Slov Vet Res 2022; 59 (3): 159–67
DOI 10.26873/SVR-1498-2022
UDC 638.12:638.144:638.124.8

Original Research Article

PERFORMANCE AND *Nosema* spp. SPORE LEVEL IN YOUNG HONEYBEE (*Apis mellifera carnica,* Pollmann 1879) COLONIES SUPPLEMENTED WITH CANDIES

Maja Ivana Smodiš Škerl[1]*, Ivana Tlak Gajger[2]

[1]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia, [2]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

*Corresponding author, E-mail: maja.smodis.skerl@kis.si

Abstract: We evaluated the efficacy of supplementation with protein, yeast, or sugar candies of young honeybee colonies originated from artificial swarms, by measuring strength and determining *Nosema* spp. spore level in adult bees in summer period. At the same time, we aimed to assess longevity of adult worker bees after feeding the same type of candies in controlled laboratory condition. The highest survival was found in Yeast and Protein candy group. On the contrary, the field study showed that artificial swarms produced significantly more pupae (2510.4 cm^2, p=0.0001) in the 1st period of measurement, and more larvae (964.8 cm^2, p=0.003) and frames with bees (5.6, p=0.008) in the 2nd period by feeding non-protein candy. In the 3rd period of evaluation of young colonies, the Sugar candy group had the highest number of frames covered by adult bees and honey stores, respectively (5.6, p=0.0009; 3432.0 cm^2, p=0.015). Sugar candy group produced the largest area of wax cells, however, the differences were not statistically significant. *Nosema* spp. spore level was checked quantitatively in adult bees. The lowest infection was statistically significant in Yeast candy group in June (4.35 million spores per bee, p=0.02), but insignificant in September. Supplementing artificial swarms Sugar candy offers the most promising potential for development of productive young colony. The findings of our study could help beekeepers to choose the effective candy supplement for optimal development of artificial swarms.

Key words: *Apis mellifera*; artificial swarms; young colonies; supplements; candies; development; *Nosema* spp.; longevity

Introduction

Honeybees are one of the most important pollinators for the agriculture and food production. The density of honeybee colonies can be very versatile in different parts of the world, depending mostly upon availability of nectar flow. Facing the overpopulated area with honeybee colonies can lead to decrease of health status, frequent infections with pathogens and exploitative competition with wild bees, like bumblebees (1).

A success and production in beekeeping sector always depends on the weather conditions, climate change, health condition of the colonies and changes in the environment due to human interventions. The essential honey bee colony needs must cover a proper quantity of quality food for productive development, reproduction, and honey yield (2), to overcome the stress caused by pesticides and pathogens. A honey bee colony needs pollen to meet the needs for protein, lipids, and vitamins (2, 3, 4). The lack of pollen diversity and diminished quantity affects the colony in a healthy brood production, increases infections due to deterioration of immune system defence mechanisms and shortens the life span of bees (4-11). Carbohydrates are the source of energy and structural storage polysaccharides (in plants there are as starch and inulin; in animals they are stored as glycogen and chitin). In pollen there are up to 41 % of sugars, but 50 % of them are starch and cell wall constituents and very hard to digest for the honey bees (4). Therefore, floral

Received: 10 March 2022
Accepted for publication: 27 July 2022

nectar and pollination drops are the richest source of carbohydrates for adult bees containing glucose, fructose, and sucrose, depending on the flowering periods and types of plants available. Honeydew is another source of carbohydrates for honey bees containing glucose, fructose, and sucrose like nectar, but also more complex sugars (maltose, melezitose etc.). It is usually available during nectar dearth and occurs on leaves of fir, larch, pine, oak, spruce etc. Nectar and honeydew are collected by bee foragers, taken to the bee hive, processed, and stored in wax cells as ripe honey. Bees need round 4 kg of honey to produce 500 g of wax or young bees at age 12 to 18 days need on average 20 kg of sugars (fructose, glucose, sucrose) and a significant amount of proteins to produce 1 kg of wax (4, 12).

Most insects can regulate protein and carbohydrates from natural food sources for optimal growth and survival (13). Different ratios of carbohydrate to protein affect social insect physiology and ability to survive. The optimal balance of nutrients can be determined by the geometric framework (13, 14). Adult honey bees lived longer on a pure carbohydrate or low protein diet (15). Paoli et al. (16) showed that honey bees of different age and behavioural have different nutritional requirements. The mortality of adult bees fed high amino acid diet was quite high. Also confirmed in ants, where Dussutour and Simpson (2012) (17) showed a reduction in ant worker lifespan when feeding a high protein diet. On contrary, Archer *et al.* (2013) (18) found that honey bees exposed to environmental stressors (e.g. low temperatures, nicotine) and fed with high protein diet (1:3=P:C) had lower mortality. However, they found that workers in this experiment did not adjust their intake to improve their survival after being exposed to the stressed condition. Feeding protein also extended the survival ability of adult bees after infection with *Nosema* sp. (19).

In beekeeping management, the establishment of young colonies is a frequently used practice in early summer to use the natural reproduction of the colony and to prevent unwanted swarming. New colonies established in May and June need round 1.5 kg of mostly young honeybees and a young, mated queen (20). As such they represent a swarm as a new young colony that must be placed by the beekeeper on another location than its primary colony. When placing the adult bees in a hive with frames and a wax comb foundation only, a newly established young colony requires constant food supplementation (20, 21) to support comb building and foragers start to bring nectar and pollen in the hive and the queen starts egg-laying. The choice of a food supplement is therefore a key factor to bring young colonies in the best shape and increase their potential of productiveness.

In our study we fed the bees with different candies in laboratory conditions to compare longevity and consumption rates. Another important aim of our study was to evaluate the performance and level of *Nosema* spp. spores in young honey bee colonies established from artificial swarms, supplemented with different candies in summer, in the apiary conditions.

Material and methods

The cage trial was established according to the standard methods for maintaining adult bees in controlled conditions (22). Combs with emerging worker bees were obtained from two colonies, placed in an incubator (34.5° C) and left overnight. On the next day, round 1.000 newly-emerged adult bees were collected and randomly put into plastic cages (8 x 12 cm) of air hoarding cages with around 80 openings of ~ 2 mm to provide circulation, and two larger holes for plastic feeders to deliver water and food. 50 bees were introduced in each cage, having 5 replicates per group. Bees were fed Yeast, Protein or Sugar candy, or sugar syrup (1:1, w:v) as a control. The cages with adult bees were kept in a darkened incubator at 28 (±1) °C. Mortality and food consumption were recorded on daily basis. The feeders were weighted on daily basis and the food replaced every 2 or 3 days.

The artificial swarms were established in spring (May) from honeybee colonies (*Apis mellifera carnica*, Pollmann 1879), weighing 1.5 kg of young, mixed-aged bees (weight of worker bees) each and transferred into boxes for swarms (Multibox®, Croatia) (Fig. 1) and left in a dark place overnight. The next day they were transferred into new LR (Langstroth hive) hives (30.5 x 50.5 x 24.3 cm) with 7 frames of AŽ (Alberti-Žnideršič, 410 x 260 mm) with a new wax foundations inserted. In total 18 hives were installed to Jable location (Middle-Slovenian region, 46°08›26.0»N 14°33›22.5»E). The young, mated queen bees were introduced, originating from the same queen breeding operation.

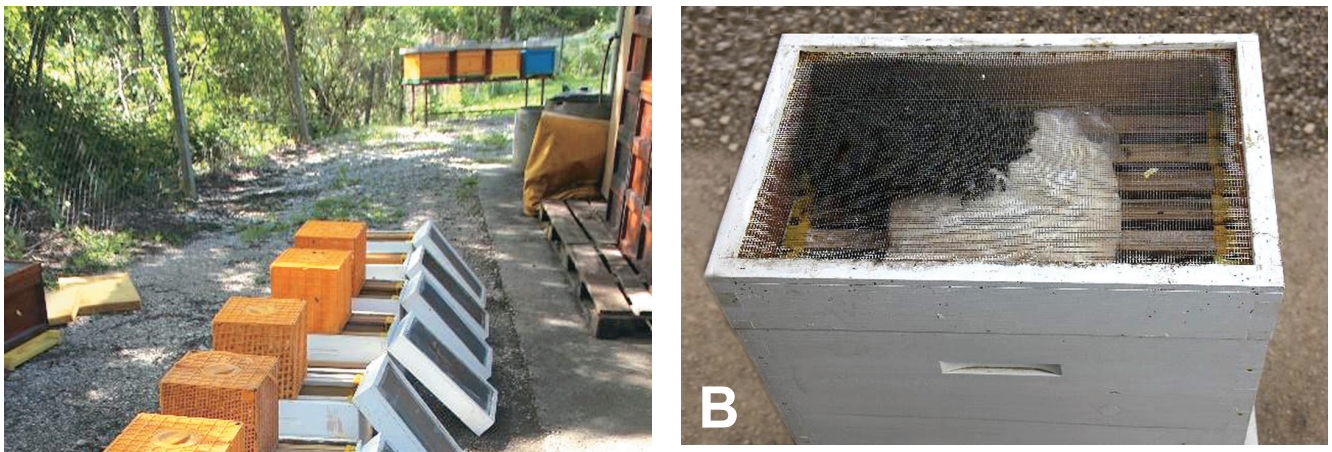

Figure 1: A – Artificial swarms in multiboxes and LR hives with 7-framed wax-foundations. B – Swarms in the hives were established and fed with candy

Artificial swarms were divided into three groups: (1) Yeast candy, (2) Protein candy and (3) Sugar candy. Colonies were continuously fed with one home-made and two commercial candies: (1) Yeast – grounded cane sugar, 5% baker's yeast and water, (2) Protein candy – Medopip plus®, Pip d.o.o., Pisarovina, Croatia, (sugar and vitamins) and (3) Sugar candy – Apifonda®, Südzucker, Germany, (sugar), Figure 1. Young colonies were evaluated three times in summer according to Liebefeld method (23).

Adult bees from the side frames were collected for *Nosema* spp. spores quantification twice, at the beginning of feeding in June and later in September. The abdomens of bees were used to estimate the *Nosema* spp. spores' prevalence and intensity as determined by light microscopy techniques, described by Cantwell (24). Briefly, bees' abdomens (20 bees, 3 replicates per colony) were macerated using a mortar and pestle in 1 ml of distilled water/bee. Further, a drop of the solution was placed on a hemocytometer and *Nosema* spp. spores were counted under a microscope, at 400 x magnification.

The experimental colonies were checked for overwintering ability by being inspected in March of the following year.

All data were analyzed using the RStudio (2021.09.0, PBC, Boston, USA). The data were expressed as mean ± standard error (SE). The lifespan was calculated using Kaplan-Meier curves of honey bee survival, and a log-rank test was performed for significant differences between curves. Measurements of colonies and *Nosema* spp. were analyzed using one-way ANOVA with Bonferroni corrected t-test.

Results

In the controlled laboratory conditions, worker bees from Yeast and Protein candy group lived longer compared to Sugar candy group (Fig. 2) having a significant difference between groups. The highest consumption was determined in group fed sugar syrup followed by Yeast candy group (Fig. 3).

In young colonies, adult bees were building wax cells most intensively in Sugar candy group in all measured periods, but the differences were not significant (Table 1). Brood area was statistically significant in Sugar and Yeast candy group (1st Period) and later on in Yeast and Protein candy group. Comparing the amount of pollen stores there were no differences, and the highest honey storage was in Sugar group (Table 1). However, we noticed that in the same group the content in the wax cells was white assuming that workers stored candy (personal observations). Number of frames with adult bees was the highest in Sugar candy group showing statistically significant differences from

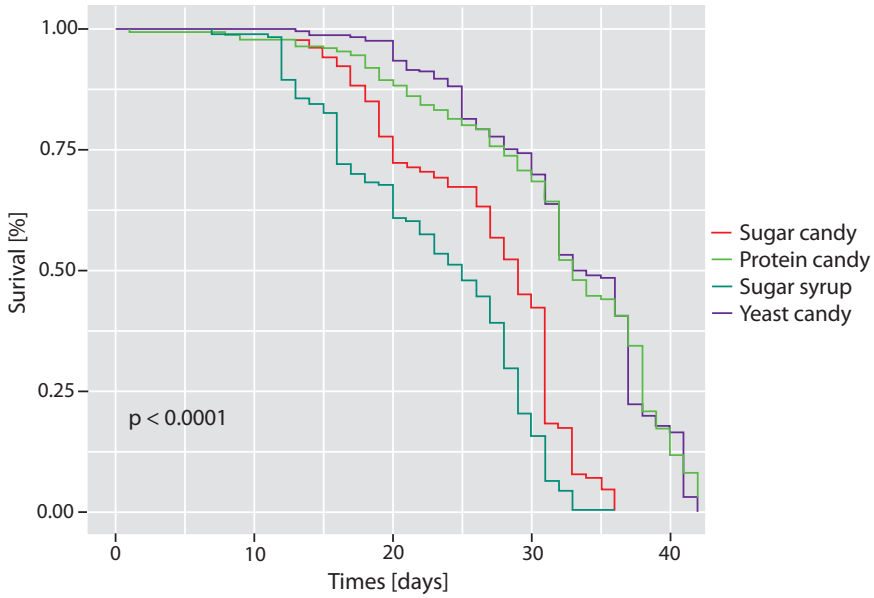

Figure 2: Survival of worker bees in controlled conditions. Kaplan-Mayer survival analysis

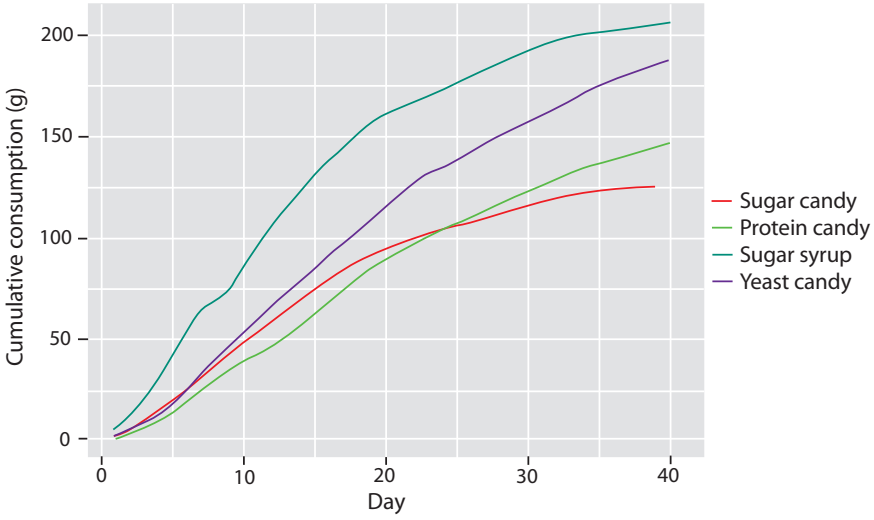

Figure 3: Cumulative consumption of candies and sugar syrup by worker bees under controlled conditions

Yeast candy group (2nd period), and later the differences were significant in all groups in the last observation period (Table 1).

In young colonies, adult bees were building wax cells most intensively in Sugar candy group in all measured periods, but the differences were not significant (Table 1). Brood area was statistically significant in Sugar and Yeast candy group (1st Period) and later on in Yeast and Protein candy group. Comparing the amount of pollen stores there were no differences, and the highest honey storage was in Sugar group (Table 1). However, we noticed that in the same group the content in the wax cells was white assuming that workers stored candy (personal observations). Number of frames with adult bees was the highest in Sugar candy group showing statistically significant differences from Yeast candy group (2nd period), and later the differences were significant in all groups in the last observation period (Table 1).

Table 1: Performance of young colonies established from artificial swarms in summer (1st period – 27.6., 2nd period – 2.7., 3rd period – 27.7.). N = 4-7, Y – Yeast candy, S – Sugar candy, P – Protein candy. SE – standard error, * significant differences (ANOVA, Bonferroni corrected t-test)

Artificial swarms	Group	1st period			2nd period			3rd period		
		Mean	±SE	p-values	Mean	±SE	p-values	Mean	±SE	p-values
Wax production (cm^2)	Y	6162	735.79	0.18	6666	909.96	0.26	8947.2	376.53	0.08
	S	7780.8	440.8		8308.8	850.61		10896	864	
	P	7073.14	453.22		8081.14	378.27		10628.57	473.42	
Eggs (cm^2)	Y	384	143.67	0.019	240	150.52	0.08	518.4	120.14	0.18
	S	840	71.6		648	109.46		624	112.83	
	P	648	54.17		480	82.48		785.14	77.45	
Larvae (cm^2)	Y	198	128.64	0.04	276*	173.48	0.003	676.8	142.47	0.85
	S	652.8	138.79		964.8	161.78		782.4	140.27	
	P	336	72.76		843.43*	50.62		754.29	105.9	
Pupae (cm^2)	Y	492*	202.35	0.0001	522*	318.45	0.001	2078.4*	531.46	0.015
	S	2510.4*	311.87		1987.2	369.19		3115.2	406.33	
	P	1690.29	138.65		2057.14*	59.88		3960*	296.72	
Bees (no. of frames)	Y	3.5	0.29	0.02	4*	0	0.008	3.8*	0.2	0.0009
	S	4.6	0.24		5.6*	0.24		5.6*	0.24	
	P	4.29	0.18		4.86	0.26		4.86*	0.26	
Pollen stores (cm^2)	Y	/			348	79.3	0.5	177.6	70.63	0.8
	S	/			235.2	98.78		249.6	84.72	
	P	/			219.43	55.78		240	76.08	
Honey stores (cm^2)	Y	/			1698	420.33	0.51	1272*	197.47	0.015
	S	/			2188.8	507.16		3432*	664.8	
	P	/			1614.86	230.42		1827.43	229.45	

In June, there were significant differences in *Nosema* spp. spore level, with the highest level in Sugar candy and the lowest in Yeast candy group ($p<0,05$). Comparing the level of *Nosema* spp. spores in September of the same year, the infection was low and non-significant (Table 2).

Table 2: Nosema spp. spores in worker bees in young colonies expressed in million per bee. N = 4-7, Y – yeast candy, S – Sugar candy, P – Protein candy. SE – standard error, *** p>0.05.** (ANOVA, Bonferroni corrected t-test).

Group	June			September		
	Mean	±SE	p-value	Mean	±SE	p-value
Yeast	4.35*	1.97	0.02	1.68	0.59	0.48
Sugar	14.96*	2.27		4.29	1.85	
Protein	12.55	2.74		4.03	2.14	

The colonies from Yeast candy group survived the winter successfully, however the other two groups lost one colony each by the time of the first inspection in early spring.

Discussion

An artificial swarm of honey bees is a very vulnerable young colony, as it possesses a small number of adult workers in comparison to the colony with brood and food stores. Young colony needs continuous food intake to allow worker bees to remain in the hive, building wax cells and later nursing young brood. Our study provides insights on how long worker bees live when fed protein or non-protein candies having no access to honey and pollen at the same time, and how

small, nucleus colonies perform during and after being fed the same type of candies.

The results of the field study show that the young colonies produced more brood feeding non-protein candy having at the same time the highest number of *Nosema* spp. spores in the same experimental group. On the contrary, adult bees in cages lived longer being fed protein or yeast candy, indicating that cage trials by itself do not provide adequate information on performance of bees. The bees in artificial swarms were of mixed age, physiologically in the stage of building wax, and therefore the nutritional needs differed from the bees in cages. However, the overwintering was less successful in groups fed commercial sugar and protein candy. This result can also be connected to some other factors that affect survival ability of honey bee colonies (Varroa, viruses etc.) (24), indicating that trials with bees in controlled conditions, among others, provide the insight in longevity and nutritional requirements (26).

For the last few decades, the apicultural sector is expected to reach a high production of honey and a strong resilience to honey bee diseases and at the same time to overwinter colonies successfully. Unfortunately, there are several factors that hinder the development of colonies and challenge the beekeepers. Changing climate affects colony development and redistribution of honey plants (27, 28, 29, 30), and adaptation cycle of plants and bees to these sudden changes is very slow. The abundance and quality of pollen and nectar is changing and is therefore very unstable natural food source for bees (27, 31). Malnourished colonies are very sensitive and susceptible to infections of pathogens and stress due to pesticide exposure (32), and even in some cases being capable to adapt, eventually those colonies will die (33). Nevertheless, beekeepers need to supply all types of colonies with food supplements and substitutes for bees to fill the gap in food shortage and according to season, health status and needs (artificial swarms, queen production etc.). There are many food supplements available on the market and beekeepers mostly prepare syrups containing white sugar (saccharose) and water (34), and some mix sugar patties with or without additives (i.e., pollen, yeast, vitamins, and minerals etc.) (34, 35, 36) to feed their colonies. In our study, we used a home-made and two commercial candies, that are commonly used by the beekeepers. We found that sugar candy (Apifonda) showed the best results in a short term to establish a young colony. Concerning *Nosema* spp. infection, several recent papers report that different additives are potentially effective to prevent or eliminate *Nosema* spp. spores and/or support development of honey bee colonies: pre/probiotics (37), EM probiotic (38), anti-nosema products (39), medicinal mushroom (40), plant extract (41, 42, 43), microalga (44), Chlorella (45), Cyanobacteria (46), pentadecapeptide BPC 157 (47), and other artificial diet (36). Sammataro and Weiss (2013) (48) compared productivity of colonies supplemented with sucrose or high fructose corn syrup (HFCS) and reported that group fed sugar syrup produced more wax, brood, and adult bee population that HFCS group. However, studies on effects of food supplements and additives on productive colonies are abundant, but there is a lack of research for artificial swarms or young colonies. Health status of worker bees in swarms and quality queen is therefore essential for optimal development and productivity of young colonies and the disease in colonies requires control (49).

Regarding the health status, young colonies originated from artificial swarms were analysed for Nosema spp. in our study. The spores were detected in adult bees due to possible previous infection of honey bee colonies that were used for the experiment, and the spore load in June was the lowest in Yeast candy group and decreased in all groups in September. Moreover, all the colonies fed Yeast candy were able to survive the winter. Microsporidia Nosema apis and N. ceranae (cause of Nosemosis type C) infect the midgut of bees and reproduce in epithelium cells of the gut (50, 51). Research of Nosema infection (52, 53, 54) shows that Nosemosis influences the strength of the colony and the honey yield (55). It should be pointed out, that through the stages of swarm manipulation (or queen production etc.) beekeepers must consider the negative impact of Nosema infection and prevent transfer of pathogen by adopting good beekeeping practice (56).

There are some differences in feeding various type of candies that might affect development and performance of young colonies. In our case we noticed differences in brood production, number of adult bees and honey stores in the hive, and longevity of workers in cages. At this point more studies of feeding supplements and effects on physiology and productivity should be done at the individual bee and colony (swarm) level to prevent colony failure.

Acknowledgments

We are grateful to Mitja Nakrst and David Kozamernik for their contribution in the field and laboratory data collection.

No potential conflict of interest was reported by the authors.

This study was funded by Slovenian Ministry for Agriculture, Forestry and Food, European Union (Uredba o izvajanju programa ukrepov na področju čebelarstva v Republiki Sloveniji v letih 2014-2016, Ur.l. RS, 6/14) and Slovenian Research Agency (P4–133).

References

1. Walther-Hellwig K., Fokul, R.Frankl G., Büchler R., Ekschmitt K., et al. Increased density of honeybee colonies affects foraging bumblebees. Apidologie 2006; 37(5): 517–32.

2. Haydak MH. Honey bee nutrition. Ann Rev Entomol 1970; 15: 143–56.

3. DeGroot A. Protein and amino acid requirements of the honey bee. Physiol Comp Oecol 1953; 3: 197–285.

4. Lipinski Z. Honey bee nutrition and feeding, in the temperate/continental climate of the northen hemisphere, Olsztyn: OZGraf S.A., 2018, pp. 430.

5. Schmidt JO, Johnson BE. Pollen feeding preference of *Apis mellifera* (Hymenoptera: Apidae), a polyectic bee. The Southwest Entomol 1984; 9: 41–7.

6. Schmidt JO, Thoenes SC, Levin MD. Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. Ann Entomol Soc Am 1987; 80: 176–83. doi: 10.1093/aesa/80.2.176

7. Schmidt LS, Schmidt JO, Rao H, Wang W, Xu L. Feeding preference of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. J Econ Entomol 1995; 88: 1591–95.

8. Naug D. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. Biol Conserv 2009; 142: 2369–72.

9. Alaux C, Brunet J-L, Dussaubat C, et al. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environ Microbiol 2010; 12: 774–82. doi:10.1111/j.1462-2920.2009.02123.x

10. Dorit A, Hendriksma H, Dag A, Uni Z, Shafir S. Nutritional aspects of honey beecollected pollen and constraints on colony development in the eastern Mediterranean. J Insect Physiol 2014; 69: 65–73. doi: 10.1016/j.jinsphys.2014.07.001

11. Wang H, Zhang S, Zeng Z-J, Yan W-Y. Nutrition affects longevity and gene expression in honey bee (*Apis mellifera*) workers. Apidologie 2014; 45: 618–25. doi:10.1007/s13592-014-0276-3

12. Whitcomb W. Feeding bees for comb production. Gleanings Bee Cult 1946; 74(4): 198–202, 247.

13. Behmer S. Insect herbivore nutrient regulation. Annu Rev Entomol 2009; 54: 165–87.

14. Raubenheimer D, Simpson SJ. Integrating nutrition: a geometrical approach. Entomol Exp Appl 1999; 91: 67–82.

15. Pirk CWW, Boodhoo C, Human H, Nicolson SW. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). Apidologie 2010; 41: 62–72.

16. Paoli PP, Donley D, Stabler D, et al. Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. Amino Acids 2014; 46(6): 1449–58.

17. Dussutour A, Simpson S. Ant workers die young and colonies collapse when fed a highprotein diet. Proc R Soc B Biol Sci 2012; 279: 2402–8. doi:10.1098/rspb.2012.0051

18. Archer C, Pirk C, Wright G, Nicolson S. Nutrition affects survival in African honeybees (*Apis mellifera scutellata*) exposed to interacting stressors. Funct Ecol 2013; 28(4): 913–23. doi: 10.1111/1365-2435.12226

19. Jack C, Lucas H, Webster T, Sagili R. Colony level prevalence and intensity of *Nosema ceranae* in honey bees (*Apis mellifera* L.). PloS One 2016; 11. e0163522. doi:10.1371/journal.pone.0163522

20. Pohl F. Moderne Imkerpraxis: Völkerpflege und Ablegerbildung. Kosmos : Stuttgart, 2010, pp. 128.

21. Zander E, Böttcher F K. Haltung und Zucht der Biene. Stuttgart : Ulmer, 1989, pp. 422.

22. Williams GR, Alaux C, Costa C, et al. Standard methods for maintaining adult *Apis mellifera* in cages under *in vitro* laboratory conditions. J Apicult Res 2013; 52(1): 1–36. doi: 10.3896/IBRA.1.52.1.04

23. Imdorf A, Buehlmann G, Gerig L, Kilchenmann V, Wille H. A test of the method of estimation of brood areas and number of worker bees in free-flying colonies [Liebefeld method] Apidologie 1987; 18: 137–46. doi: 10.1051/apido:19870204

24. Cantwell GE. Standard methods for counting Nosema spores. Am Bee J 1970; 110: 222–3.

25. Desai SD, Currie RW. Effects of wintering environment and parasite-pathogen interactions on honey bee colony loss in north temperate regions. PloS One 2016; 11(7): e0159615. doi:10.1371/journal.pone.0159615NLM

26. Altaye SZ, Pirk CWW, Crewe RM, Nicolson SW. Convergence of carbohydratebiased intake targets in caged worker honeybees fed different protein sources. J Exp Biol 2010; 213(19): 3311–8. doi: 10.1242/jeb.046953

27. Le Conte Y, Navajas M. Climate change: impact on honey bee populations and diseases. OIE Rev Sci Tech 2008; 27: 485–97, 499.

28. Ricigliano VA, Mott BM, Floyd AS, et al. Honey bees overwintering in a southern climate: longitudinal effects of nutrition and queen age on colony-level molecular physiology and performance. Sci Rep 2018; 8(1): e10475. doi: 10.1038/s41598-018-28732-z

29. Wilson Rankin EE, Barney SK, Lozano GE. Reduced water negatively impacts social bee survival and productivity via shifts in floral nutrition, J Insect Sci 2020; 20(5): e15. doi: 10.1093/jisesa/ieaa114.

30. Descamps C, Quinet M, Jacquemart AL. Climate changeinduced stress reduce quantity and alter composition of nectar and pollen from a beepollinated species (*Borago officinalis*, Boraginaceae). Front Plant Sci 2021; 12: e755843. doi: 10.3389/fpls.2021.755843

31. Ziska LH, Pettis JS, Edwards J, et al. Rising atmospheric CO2 is reducing the protein concentration of a floral pollen source essential for North American bees. Proc Biol Sci B 2016; 283(1828): e20160414. doi: 10.1098/rspb.2016.0414.

32. Castle D, Alkassab AT, Bischoff G, Steffan-Dewenter I, Pistorius J. High nutritional status promotes vitality of honey bees and mitigates negative effects of pesticides. Sci Total Environ 2022; 806(4): e151280. doi: 10.1016/j.scitotenv.2021.151280.

33. VanEngelsdorp D, Evans JD, Saegerman C, et al. Colony collapse disorder: a descriptive study. PLoS One 2009; 4(8): e6481. doi: 10.1371/journal.pone.0006481.

34. Somerville DC. Fat bees, skinny bees. Manual on honey bee nutrition for beekeepers. Kingston : Rural Industries Research and Development Corporation, 2005. RIRDC Publication No. 05/054, 2005. Available at: www.agrifutures.com.au/product/fat-bees-skinny-bees-a-manual-on-honeybee-nutrition-for-beekeepers/ 20th February 2022)

35. Ahmad S, Khan SA, Khan KA, Li J. Novel insight into the development and function of hypopharyngeal glands in honey bees. Front Physiol 2021; 11: e615830. doi: 10.3389/fphys.2020.615830

36. Ricigliano VA, Williams ST, Oliver R. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. BMC Vet Res 2022; 18(1): e52. doi: 10.1186/s12917-022-03151-5.

37. Klassen SS, VanBlyderveen W, Eccles L, et al. *Nosema ceranae* infections in honey bees (*Apis mellifera*) treated with pre/probiotics and impacts on colonies in the field. Vet Sci 2021; 8(6): e107. doi: 10.3390/vetsci8060107

38. Tlak Gajger I, Vlainić JP, Šoštarić P, Prešern J, Bubnič J, Smodiš Škerl MI. Effects on some therapeutical, biochemical, and immunological parameters of honey bee (*Apis mellifera*) exposed to probiotic treatments, in field and laboratory conditions. Insects 2020; 11(9): e638. doi: 10.3390/insects11090638.

39. Tlak Gajger I, Smodiš Škerl MI, Šoštarić P, Šuran J, Sikirić P, Vlainić J. Physiological and immunological status of adult honeybees (*Apis mellifera*) fed sugar syrup supplemented with pentadecapeptide BPC 157. Biology 2021; 10(9): e891. doi: 10.3390/biology10090891.

40. Stevanovic J, Stanimirović Z, Simeunović P, et al. The effect of *Agaricus brasiliensis* extract supplementation on honey bee colonies. An Acad Bras Cienc 2018; 90: 219–29.

41. Schulz M, Łoś A, Grzybek M, Ścibior R, Strachecka A. Piperine as a new natural supplement with beneficial effects on the life-span and defence system of honeybees. J Agric Sci 2019; 157(2): 140–9.

42. Hussain A, Shehata I, Laban G, Al-Ayat A. Influence of feeding on the production of honey bee queens during the dearth period under Nasr City conditions – Cairo. Al-Azhar J Agric Res 2020; 45(1): 1–7. doi:10.21608/ajar.2020.126605

43. Al-Ghamdi AA, Abou-Shaara HF, Ansari MJ. Effects of sugar feeding supplemented with three plant extracts on some parameters of honey bee colonies. Saudi J Biol Sci 2021; 28: 2076–82.

44. Ricigliano V, Simone-Finstrom M. Nutritional and prebiotic efficacy of the microalga *Arthrospira platensis* (spirulina) in honey bees. Apidologie 2020; 51: 898–910. doi: 10.1007/s13592-020-00770-5

45. Jehlík T, Kodrík D, Krištůfek V, et al. Effects of Chlorella sp. on biological characteristics of the honey bee *Apis mellifera*. Apidologie 2019; 50(4): 564–77. doi: 10.1007/s13592-019-00670-3

46. Ricigliano VA, Ihle KE, Williams ST. Nutrigenetic comparison of two Varroaresistant honey bee stocks fed pollen and spirulina microalgae. Apidologie 2021; 52: 873–86.

47. Tlak Gajger I, Ribarić J, Smodiš Škerl M, Vlainić J, Sikiric P. Stable gastric pentadecapeptide BPC 157 in honeybee (*Apis mellifera*) therapy, to control *Nosema ceranae* invasions in apiary conditions. J Vet Pharmacol Ther 2017; 41(4): 614–21. doi:10.1111/jvp.12509

48. Sammataro D, Weiss M. Comparison of productivity of colonies of honey bees, *Apis mellifera*, supplemented with sucrose or high fructose corn syrup. J Insect Sci 2013; 13: e19. doi: 10.1673/031.013.1901

49. Burnham AJ. Scientific advances in controlling *Nosema ceranae* (Microsporidia) infections in honey bees (*Apis mellifera*). Front Vet Sci 2019; 6: e79. doi: 10.3389/fvets.2019.00079

50. Mutinelli F. The spread of pathogens through trade in honey bees and their products (including queen bees and semen): overview and recent developments. Rev Sci Tech 2011; 30: 257–71.

51. Higes M, Martín-Hernández R, Meana *A. Nosema ceranae* in Europe: an emergent type C nosemosis. Apidologie 2010; 41: 375–92.

52. Higes M, Martín-Hernández R, Botías C, et al. How natural infection by *Nosema ceranae* causes honey bee colony collapse. Environ Microbiol 2008; 10: 2659–69.

53. Genersch E, Ohe W, Kaatz H, et al. The German bee monitoring: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 2010; 41(3): 332–52. doi: 10.1051/apido/201 0014

54. Gisder S, Hedtke K, Möckel N, Frielitz MC, Linde A, Genersch E. Five-year cohort study of *Nosema* spp. in Germany: does climate shape virulence and assertiveness of *Nosema ceranae*? Appl Environ Microbiol 2010; 76: 3032–8.

55. Botías C, Martín-Hernández R, Barrios L, Meana A, Higes M. Nosema spp. infection and its negative effects on honey bees (Apis mellifera iberiensis) at the colony level. Vet Res 2013; 44(1): e25. doi: 10.1186/1297-9716-44-25

56. Rivera-Gomis J, Bubnic J, Ribarits A, et al. Good farming practices in apiculture. Rev Sci Tech 2019; 38(3): 879–90. doi: 10.20506/rst.38.3.303

RAZVOJ DRUŽIN IN ŠTEVILO SPOR *Nosema* spp. PRI MLADIH DRUŽINAH MEDONOSNE ČEBELE (*Apis mellifera carnica,* Pollmann 1879), KRMLJENIH S POGAČAMI

M. I. Smodiš Škerl, I. Tlak Gajger

Izvleček: Mlade čebelje družine iz umetnih rojev smo krmili z različnimi pogačami z dodatkom beljakovin, kvasa ali sladkorja. Ocenjevali smo razvoj družin in določali število spor *Nosema* spp. pri odraslih čebelah v poletnem obdobju. V laboratorijskih pogojih smo krmili čebele delavke z isto vrsto pogač. Najboljše preživetje smo ugotovili v skupinah, ki so prejele pogačo s kvasom oziroma beljakovinami. Nasprotno pa je poskus v družinah pokazal, da je bilo v 1. obdobju merjenja bistveno več bub (2510,4 cm^2, p = 0,0001), v 2. obdobju pa več ličink (964,8 cm^2, p = 0,003) in okvirjev s čebelami (5,6, p = 0,008) pri krmljenju s sladkorno pogačo. V 3. obdobju ocenjevanja mladih družin je imela skupina s sladkorno pogačo največ pokritih okvirjev z odraslimi čebelami in zalog medu (5,6, p = 0,0009; 3432,0 cm^2, p = 0,015). Skupina s sladkorno pogačo je zgradila največjo površino satja, vendar razlike niso bile statistično značilne. Število spor *Nosema* spp. je bilo kvantitativno preverjeno pri odraslih čebelah. Najnižja okužba je bila statistično značilna v skupini s pogačo s kvasom v juniju (4,35 milijona spor na čebelo, p = 0,02), septembra pa spremembe niso bile signifikantne. Dodajanje sladkorne pogače umetnim rojem se je pokazalo kot najbolj obetavno za razvoj produktivnih mladih čebeljih družin. Ugotovitve naše študije bi lahko pomagale čebelarjem pri izbiri učinkovitega dodatka pogač za optimalen razvoj umetnih rojev.

Ključne besede: *Apis mellifera*; umetni roji; mlade družine; dodatki; pogače; razvoj; *Nosema* spp.; dolgoživost