold for 15 min, then centrifuged at *t* 4 C for 15 min at 2500 rpm. The supernatant liquid was drained, and 2 cm^3 of H_2O and 1 cm^3 of 0.8% TBA were added to the precipitate, mixed, covered, and incubated for 1 h in a heated bath at 100°C, then cooled in running water and centrifuged for 10 min at 6000 rpm. In the centrifuge, the optical density was measured on a spectrophotometer at 535 and 580 nm to prevent the absorption of stained complexes by TBA substances of non-lipid origin.

Determination of lipid hydroperoxide content

The precipitation of proteins determines lipid hydroperoxides' content in biological material with a solution of trichloroacetic acid and extraction of lipids with ethanol, followed by the interaction of the studied extracts with ammonium thiocyanate. 2.8 cm^3 of ethanol and 0.05 cm^3 of a 50% TChA solution were added to 0.2 cm^3 of the homogenate. The test tube was closed and shaken for 5–6 min. The resulting protein precipitate was isolated by centrifugation for 10 min at 3000 rpm. 1.5 cm^3 of the ethanol extract was taken and brought up to 2.7 cm^3 with ethanol, shaken, and 0.02 cm^3 of conc. HCl and 0.03 cm^3 of a 1% solution of Mohr's salt in a 3% solution of HCl. It was shaken, and after 30 s, 0.2 cm^3 of 20% ammonium thiocyanate was added. The optical density was measured for 10 min after adding ammonium thiocyanate on a spectro-

photometer at a wavelength 480 nm. The control sample was placed as a test sample, but 0.2 cm^3 of bidistilled water was taken instead of the homogenate.

No vertebrate animals were used in the experiments.

Statistical analysis

All obtained digital data were processed using the *Statistica* computer program using the method of variational statistics and the *Excel* program from the *Microsoft Office* 2007 and 2010 service packages. Differences between groups were considered statistically significant at P<0.05.

Results and Discussion

The analysis of the obtained research results indicates that the content of total lipids and the relative ratio of lipid classes in homogenates of body tissues of bees of the experimental groups changed compared to both the control group and the preparatory period (fig. 1, table).

An increase in the content of total lipids in the R1 and R2 groups was found, respectively, by 11.14% (P<0.05) and 7.65% (P<0.05) compared to the preparatory period (fig. 1). A significant increase in total lipids may indicate the stimulating effect of applied doses of Ge citrate and *L. casei* B-7280 on their exchange and synthesis in the tissues of honey bees. However, the absence of potential differences in the content of total lipids may indicate a minor effect of Ge in the form of citrate and the pH of the probiotic *L. casei* B-7280 on the synthesis and deposition of lipids in the body of bees.

It has been proven that the central mass of lipids in the body of bees comes from the alimentary canal and is deposited in the fat body. The chemical composition of these reserve fats depends on both the feed's composition and the body's physiological state [6].

Our research revealed changes in the ratio of lipid classes in bees' body tissues. In particular, such changes concern phospholipids, mono- and diacylglycerol (MDAG), free cholesterol, non-esterified fatty acids (NEFA), triacylglycerol, and esterified cholesterol (table). Phospholipids make up 24–28% of the total amount of lipids and predominate in the bees' body tissues in the control and experimental groups. In bees' body tissues homogenates of R1 and R2 groups, an increase in the phospholipids relative content compared to bees of the preparatory period and the control group was established by 16.79% and 17.08% (P<0.05) and 15.73% and 16.02% (P<0.05), respectively.

This class of lipids may be more synthesized in the body of bees under the action of NTC Ge and *L. casei* to enhance the functions of lipid membranes. It is known that the fatty acid composition of phospholipids of cell membranes is the main factor affecting the intensity of the transition of fatty acid nutritional components, through their active and passive transport, into bee tissues. In turn, the functioning of their nervous, immune, and reproductive systems and the oxidation process depends on the content of phospholipids and their fatty acid composition in bee tissues.

Table. Fractional composition of total lipids in bee body, % (M±SE, n=5)

Lipid classes	Groups			
	P	K	R1	R2
Phospholipids	24.12± ±0.74	24.34± ±0.38	28.17± ±0.51*#	28.24± ±0.57*#
Mono- and triacylglycerols	16.84± ±0,74	15.76± ±0.49	15.45± ±0.61	19.69± ±1.01*##
Free cholesterol	16.44± ±0.27	16.64± ±0.50	11.77± ±0.62*#	11.55± ±0.43***###
Non-esterified fatty acids	16.14± ±0.60	14.56± ±0.57	11.33± ±0.38*#	11.27± ±0.82*#
Triacylglycerols	13.98± ±0.31	16.46± ±0.53	14.33± ±0.37#	15.89± ±0.37*
Esterified cholesterol	12.49± ±0.86	12.24± ±0.57	18.95± ±0.55*#	13.36± ±0.87

Note. In the table and the fig. 1: * — P<0.05, ** — P<0.01, *** — P<0.001: significant differences between the preparatory and experimental periods by groups. # — P<0.05, ## — P<0.01, ### — P<0.001: significant differences between control and experimental groups.

It should be noted that membrane phospholipids are necessary to stabilize the aggregation and conformation of individual components in enzymatic protein complexes and create a hydrophobic environment to form a continuous structure with all the properties inherent to them [16].

An increase in the MDAG fraction in the R2 group by 16.92% (P<0.05) concerning the preparatory period and by 24.94% (P<0.01) in the control group was also established. It is known that the lipid transport system is a feature of fat metabolism in bees. The main feed lipids in the body of bees are transformed into diacylglycerols, performing, like glucose, the function of energy supply. Therefore, the increase in the level of MDA in the tissues of the body of bees of the R2 group may indicate a more intensive energy supply of their tissues due to the addition of 0.2 μg of Ge in the form of citrate and the pH probiotic *L. casei* B-7280 at a concentration of 10^6 cell/cm^3 to the sugar syrup.

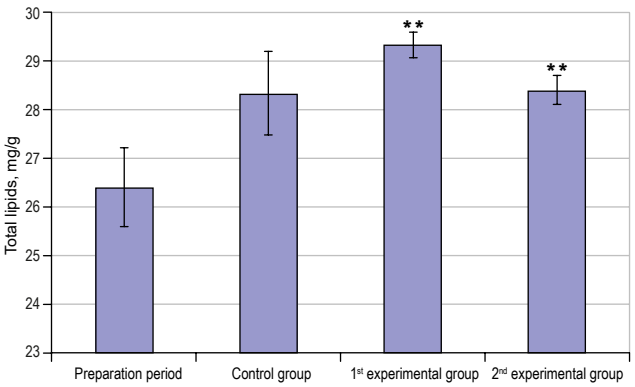

Fig. 1. Content of total lipids in homogenates of bee body tissues

The content of free cholesterol decreased in bees of R1 and R2 groups by 28.41% (P<0.05) and 29.74% (P<0.001) concerning the preparatory period and by 29.27% (P<0.05) and 30.59% (P<0.001) relative to the control group. The supply of Ge citrate in the body of bees contributes to the reduction of free cholesterol by increasing its use in metabolic reactions. Perhaps cholesterol is used to synthesize vitellogenin in the cholesterol-hydroxyecdysone-Vg pathway in trophocytes and enocytes of worker bees [12, 26].

A decrease in the content of NEFA in bees of R1 and R2 groups was established by 29.80% and 30.17% (P<0.05) concerning the preparatory period and by 22.18% and 22.60% (P<0.05) relative to the control group. The obtained data on the content of non-esterified fatty acids in the lipids of the tissues of bees of the experimental groups indicate the activation of lipolysis processes in the body of bees of these groups since a significant decrease in the relative content of non-esterified fatty acids, as precursors of lipid synthesis, was established compared to the control. It is known that lipolysis is physiologically reduced to maintaining the homeostatic concentrations of individual lipid components necessary for aerobic cellular respiration and the formation of PUFA to compensate for the energy needs of tissues in bees [31].

The content of esterified cholesterol increased only in R1 group by 51.72% (P<0.05) during the preparatory period and by 54.82% (P<0.05) following the control group. The increase in the content of cholesterol ethers in the tissues of bees in the first research group may indicate a higher antilipolytic activity of enzymes that regulate the process of its esterification under the action of SS and 0.1 μg of Ge citrate and the pH of the probiotic *L. casei* B-7280 at a concentration of 10^6 cell/cm^3 and no such effect at a higher dose of Ge citrate.

An increase in the content of triacylglycerols in the R2 group by 15.89% (P<0.05) concerning the preparatory period and a decrease by 12.94% (P<0.05) compared to the control group was established. This indicates the optimizing effect of complex feeding of bees with SS and a dose of 0.2 μg of Ge citrate and pH probiotic *L. casei* B-7280 at a 10^6 cell/cm^3 concentration and the absence of such an effect with a Ge citrate lower dose.

It is known that active forms of oxygen are formed due to aerobic respiration and oxidation of substrates. In body cells exposed to various stresses, the production of reactive oxygen species increases, which directly affect enzymes and damage cells [34]. At the same time, an analysis of the literature shows that Germanium promotes the removal of toxins from the body and neutralizes the negative impact of environmental factors, has a wide range of biological effects, which confirm our results [10], and prevents aging and cell death. This element is essential in forming the body's resistance and can restore and prevent many diseases [10].

Malondialdehyde is one of the end products of the peroxidation of polyunsaturated fatty acids in cells. An increase in the content of free radicals causes excessive production of MDA. The malondialdehyde level is a marker of oxidative stress and the body's antioxidant status [30].

Excessive activation of LPO processes with reduced activity of the body's antioxidant system can lead to significant pathological changes, primarily accompanied by damage to subcellular and cellular membranes. LPO products cause disruption of not only lipid bonds in biomembranes and their protein component — due to binding with amine groups, which leads to disruption of protein-lipid interaction. Free radical oxidation of lipids causes changes in fiber elasticity and initiates fibroplastic processes and collagen aging [2, 15].

As a result of the conducted studies, it was established that in the homogenates of bee body tissues, the concentration of lipid hydroperoxides in the R2 experimental group decreased by 16.67% (P<0.05) and TBARS by 41.85% (P<0.001) compared to the control group (fig. 2, 3).

These results indicate the antioxidant effect of Ge citrate in the body of bees in the applied doses, which was accompanied by a decrease in the content of TBARS (MDA) and lipid hydroperoxides in homogenates of bee body tissues of all experimental groups, which is consistent with the data of other authors [5, 9, 28].

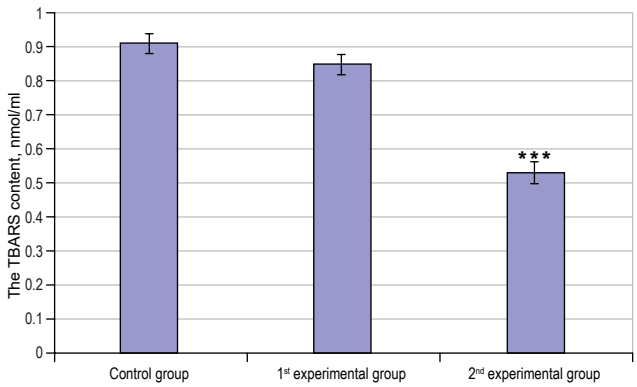

Fig. 2. Content of TBARS in homogenates of bee body tissues

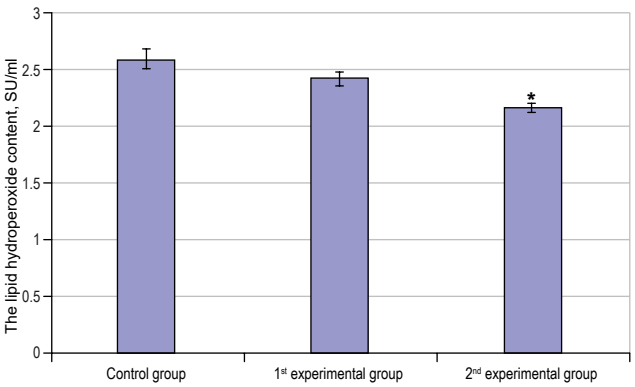

Fig. 3. Content of lipid hydroperoxides in homogenates of bee body tissues

Note. In these figures: * — P<0.05, ** — P<0.01, *** — P<0.001 — significant differences between the control and experimental periods by groups.

Conclusions

Supplementation of bees with NTC Ge at a dose of 0.1 and 0.2 μg/ml sugar syrup and 10^6 cell/cm^3 sugar syrup of *L. casei* was characterized by differences in the distribution of individual classes of lipids in homogenates of body tissues with a higher relative content of phospholipids, mono- and diacylglycerols (only in R2 group), of esterified cholesterol (R1), but a decrease in free cholesterol, NEFA compared to the control group and the experimental period, which indicates a dose-dependent effect of NTC Ge on lipid metabolism. An increase in the level of triacylglycerols in the lipids of bee tissues of all groups during the experimental period was noted.

The biological effect of nanotechnological Ge citrate and *L. casei* led to a decrease in the lipid peroxidation processes (lipid hydroperoxides, TBARS) in the body tissues of the bees of the experimental groups compared to the control group under the action of a higher dose of Ge citrate.

References

1. Almasri H, Tavares DA, Diogon M, Pioz M, Alamil M, Sené D, Tchamitchian S, Cousin M, Brunet JL, Belzunces LP. Physiological effects of the interaction between *Nosema ceranae* and sequential and overlapping exposure to glyphosate and difenoconazole in the honey bee *Apis mellifera*. *Ecotoxicol. Environ. Saf*. 2021; 217: 112258. DOI: 10.1016/j.ecoenv.2021.112258.
2. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new. *J. Eur. Acad. Dermatol. Venereol.* 2003; 17 (6): 663–669. DOI: 10.1046/j.1468-3083.2003.00751.x.
3. Cho JM, Chae J, Jeong SR, Moon MJ, Shin DY, Lee JH. Immune activation of Bio-Germanium in a randomized, double-blind, placebo-controlled clinical trial with 130 human subjects: Therapeutic opportunities from new insights. *PLoS ONE*. 2020; 15 (10): e0240358. DOI: 10.1371/journal.pone.0240358.
4. Daisley BA, Chmiel JA, Pitek AP, Thompson GJ, Reid G. Missing microbes in bees: How systematic depletion of key symbionts erodes immunity. *Trends Microbiol*. 2020; 28 (12): 1010–1021. DOI: 10.1016/j.tim.2020.06.006.
5. Dolaychuk OP, Fedoruk RS, Kovalchuk II, Kropyvka SY. Physiological and biochemical processes in the organisms of rats that were fed with different amounts of germanium citrate. *Biol. Tvarin*. 2015; 17 (2): 50–56. DOI: 10.15407/animbiol17.02.050. (in Ukrainian)
6. Dvylyuk I. Mineral and lipid composition the body of the honeybees organism and the biological value of honey in the summer-autumn period under the conditions of feeding honey bees by citrate-capped silver and copper nanoparticles. *Sci. Mess. LNUVMBT. Ser. Agricult. Sci*. 2018; 20 (89): 89–94. DOI: 10.32718/nvlvet8917.
7. Dvylyuk II, Kovalchuk II. Reproductive ability of bee queens at the conditions of feeding citrates of Argentum and Cuprum. *Biol. Tvarin*. 2017; 19 (2): 30–36. DOI: 10.15407/animbiol19.02.030.
8. Falalyeyeva TM, Leschenko IV, Beregova TV, Lazarenko LM, Savchuk OM, Sichel LM, Tsyryuk OI, Vovk TB, Spivak MY. Probiotic strains of lactobacilli and bifidobacteria alter pro- and anti-inflammatory cytokines production in rats with monosodium glutamate-induced obesity. *Fiziol. Zh*. 2017; 63 (1): 17–25. DOI: 10.15407/fz63.01.017.
9. Fedoruk RS, Kovalchuk II, Mezentseva LM, Tesarivska UI, Pylypets AZ, Kaplunenko VH. Germanium compounds and their role in animal body. *Biol. Tvarin*. 2022; 24 (1): 50–60. DOI: 10.15407/animbiol24.01.050. (in Ukrainian)
10. Fedoruk RS, Kovalchuk II, Romaniv LI, Xrabko MI. Influence of germanium citrate and selenium on lipid and heavy metals content in melliferous bees' organism. *Biol. Tvarin*. 2014; 16 (2): 141–149. Available at: http://aminbiol.com.ua/index.php/archive/100-archive/bt-16-2-2014/1487 (in Ukrainian)
11. Fedoruk RS, Tesarivska UI, Kovalchuk II, Tsap MM, Kaplunenko VH, Koleschuk OI, Khrabko MI. Biological effects of iodine, selenium, sulfur citrates in broiler chickens. *Reg. Mech. Biosys*. 2021; 12 (3): 523−530. DOI: 10.15421/022172.
12. Feldlaufer MF, Harrison DJ. Neutral sterols in honey bee (*Apis mellifera*) feces. *J. Apicult. Res.* 2020; 59 (5): 1033–1036. DOI: 10.1080/00218839.2020.1753917.
13. Folch J, Lees M, Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 1957; 226 (1): 497–509. DOI: 10.1016/S0021-9258(18)64849-5.
14. Frias BED, Barbosa CD, Lourenço AP. Pollen nutrition in honey bees (*Apis mellifera*): impact on adult health. *Apidologie*. 2016; 47 (1): 15–25. DOI: 10.1007/s13592-015-0373-y.
15. Galiniak S, Mołoń M, Biesiadecki M, Bożek A, Rachel M. The role of oxidative stress in atopic dermatitis and chronic urticaria. *Antioxidants*. 2022; 11 (8): 1590. DOI: 10.3390/antiox11081590.
16. Hartfelder K, Bitondi MMG, Brent CS, Guidugli-Lazzarini KR, Simões ZLP, Stabentheiner A, Tanaka ÉD, Wang Y. Standard methods for physiology and biochemistry research in *Apis mellifera*. *J. Apicult. Res*. 2013; 52 (1): 1−48. DOI: 10.3896/IBRA.1.52.1.06.
17. Karavan VV, Kachmaryk DY, Cherevatov VF, Yazlovytska LS. Influence of wintering temperature on the state of the antioxidative system in *Apis mellifera* L. *Biol. Tvarin*. 2021; 23 (4): 32–42. DOI: 10.15407/animbiol23.04.032. (in Ukrainian)
18. Kosinov MV, Kaplunenko VH. Method of obtaining metal carboxylates "Nanotechnology of obtaining metal carboxylates". Patent UA no 38391. from 12.01.2009. Available at: https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=128062 (in Ukrainian)
19. Kovalchuk II, Fedoruk RS. Content of heavy metals in the bees tissues and products depending on agroecological conditions of the Carpathians region. *Biol. Tvarin*. 2013; 15 (4): 54–65. Available at: http://aminbiol.com.ua/index.php/archive/94-archive/bt4-15-2013/1536 (in Ukrainian)
20. Kovalchuk II, Fedoruk RS, Rivis YF, Romaniv LI. Lipid and fatty acid composition of body tissues of honey bees and pollen under conditions of supplementation with germanium and selenium nanoaquacitrates. *Sci. Tech. Bull. SRCIVMFA IAB*. 2014; 15 (2−3): 31−36. Available at: http://nbuv.gov.ua/UJRN/Ntbibt_2014_15_2-3_7 (in Ukrainian)
21. Kovalchuk II, Fedoruk RS, Spivak MY, Romanovych MM, Iskra RY. *Lactobacillus casei* IMV B-7280 immunobiotic strain influence on the viability of honey bees and the content of microelements in the organism. *Mikrobiol. Z.* 2021; 83 (2): 42–50. DOI: 10.15407/microbiolj83.02.042.
22. Kovalchuk II, Kykish IB, Kaplunenko VH. Influence of citrate microelements on the reproductive capacity of queen bees. In: *Actual problems of natural sciences: modern scientific discussions*. A collective monograph. Riga, Latvia, Baltija Publ., 2020; 87–110. DOI: 10.30525/978-9934-26-025-4-6.
23. Lazarenko LM, Babenko LP, Gichka SG, Sakhno LO, Demchenko OM, Bubnov RV, Sichel LM, Spivak MY. Assessment of the safety of *Lactobacillus casei* IMV B-7280 probiotic strain on a mouse model. *Probiot. Antimicr. Prot.* 2021; 13 (6): 1644–1657. DOI: 10.1007/s12602-021-09789-1.
24. Lazarenko LM, Babenko LP, Mokrozub VV, Demchenko OM, Bila VV, Spivak MY. Effects of oral and vaginal administration of probiotic bacteria on the vaginal microbiota and cytokines production in the case of experimental staphylococcosis in mice. *Mikrobiol Z.* 2017; 79 (6): 105–119. DOI: 10.15407/microbiolj79.06.105.

25. Li Z, Hou M, Qiu Y, Zhao B, Nie H, Su S. Changes in antioxidant enzymes activity and metabolomic profiles in the guts of honey bee (*Apis mellifera*) larvae infected with *Ascosphaera apis*. *Insects*. 2020; 11 (7): 419. DOI: 10.3390/insects11070419.

26. Lu CY, Huang PJ, Hsu CY. The cholesterol-hydroxyecdysone-vitellogenin pathway is involved in the longevity of trophocytes and oenocytes of queen honey bees (*Apis mellifera*). *Apidologie*. 2018; 49: 721–733. DOI: 10.1007/s13592-018-0596-9.

27. Mucci CA, Ramirez L, Giffoni RS, Lamattina L. Cold stress induces specific antioxidant responses in honey bee brood. *Apidologie*. 2021; 52: 596–607. DOI: 10.1007/s13592-021-00846-w.

28. Mylostiva D. Influence of germanium citrate on the defensive anti-oxidative system of rats organism. *Sci. Tech. Bull. SRCIVMFA IAB*. 2017; 18 (2): 34–37. Available at: http://nbuv.gov.ua/UJRN/Ntbibt_2017_18_2_7 (in Ukrainian)

29. Neov B, Georgieva A, Shumkova R, Radoslavov G, Hristov P. Biotic and abiotic factors associated with colonies mortalities of managed honey bee (*Apis mellifera*). *Diversity*. 2019; 11 (12): 237. DOI: 10.3390/d11120237.

30. Prezenská M, Sobeková A, Sabová L. Antioxidant enzymes of honeybee larvae exposed to oxamyl. *Folia Vet*. 2019; 63 (4): 9–14. DOI: 10.2478/fv-2019-0032.

31. Romaniv LI, Kovalchuk II, Pashchenko AG, Fedoruk RS. Content of lipids in tissues of the melliferous bees additionally fed with soybean meal, sugar syrup and citrates of Co and Ni. *Biol. Tvarin*. 2018; 20 (3): 84–92. DOI: 10.15407/animbiol20.03.084. (in Ukrainian)

32. Tauber JP, Collins WR, Schwarz RS, Chen Y, Grubbs K, Huang Q, Lopez D, Peterson R, Evans JD. Natural product medicines for honey bees: Perspective and protocols. *Insects*. 2019; 10 (10): 356. DOI: 10.3390/insects10100356.

33. Tawfik AI, Ahmed ZH, Abdel-Rahman MF, Moustafa AM. Effect of some bee bread quality on protein content and antioxidant system of honeybee workers. *Int. J. Trop. Insect. Sci.* 2022; 43: 93–105. DOI: 10.1007/s42690-022-00888-2.

34. Tezuka T, Higashino A, Akiba M, Nakamura T. Organogermanium (Ge-132) suppresses activities of stress enzymes responsible for active oxygen species in monkey liver preparation. *Adv. Enz. Res*. 2017; 5 (2): 13–23. DOI: 10.4236/aer.2017.52002.

35. Vlizlo VV. (ed.). *Laboratory Methods in Biology, Stockbreeding and Veterinary Medicine*. Lviv, Spolom. 2012: 764 p. (in Ukrainian)

36. Yefimenko T, Odnosum H, Vorobiy O. Flow of sacbrood disease in creation of infertile period at bee colonies in comparison with bee colonies treatment with eucalyptus and hypericum extracts and analogue means. *Beekeep. Ukr*. 2021; 1 (6): 18–23. DOI: 10.46913/beekeepingjournal.2021.6.03.

Ліпідний склад та продукти перекисного окиснення тканин організму бджіл за впливу різних доз нанотехнологічного цитрату Ge та пробіотика *Lactobacillus casei* B-7280

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Застосування сучасних засобів збереження медоносних бджіл спрямоване на підвищення їх життєздатності, рівня живлення та продуктивності у різні періоди розвитку. Відзначено тенденцію до використання нових ефективних засобів натурального походження, механізми дії яких відрізняються від синтетичних речовин і препаратів за рахунок активації захисних реакцій організму на фізіологічному рівні. Пробіотик *Lactobacillus casei* IMV B-7280 має ефективну стимулювальну дію на фізіологічні процеси за різних екологічних та експериментальних умов життєдіяльності бджіл. Фізіологічний вплив цього пробіотика пов'язаний з нормалізацією кишкової бактеріальної мікрофлори та участю в модуляції захисних реакцій організму. Опірність медоносних бджіл також залежить від мінерального живлення, що впливає на обмінні процеси на рівні тканин, органів і систем та визначає життєздатність і резистентність організму. Мінеральні елементи беруть участь у білковому, ліпідному, вуглеводному та енергетичному обмінах, активують ферментні системи. Тому метою досліджень було визначення впливу пробіотичного препарату класу *L. casei* B-7280 у поєднанні з різними дозами нанотехнологічного цитрату (НТЦ) Ge на ліпідний склад та вміст продуктів перекисного окиснення у тканинах організму бджіл. Бджоли контрольної групи отримували підгодівлю з 60% цукрового сиропу (ЦС) в кількості 1 мл/групу/добу. Перша дослідна група (Е1) бджіл додатково до 1 мл цукрового сиропу отримувала 0,1 мкг Ge у вигляді нанотехнологічного цитрату та розчин пробіотика *L. casei* B-7280 у концентрації 10^6 КУО/мл; друга дослідна (Е2) — 0,2 мкг Ge у вигляді цитрату та пробіотик *L. casei* B-7280 у концентрації 10^6 КУО/мл. Тривалість випоювання цукрового сиропу, Ge цитрату та пробіотика — 34 дні. Встановлено, що підгодівля бджіл цукровим сиропом, *L. casei* 10^6 КУО/мл та різними дозами Ge цитрату характеризувалась вищим відносним вмістом фосфоліпідів, моно- і диацилгліцеролів в Е2 групі та етерифікованого холестеролу — в Е1, але зниженням вільного холестеролу, неетерифікованих жирних кислот стосовно контрольної групи і дослідного періоду. Ці результати вказують на дозозалежний вплив нанотехнологічного цитрату Ge на обмін ліпідів в гомогенатах тканин організму. Відзначено підвищення рівня триацилгліцеролів у ліпідах тканин бджіл всіх груп у дослідний період. Біологічний вплив нанотехнологічного цитрату Ge і *L. casei* зумовлював у тканинах організму бджіл дослідних груп зниження рівня процесів пероксидації ліпідів за дії вищої дози цитрату Ge.

Ключові слова: бджоли**,** Ge цитрат, пробіотик, ліпіди, продукти перекисного окиснення

Pylypets AZ, Spivak MY, Fedoruk RS, Tsap MM, Kovalchuk II, Romanovych MM. Lipid composition and peroxidation products in the body tissues in bees under the action of different doses of nanotechnological Ge citrate and the probiotic *Lactobacillus casei* B-7280. *Biol. Tvarin*. 2023; 25 (1): 20–26. DOI: 10.15407/animbiol25.01.020.