Bulgarian Journal of Agricultural Science, 29 (No 1) 2023, 131–137

The use of probiotics in spring supplementary feeding of bee colonies

Galina Mishukovskaya[1*], Marat Giniyatullin[2], Dmitry Shelekhov[2], Airat Khabirov[1], Elena Smolnikova[1] and Aygul Naurazbaeva[1]

[1] *Bashkir State Agrarian University, Department of Animal Physiology, Biochemistry and Feeding, Federal State Budgetary Educational Institution of Higher Education, 450001 Ufa, Russian Federation*
[2] *Bashkir State Agrarian University, Department of Beekeeping, Private Animal Husbandry and Animal Breeding, Federal State Budgetary Educational Institution of Higher Education, 450001 Ufa, Russian Federation*
[*]*Corresponding author:* mishukovskaya636@rambler.ru

Abstract

Mishukovskaya, G., Giniyatullin, M., Shelekhov, D., Khabirov, A., Smolnikova, E. & Naurazbaeva, A. (2023). The use of probiotics in spring supplementary feeding of bee colonies. *Bulg. J. Agric. Sci., 29 (1)*, 131–137

Long wintering in countries with temperate and cold climates significantly undermines bee colonies, making them more susceptible to various diseases. The use of beneficial bacteria in spring supplementary feeding can rapidly recover bee colonies after wintering and increase their productivity during the honey harvest period. The paper presents the results of a comparative study of the effect of probiotic feed additives created based on different groups of microorganisms on the bee colonies spring development. The PcheloNormosil feed additive consists of lactic acid bacteria and saccharomycetes. The SpasiPchel additive includes three strains of the *Bacillus subtilis* bacterium. The subject of the study were bee colonies of the dark forest bee *Apis mellifera mellifera* L. The bees were kept in an apiary located in the northern forest-steppe zone of the Republic of Bashkortostan (Russian Federation). The research revealed a beneficial effect of probiotics on bee colonies wellbeing. Adding them to sugar syrup when feeding bees stimulates the oviposition of queens, increases the sealed brood amount and strengthens the colony when it prepares for the main honey harvest from small-leaved linden. Colonies receiving lactobacilli and saccharomycetes showed significantly higher productivity exceeding the control values. Feeding based on *Bacillus subtilis* did not significantly impact the bee colonies honey productivity. Optimization of dosage and selection of strains of beneficial bacteria for use in beekeeping need further research.

Keywords: Apis mellifera mellifera; feed additives; probiotics; bee colonies strength; sealed brood

Introduction

In a temperate continental climate, the life of a bee colony during the year consists of two periods: active state and winter rest. The first period coincides with the honey plants flowering. There is an active colony growth and development in this period, nest construction, and food accumulation. During this period, the care of colonies needs special attention since the collection of nectar by bees during the main honey harvest and the effectiveness of pollination of entomophilic crops largely depend on the colony growth and development in spring. Beekeepers feed bees with sugar syrup to replenish feed reserve in early spring when the honey harvest is low. They often enrich the syrup with biologically active substances to stimulate the bee colonies development. In this regard, the use of feed additives based on probiotics is of interest since they contain beneficial bacteria, components of the intestinal flora of bees.

The digestive tract of honey bees is inhabited by a microbial community with quite a stable composition (Guo et al., 2015; Romero et al., 2019). Morphofunctional indicators of the bee organism such as body weight, hormonal status, behaviour de-

pend on the intestinal microbiome state (Zheng et al., 2017). When isolating enzymes that break down macromolecules of polysaccharides and polypeptides, intestinal bacteria participate in the feed digestion and affect the honey bees metabolism (Zheng et al., 2017; Lee et al., 2015; Zheng et al., 2016). The bees immune status is also primarily determined by the intestinal microflora (Emery et al., 2017; Kwong and Moran, 2016; Schwarz et al., 2016). A microbiota composition disorder increases bees mortality and slowdowns the development of the whole colony (Maes et al., 2016; Raymann & Moran, 2018). There are many factors, which can cause disorders. They are lack or poor quality of feed (Maes et al., 2016), the use of antibiotics in beekeeping (Raymann et al., 2017; Tian et al., 2012) and pesticides in crop production (Kakumanu et al., 2016). The use of beneficial bacteria inhabiting the intestines of healthy bees has been proposed to minimize the effects of adverse factors and restore the normal microflora balance. The main purpose of their use is to strengthen the bees' immunity. The research revealed the cytotoxic effect of *Lactobacillus* spp. on the causative agent of the American foulbrood *Paenibacillus larvae*. Probiotics proved to increase the gene expression of antimicrobial peptides that play a key role in protecting bees from foulbrood infection (Daisley et al., 2020). There was a decrease in the number of *Nosema Apis* spores in the bee's intestines under the action of probiotics (Arredondo et al., 2020; Borges et al., 2021; Ptaszyńska et al., 2016). Some *Bacillus subtilis* strains with anti fungicidal activity are recommended for use to prevent or treat bee ascospherosis (Omar et al., 2014).

One of the reasons for the mass bee colonies death in recent years has been the treatment of agricultural honey plants with pesticides. Pesticides harm the bees' physiology and behaviour. Moreover, they influence the insects' intestinal microbiota (Cuesta-Maté et al., 2021) negatively. In their research, Daisley et al. (2017) and Chmiel et al. (2020) prove that probiotics increase the bees' resistance to pesticide intoxication and extend their lifespan when exposed to neonicotinoids (Emery et al., 2017).

Probiotics do not significantly affect the bees' intestinal microbiota composition, and their effect is probably associated with the stimulation of the immune response. However, this fact needs further studies to be confirmed (Alberoni et al., 2021).

Much research deals with the effect of probiotics on the bees' resistance to various diseases. However, few studies deal with their influence on the productive indicators of bee colonies. Besides, these studies were mainly conducted in countries with hot climates (Alberoni et al., 2018; Fanciotti et al., 2018; Sabaté et al., 2012). The Republic of Bashkortostan is located in the Southern Urals region, where forests have rich honey resources (Sultanova et al., 2019; Ermakov et al., 2021; Safonov, 2022). However, bee colonies have to stay in closed winter hives for 5-6 months since the climatic features of the region are long and cold winters. Low temperatures, a feed shortage, and the inability to empty the intestines lead to a significant weakening of bee colonies, making them more susceptible to various diseases. Beneficial bacteria can contribute to the speedy recovery of bee colonies after wintering and increase their productivity during the honey harvest period.

The research aims to make a comparative study of the effect of probiotic feed additives created based on different groups of microorganisms, lactobacilli and bacteria of the genus *Bacillus*, on the processes of spring development of bee colonies.

Material and Methods

Bee colonies

The research used *Apis mellifera mellifera* honey bee colonies kept at the educational and experimental apiary of the Bashkir State Agrarian University. The bee garden is in the northern forest-steppe zone of the Republic of Bashkortostan (Russian Federation). The main honey plant in the bee garden neighbourhood is small-leaved linden, and the additional ones are willow, viburnum, rowan, and other trees. Bee colonies were kept in 12-frame Dadan-Blatt hives in equal care, feeding, and honey collection conditions.

For obtaining even-aged bees, one frame with sealed brood from each colony of the control and experimental groups was placed in a thermostat and kept at a temperature of 35°C and humidity of 60%. Bees that left the cell (n = 100) were labelled and returned to the colony.

Supplementary feeding of bees

To study the effect of probiotics on bees spring development, 3 groups of bee colonies were formed. Each group consisted of 10 colonies. The control group received pure sugar syrup (50%) as feed. Experimental group 1 received sugar syrup with PcheloNormosil probiotic, containing lactic acid bacteria and saccharomycetes. Experimental group 2 received sugar syrup with SpasyPchel feed additive created based on three strains of *Bacillus subtilis* bacterium. Per 1 litre of sugar syrup, 4 ml of one of the two probiotics were added. After careful mixing, bees received carbohydrate feed poured into side feeders on the same day in the evening. Supplementary feeding was carried out three times with an interval of 3 days. Each bee colony received 0.5 litres of syrup.

Determination of the degree of the development of the pharyngeal glands

Six 9-day-old bees (nurse bees) were selected from the colonies to determine the degree of development of the pharyn-

geal glands. The bees were frozen and taken to the laboratory. After defrosting, the bees were decapitated. The pharyngeal glands were extracted, placed on a glass slide in a drop of 0.25 M sodium chloride solution and examined under a microscope at 100x magnification. The diameter of the acini with clear boundaries was measured using an eyepiece-micrometre.

Assessment of the bee colonies wellbeing

To study the effect of probiotics on the spring development of bee colonies, their wellbeing was assessed 5 times with an interval of 12 days, starting from the supplementary feeding finish date (April 27) to mid-June. When assessing the bee colonies wellbeing, the amount of sealed brood, the colony strength, the foraging bees flight activity and the productivity of bee colonies of the control and experimental groups at the end of the season were taken into account.

The amount of sealed brood in bee colonies was measured using a standard 435x300 mm grid divided into squares of 5x5 cm. Each square holds 100 bee cells. The grid was applied on both sides of the frames with the brood. Then, the total number of cells occupied by the sealed brood was calculated. The number of frames occupied by bees on both sides determined the bee colony strength.

The bees flight activity was determined on May 18-20 by counting bees returning to the hive for 3 min (three-fold repetition) at different times – at 9, 13 and 19 o'clock. A Sony FDR-AH100E digital video camera was used to count bees. During the research, the weather was calm, cloudy; the air temperature was 20-22°C. The counting lasted three days, and then the average value of the bees' flight activity was calculated.

The colony honey productivity was determined by weighing the frames with honey and deducting the frame's mass with an empty honeycomb. The number of built-up honeycombs determined wax productivity.

Statistical analysis

Statistical data processing involved accepted variational statistics methods using Microsoft Excel 2019 software. The reliability of the difference in the arithmetic mean was evaluated using the Student's t-test; the differences were considered statistically significant at $p < 0.05$.

Results

At the beginning of the experiment in April 2019, bee colonies were still weak after wintering. Brood appeared by that time, but its amount was still small – 74-75 hundred cells per colony (Figure 1).

The flowering of early honey plants provides bee colonies with protein feed, i.e. pollen, and stimulates the process-

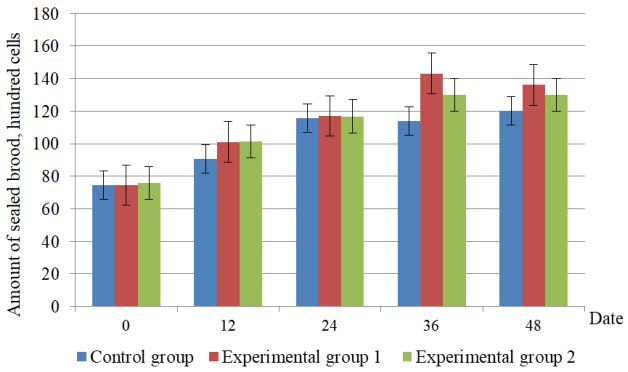

Fig. 1. Dynamics of the sealed brood amount in bee colonies during the observation period with different feeding options: sugar syrup (SS) in the control group; SS + PcheloNormosil in experimental group 1; SS + SpasiPchel in experimental group 2

es of the queen bee oviposition. On May 8, the amount of sealed brood increased by 22% in the control group and 33% in the experimental group. By Jun 3, there is a peak in the queen's egg production. The same indicator in experimental colonies is maximum. In the first experimental group, this indicator exceeded the control value by 25% ($p < 0.05$). In the second group, the exceedance made 14%.

In the second half of June, colonies prepare for gathering honey from small-leaved linden. In this period, the egg production of queens decreases slightly, decreasing the amount of sealed brood in bee colonies. This indicator still increases in the control group, and however, it does not reach the level of the experimental groups.

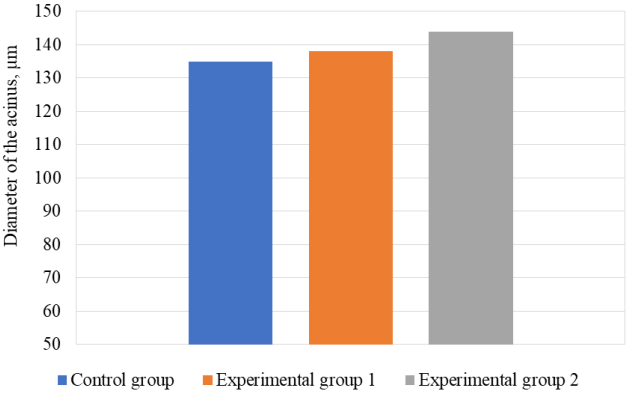

Fig. 2. Diameter of the acinus of the hypopharyngeal glands of 9-day-old bees receiving different supplementary feeding: sugar syrup (SS) in the control group; SS + PcheloNormosil in experimental group 1; SS + SpasiPchel in experimental group 2

Bees use royal jelly to feed the brood, which is secreted by the hypopharyngeal glands. Bees hypopharyngeal glands reach maximum development at the age of 9-12 days. The study of the supplementary feeding effect on the morphofunctional parameters of bees did not reveal any significant differences in the diameter of the acini of the hypopharyngeal glands of nurse bees (aged nine days) of different groups (Figure 2).

At the beginning of the experiment, the strength of bee colonies of different groups was at the level of 5.7 frames; the amount of feed averaged 5.4 kg. By the next accounting date, 12 days after the start of feeding, the strength of bee colonies in the control group increased by 19.3% (Table 1). The increase in the 1st and 2nd experimental groups made 32.1% and 24.6%, respectively. On the following dates, despite the cool and rainy weather, there was a significant increase in the strength of bee colonies of the control and experimental groups.

By the end of the observations on June 16, the indicator increased by 219.3% in the control group and 262.5% and 240.4% in the experimental groups 1 and 2, respectively, compared to the beginning of the experiment. The maximum strength of bee colonies on that date was revealed in the 1st experimental group making 14.7 frames.

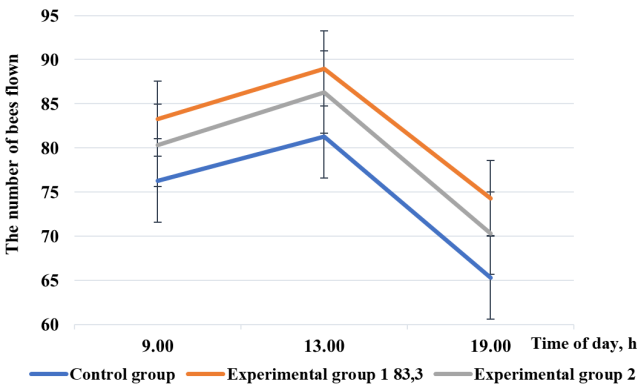

Fig. 3. Flight activity of bees receiving different supplementary feeding: sugar syrup (SS) in the control group; SS + PcheloNormosil in experimental group 1; SS + SpasiPchel in experimental group 2

The difference with the control was reliable ($p < 0.05$) and made 17.6%. The amount of feed on that date in the 1st experimental group exceeded the control by 15.3%. The exceedance of 10.2% was revealed in the 2nd experimental group.

Table 1. Dynamics of the bee colonies strength and the amount of feed according to different supplementary feeding options: sugar syrup (SS) in the control group; SS + PcheloNormosil in experimental group 1; SS + SpasiPchel in experimental group 2

Group of bee colonies	Colony strength, frames			Feed amount, kg		
	Lim	M ± m	% compared to the control group	Lim	M ± m	% compared to the control group
Apr 27						
Control group	5-7	5.7±0.2	100	2.4-9.4	5.32±0.9	100
Experimental group 1	4-8	5.6±0.4	98.2	2.4-8.4	5.45±0.7	102.4
Experimental group 2	4-8	5.7±0.4	100	2.3-10.0	5.40±0.9	101.5
May 8						
Control group	5-8	6.8±0.6	100	3.9-9.2	6.5±0.4	100
Experimental group 1	6-9	7.4±0.3	108.8	3.6-10.4	7.1±0.7	109.2
Experimental group 2	6-9	7.1±0.3	110.3	3.3-10.1	6.7±0.7	103.1
May 21						
Control group	8-12	9.9±0.7	100	3.1-13.3	7.3±1.0	100
Experimental group 1	10-13	10.7±0.3	108.1	2.6-11.5	7.3±0.8	100
Experimental group 2	9-12	10.2±0.3	103.0	2.4-12.0	7.2±0.8	98.6
Jun 3						
Control group	10-14	11.8±0.66	100	2.8-10.5	5.4±0.7	100
Experimental group 1	11-15	13.2±0.52	111.8	2.4-11.1	6.4±0.8	118.5
Experimental group 2	11-14	12.5±0.41	105.9	4.2-9.9	6.5±0.5	120.4
Jun 16						
Control group	10-15	12.5±0.87	100	3.3-9.0	5.9±0.7	100
Experimental group 1	10-16	14.7±0.47	117.6	3.9-11.1	6.8±0.8	115.3
Experimental group 2	11-15	13.7±0.40	101.6	2.4-10.1	6.6±0.8	110.2

Table 2. Bee colonies productivity when receiving different supplementary feeding: sugar syrup (SS) in the control group; SS + PcheloNormosil in experimental group 1; SS + SpasiPchel in experimental group 2

Value	Statistical value	Group of bee colonies		
		control group	experimental group 1	experimental group 2
Honey productivity, kg	Lim	37.0-54.8	41.5-63.5	40.5-60.0
	M ± m	46.6±2.6	54.1±1.9	48.1±2.3
	% compared to the control group	100	116.1	103.2
Wax productivity, g	Lim	732-956	652-1060	708-980
	M ± m	823.2±92.7	863.5±68.4	848.0±43.8
	% compared to the control group	100	104.9	103.0

The number of worker bees in a bee colony and their functional activity determine the colonies productivity. In the morning, the number of bees descended on the hive ranged from 76 to 83 over three days of observations (Figure 3).

At noon the number of bees increased by 6.5-7.0%. In the evening, the bees flight activity decreased again. There were no significant differences in the flight activity of foraging worker bees in the experimental and control groups.

The evaluation of honey productivity of bee colonies proves the influence of the feed composition and feed additives on this indicator. The colonies of the control group collected 46.6 kg of honey during the season (Table 2).

Compared to the control, the reliable increase in honey productivity ($p < 0.05$) was revealed in experimental group 1. In this group, bees received probiotic PcheloNormosil, which contains lactic acid bacteria and saccharomycetes. Each colony of this group gave 7.5 kg more honey on average than in the control group. SpasiPchel feed additive created based on three strains of *Bacillus subtilis* increased the bee colonies honey productivity by 1.5 kg compared to the control; the difference between the indicators is unreliable. Wax productivity in the 1st and 2nd experimental groups exceeded the control by 4.9% and 3.0%, respectively.

Discussion

In the spring, after long wintering, bee intestines are overfull, creating favourable conditions for developing pathogenic and conditionally pathogenic microflora. Thus, colonies are especially susceptible to various infectious and invasive diseases after leaving the winter hives. Weak colonies develop and increase their strength slowly in the spring. Therefore, probiotics as spring supplementary feeding are essential since they accelerate intestinal microbiocenosis. Beneficial bacteria have an antagonistic effect against pathogenic microflora and increase bees' resistance to diseases. Besides, they stimulate bees' bodies, producing biologically active substances.

The research provides a comparative assessment of two types of feed additives based on PcheloNormosil and SpasiPchel probiotics. PcheloNormosil includes lactic acid bacteria and saccharomycetes. SpasiPchel is created based on three strains of the bacterium *Bacillus subtilis*. PcheloNormosil and SpasiPchel as the spring supplementary feeding of bee colonies affected the colony reproduction rate. They increased the amount of sealed brood at the peak of egg production of queens by 25% and 14%, respectively. Probiotics sped the development of bee colonies and increased their strength by 19% and 10% before the main honey harvest. These data are consistent with the studies of Alberoni et al. (2018), who revealed a more significant stimulating effect of beneficial bacteria. Audisio (2017), Fanciotti et al. (2018) also reported the positive effect of probiotics on the development of bee colonies. Besides, according to Audisio (2017), probiotic supplementary feeding decreases the manifestations of bees' two major diseases – nosematosis and varroatosis.

One of the indicators of the physiological status of worker bees is the degree of development of pharyngeal glands of nurse bees. Some data prove that an increase in the size of pharyngeal glands is not always an indicator of good bee health (Maes et al., 2016). However, Tlak Gajger et al. (2020) prove a stimulating effect of probiotics on the development of hypopharyngeal glands and consider it a positive reaction of the bees' body to an increase in the concentration of beneficial microorganisms in the feed. This research did not reveal the effect of probiotics on this indicator. Differences in the diameter of the acini of the hypopharyngeal glands of nurse bees in the experimental and control groups were unreliable. Differences in the flight activity of foraging bees are also unreliable.

Horton et al. (2015) did not reveal the direct correlation between colony productivity and the composition of the bees intestinal microflora. However, some authors reported a significant increase in honey production in colonies receiving probiotics (Alberoni et al., 2018; Fanciotti et al., 2018;

Sabaté et al., 2012). In this research, the improvement in the reproductive qualities of bee colonies and the strength increase before the main honey harvest affected the honey productivity indicator. Moreover, the effect depended on the composition of feed additives. Thus, bee colonies receiving lactic acid bacteria and saccharomycetes with supplementary feeding gave 16% more honey than colonies receiving pure sugar syrup. The productivity increase was only 3% when using *Bacillus subtilis* bacteria. However, Audisio (2017), Sabaté et al. (2012) note a positive effect of supplementary feeding based on this particular type of bacteria on the economic traits of bee colonies. Perhaps additional studies to increase the effect of *Bacillus subtilis* and optimize the dosages of the feed additive are necessary.

Conclusions

The research results prove the beneficial effect of probiotics on the processes of spring development of bee colonies. Probiotics added to sugar syrup stimulate the oviposition of queens, which increases the amount of brood and strengthen the colony when preparing for the main honey harvest. Finally, probiotics increase the productivity of bee colonies, which is the primary indicator of bee garden work. Optimization of dosage and selection of strains of beneficial bacteria for use in beekeeping need further research.

References

Alberoni, D., Baffoni, L., Braglia, C., Gaggìa, F. & Di Gioia, D. (2021). Honeybees exposure to natural feed additives: how is the gut microbiota affected? *Microorganisms, 9(5),* 1009. https://doi.org/10.3390/microorganisms9051009

Alberoni, D., Baffoni, L., Gaggìa, F., Ryan, P. M., Murphy, K., Ross, P. R., Stanton, C. & Di Gioia, D. (2018). Impact of beneficial bacteria supplementation on the gut microbiota, colony development and productivity of *Apis mellifera* L. *Beneficial Microbes, 9(2),* 269–278. https://doi.org/10.3920/BM2017.0061

Arredondo, D., Castelli, L., Porrini, M. P., Garrido, P. M., Eguaras, M. J., Zunino, P. & Antúnez, K. (2017). *Lactobacillus kunkeei* strains decreased the infection by honey bee pathogens *Paenibacillus larvae* and *Nosema ceranae*. *Beneficial Microbes, 9(2),* 279–290. https://doi.org/10.3920/BM2017.0075

Audisio, M. C. (2017). Gram-positive bacteria with probiotic potential for the *Apis mellifera* L. honey bee: the experience in the northwest of Argentina. *Probiotics and Antimicrobial Proteins, 9(1),* 22-31. https://doi.org/10.1007/s12602-016-9231-0

Borges, D., Guzman-Novoa, E. & Goodwin, P. H. (2021). Effects of prebiotics and probiotics on honey bees (*Apis mellifera*) infected with the microsporidian parasite *Nosema ceranae*. *Microorganisms, 9(3),* 481. https://doi.org/10.3390/microorganisms9030481

Chmiel, J. A., Daisley, B. A., Pitek, A. P., Thompson, G. J. & Reid, G. (2020). Understanding the effects of sublethal pesticide exposure on honey bees: a role for probiotics as mediators of environmental stress. *Frontiers in Ecology and Evolution, 8,* 22. https://doi.org/10.3389/fevo.2020.00022

Cuesta-Maté, A., Renelies-Hamilton, J., Kryger, P., Jensen, A. B., Sinotte, V. M. & Poulsen, M. (2021). Resistance and Vulnerability of Honeybee (*Apis mellifera*) Gut Bacteria to Commonly Used Pesticides. *Frontiers in Microbiology, 12,* 717990. https://www.frontiersin.org/article/10.3389/fmicb.2021.717990

Daisley, B. A., Pitek, A. P., Chmiel, J. A., Al, K. F., Chernyshova, A. M., Faragalla, K. M., Burton, J. P., Thompson, G. J. & Reid, G. (2020). Novel probiotic approach to counter *Paenibacillus larvae* infection in honey bees. *The ISME Journal, 14(2),* 476-491. https://doi.org/10.1038/s41396-019-0541-6

Daisley, B. A., Trinder, M., McDowell, T. W., Welle, H., Dube, J. S., Ali, S. N., Leong, H. S., Sumarah, M. W. & Reid, G. (2017). Neonicotinoid-induced pathogen susceptibility is mitigated by *Lactobacillus plantarum* immune stimulation in a Drosophila melanogaster model. *Scientific Reports, 7,* 2703. https://doi.org/10.1038/s41598-017-02806-w

Emery, O., Schmidt, K. & Engel, P. (2017). Immune system stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*). *Molecular Ecology, 26(9),* 2576–2590. https://doi.org/10.1111/mec.14058

Ermakov, V., Safonov, V. & Dogadkin, D. (2021). Characteristic features of molybdenum, copper, tungsten and rhenium accumulation in the environment. *Innovative Infrastructure Solutions*, *6*(2), 104. https://doi.org/10.1007/s41062-021-00481-5

Guo, J., Wu, J., Chen, Y., Evans, J. D., Dai, R., Luo, W. & Li, J. (2015). Characterization of gut bacteria at different developmental stages of Asian honey bees, Apis cerana. *Journal of Invertebrate Pathology, 127,* 110-114. https://doi.org/10.1016/j.jip.2015.03.010

Horton, M. A., Oliver, R. & Newton, I. L. (2015). No apparent correlation between honey bee forager gut microbiota and honey production. *Peer J., 3,* e1329. https://doi.org/10.7717/peerj.1329

Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R. & Williams, M. A. (2016). Honey bee gut microbiome is altered by in-hive pesticide exposures. *Frontiers in Microbiology, 7,* 1255. https://doi.org/10.3389/fmicb.2016.01255

Kwong, W. K. & Moran, N. A. (2016). Gut microbial communities of social bees. *Nature Reviews Microbiology, 14(6),* 374-384. https://doi.org/10.1038/nrmicro.2016.43

Lee, F. J., Rusch, D. B., Stewar, F. J., Mattila, H. R. & Newton, I. L. G. (2015). Saccharide breakdown and ferment at ion by the honey bee gut microbiome. *Environmental Microbiology, 17(3),* 796-815. https://doi.org/10.1111/1462-2920.12526

Maes, P. W., Rodrigues, P. A. P., Oliver, R., Mott, B. M. & Anderson, K. E. (2016). Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and Nosema disease in the honeybee (*Apis mellifera*). *Molecular Ecology, 25(21),* 5439-5450. https://doi.org/10.1111/mec.13862

Novicov Fanciotti, M., Tejerina, M., Benítez-Ahrendts, M. R. & Audisio, M. C. (2018). Honey yield of different commercial apiaries treated with *Lactobacillus salivarius* A3iob, a new bee-probiotic strain. *Beneficial Microbes, 9(2),* 291–298. https://doi.org/10.3920/BM2017.0089

Omar, M. O. M., Moustafa, A. M., Ansari, M. J., Anwar, A. M., Fahmy, B. F., Al-Ghamdi A. & Nuru, A. (2014). Antagonistic effect of gut bacteria in the hybrid carniolan honey bee, *Apis Mellifera Carnica*, against *Ascosphaera Apis*, the causal organism of chalkbrood disease. *Journal of Apicultural Science, 58(1),* 17-27. https://doi.org/10.2478/jas-2014-0002

Ptaszyńska, A. A., Borsuk, G., Mułenko, W. & Wilk, J. (2016). Impact of vertebrate probiotics on honeybee yeast microbiota and on the course of nosemosis. *Veterinary Medicine, 72(7),* 430-434.

Raymann, K. & Moran, N. A. (2018). The role of the gut microbiome in health and disease of adult honey bee workers. *Current Opinion in Insect Science, 26,* 97-104. https://doi.org/10.1016/j.cois.2018.02.012

Raymann, K., Shaffer, Z. & Moran, N. A. (2017). Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biology, 15*(3), e2001861. https://doi.org/10.1371/journal.pbio.2001861

Romero, S., Nastasa, A., Chapman, A., Kwong, W. K. & Foster, L. J. (2019). The honey bee gut microbiota: strategies for study and characterization. *Insect Molecular Biology, 28(4),* 455-472 https://doi.org/10.1111/imb.12567

Sabaté, D. C., Cruz, M. S., Benítez-Ahrendts, M. R. & Audisio, M. C. (2012). Beneficial effects of *Bacillus subtilis* subsp. subtilis Mori2, a honey-associated strain, on honeybee colony performance. *Probiotics and Antimicrobial Proteins, 4(1),* 39-46. https://doi.org/10.1007/s12602-011-9089-0

Safonov, V. (2022). Dependence of antioxidant and biochemical status on selenium content in the blood of animals. *Advances in Animal and Veterinary Sciences, 10*(2), 263-269.http://dx.doi.org/10.17582/journal.aavs/2022/10.2.263.269

Schwarz, R. S., Moran, N.A. & Evans, J. D. (2016). Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. *Proceedings of the National Academy of Sciences, 113(33),* 9345-9350. https://doi.org/10.1073/pnas.1606631113

Sultanova, R. R., Gabitov, I. I., Yanbaev, Y. A., Yumaguzhin, F. G., Martynova, M. V., Chudov, I. V. & Tuktarov, V. R. (2019). Forest melliferous resources as a sustainable development factor of beekeeping. *Israel Journal of Ecology and Evolution, 65(3-4),* 77-84.

Tian, B., Fadhil, N. H., Powell, J. E., Kwong, W. K. & Moran, N. A. (2012). Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honey bees. *mBio, 3(6),* e00377–e412. https://doi.org/10.1128/mBio.00377-12

Tlak Gajger, I., Vlainić, J., Šoštarić, P., Prešern, J., Bubnič, J. & Smodiš Škerl, M. I. (2020). Effects on some therapeutical, biochemical, and immunological parameters of honey bee (*Apis mellifera*) exposed to probiotic treatments, in field and laboratory conditions. *Insects, 11(9),* 638. https://doi.org/10.3390/insects11090638

Zheng, H., Nishida, A., Kwong, W. K., Koch, H., Engel, P., Steele, M. I. & Moran, N. A. (2016). Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. *mBio, 7(6),* e01326-16. https://doi.org/10.1128/mBio.01326-16

Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C. & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proceedings of the National Academy of Sciences, 114(18),* 4775-4780. https://doi.org/10.1073/pnas.1701819114

Received: **March, 10, 2022**; *Approved*: **July, 08, 2022**; *Published*: **February, 2023**