

Use Supplementary Feedings on Bee Colony Development and Venom Production

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Abstract

The paper presents the influence of the use of supplementary feedings on the development of bee colonies and venom production. We evaluated the venom production during harvests of rapeseed, acacia and sunflower. The research was carried out in the apiary of the Faculty of Bioengineering of Animal Resources within the ULST. The biological material was represented by 40 colonies of bees (*Apis mellifera carpatica*), divided into 8 experimental groups, each group consisting of 5 colonies with equal strength and a queen of the same age. During the spring, bee colonies were fed sugar syrup into which we introduced probiotic products and/or essential oils. Counting of brood cells was performed at 7, 14 and 21 days during supplementary feeding. At the end of the additional feeding period, as well as during rapeseed, acacia and sunflower harvests, venom was collected using the BeeWhisper v.5.1 venom collector, model 2016. Regarding venom production, the best results were observed in the case of additional feedings with the products used. Batches using Colobiotic (2.2 g venom), Oregano essential oil (2.1 g venom) and Lacium (2.1 g venom) had the highest amounts of venom.

Keywords: bee colonies, brood, supplementary feeding, venom production

1. Introduction

Supplementary feeding has a well-established role when food resources are limited, when natural sources are few and a replenishment of food supplies is required [1]. Research has shown that, in the digestive tract of bees, there is a consistent microbial community with a fairly stable composition [2, 3]. Gut microflora determines the immune status of bees [4]. Slowing down the development of the bee colony and bee death occurs when the composition of the microbiota is disturbed [5]. Some of the factors that cause the disorder are: poor quality of food or the lack of it [6], the use of antibiotics in beekeeping [7] and the use of pesticides [8]. Essential oils have a

number of antibacterial, antifungal, antioxidant, antiviral properties and are an alternative source to the use of antibiotics [9].

Probiotics are microorganisms that have a favorable influence on the digestive tract, contributing to the balance of intestinal microflora [10, 11]. Research by Chmiel et al. (2020) [12] showed that probiotics increase the resistance of bees to pesticide poisoning.

Bee venom is produced in the two abdominal glands (venom gland and Dufour gland) of worker bees [13] and known by the name of apitoxin, used since ancient times, the venom even being mentioned in some religious books (Bible and Koran) [14].

Extracting a substantial amount of venom is complicated by the small size of the bee. Venom collectors are used for this purpose [15, 16].

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2. Materials and methods

In order to see how essential oils and probiotic products influence the development of the bee colony during the stimulation feeding period and at the same time the production of venom that we obtain at certain production harvests, we carried out experiments in the apiary of the Faculty of Bioengineering of Animal Resources, within the discipline of Apiculture. The experiments were carried out on colonies of bees from the *Apis mellifera carpatica* breed, divided into 8 experimental batches, each group consisting of 5 families of bees with medium strength and a queen of the same age. The essential oils used in the experiment were purchased from the same manufacturer, namely SC BIONOVATIV SRL and were represented by: thyme, wild oregano and basil. The probiotic products used were: Laciium, Colobiotic and Enterolactis Plus. During supplementary feeding, bee colonies were given 1:1 sugar syrup (1kg sugar:1 liter water) to which probiotic products and/or essential oils were added for 3 weeks according to the scheme of the experiment (Table 1). To evaluate the degree of development of the bee families, the Netz frame was used, with which we measured the area occupied by both hatched and non-hatched brood. Measurement of the brood was made at the beginning of the experiment, at 7 days, 14 days and at 21 days at the completion of the experiment. In 2023, 4 venom collections were

made: the first collection was carried out after the completion of the additional feeding period with sugar syrup, probiotic products and/or essential oils, during April 20-21; harvest 2 was carried out after the rape harvest during May 10-11; harvest 3 was carried out after the acacia harvest on June 26-27 and harvest 4 was done after the sunflower harvest on July 22-23. The collection of bee venom was carried out with the BeeWhisper v.5.1 model 2016 collector according to the following collection protocol: the collector was placed at the beehive and turned on, a low intensity current passed through the filaments of the collector and when the bees came into contact with they stung and the venom ended up on the collector glass. The bees' needle remained intact, they were able to continue their activities within the colony. In each colony of bees, the collector was left functioning for 30 minutes after which it was stopped and kept for 2 minutes in order to allow the bees to withdraw to the hive. The venom was scraped from the collector glass and placed in 1 ml containers. After being placed in containers, the venom was placed in a refrigerated box for transport and placed in the freezer at a temperature of -20°C . After each collection the resulting venom was weighed with a Shimadzu AW220 analytical balance with 4 decimals. The statistical analysis of the results was carried out using the Minitab program, version Minitab®20.3.

Table 1. Organization scheme of the experiment

Batch	Sugar syrup (l)	Food administered	
		Essential oil (drops)	Probiotic product (capsules)
Control	1.5	-	-
Batch I	1.5	Basil 3	-
Batch II	1.5	Thyme 3	-
Batch III	1.5	Oregano 3	-
Batch IV	1.5	-	Laciium 1
Batch V	1.5	-	Colobiotic 1
Batch VI	1.5	-	Enterolactis Plus 1
Batch VII	1.5	Oregano 3	Laciium 1

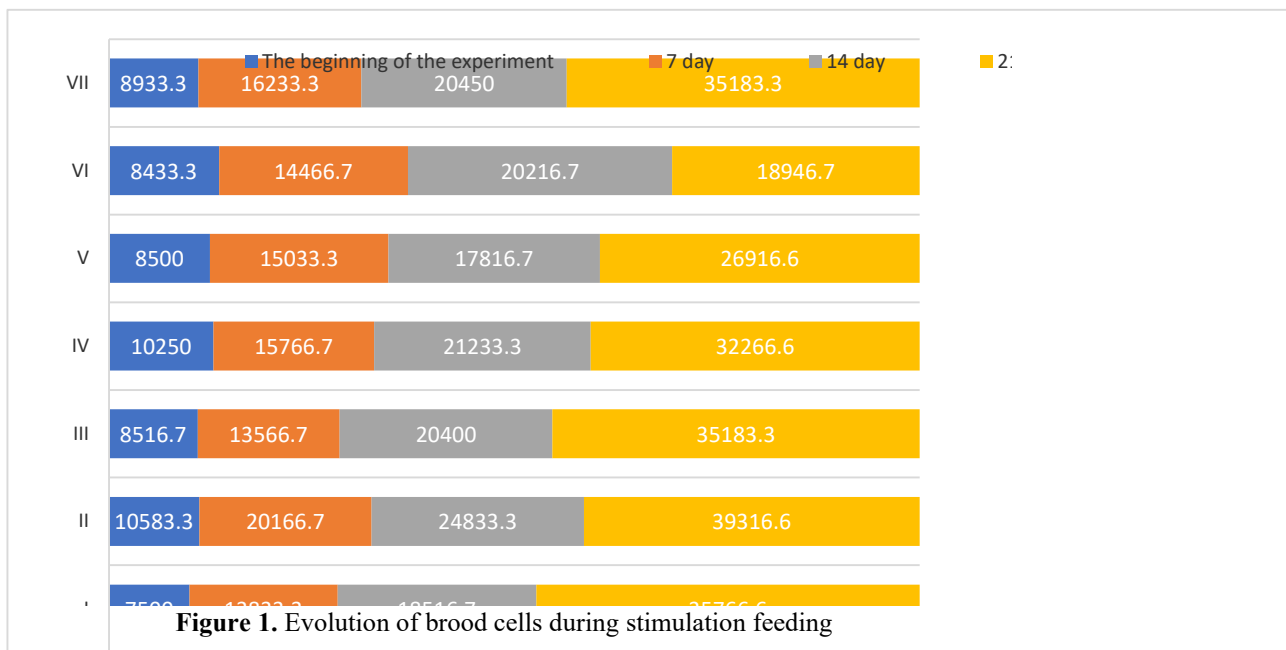
3. Results and discussion

The use of essential oils and probiotic products during the booster feeding period resulted in increased numbers of brood cells. A brood cell

count was performed at the beginning of the Netz frame experiment, after which counts were made every 7 days until day 21 when the experiment was completed. The highest average number of cells with brood throughout the experiment was in

batch II where sugar syrup and essential oil of thyme were administered, followed by batch VII where sugar syrup, Laciium and essential oil of oregano were administered. Batch II had the best evolution of the number of brood, having at the beginning of the experiment 10583 cells with brood and on day 21 a number of 39317 cells with brood (Figure 1). The slowest development was recorded in batch VI which had 8433 cells with brood at the beginning of the experiment and on

day 21 a number of 20217 cells with brood. In the researches performed, Pătruica et al. (2018) [17] concluded that the use of essential oils led to the stimulation of queen laying, especially in the case of using sugar syrup supplemented with basil essential oil. In a recent experiment Pătruica et al. (2023) obtained similar results in terms of the number of brood cells, with the best results reported using essential oils of thyme and basil followed by oregano and peppermint.



At the end of the additional feedings, following the collection of venom, the largest amount was obtained from batch V which was administered sugar syrup and Colobiotic, obtaining 1.3613 g, and the smallest amount of venom from the control batch, which was offered only sugar syrup, obtaining 0.7822 g (Figure 2). After picking the rapeseed, the highest amount of venom obtained was from the control batch, followed by batch VII (sugar syrup with oregano and Laciium essential oil), and the lowest amount from batch I, stimulated with sugar syrup and basil essential oil. After the acacia harvest, the largest amount of venom collected was from batch III, which was given sugar syrup and oregano essential oil during the stimulation feeding. The last venom collection was made after the sunflower harvest, the largest

amount being obtained from batch IV, where sugar syrup and Laciium were administered during the stimulation feeding. Regarding the amount of venom during the feeding period and during the 3 collections, the highest total amount of venom was recorded in batch V. - sugar syrup and Colobiotic (2.2 g venom), batch III. – sugar syrup and oregano essential oil (2.1 g venom) and batch IV. – sugar syrup and Laciium (2.1 g venom). The results obtained after the collection show that when either essential oils or probiotic products were administered, a greater amount of venom was obtained compared to the amount that was obtained in the control group (Figure 2). The benefits on the development and health of bee colonies arising from the use of probiotic products have been confirmed by other authors [1, 19].

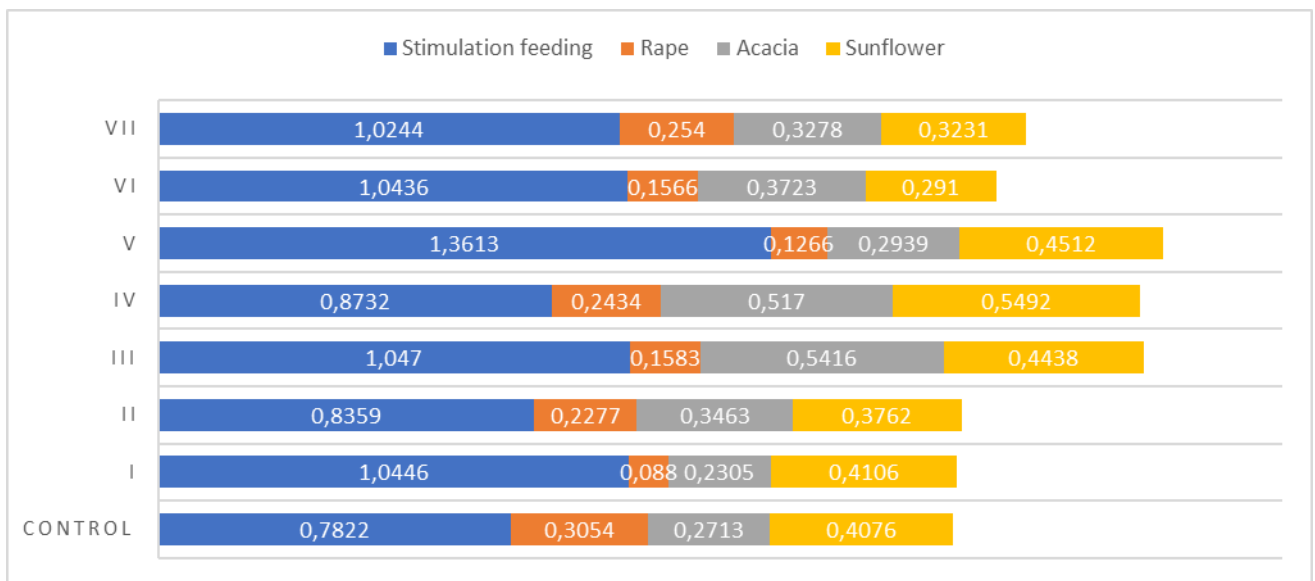


Figure 2. Amount of venom obtained after stimulation feedings and the 3 harvests per batch

4. Conclusions

Stimulation feeding is very important for the development of the bee colony as well as for the synthesis of bee venom. For the development of the bee colony, the use of thyme essential oil had the best effect. After additional feeding, the highest amount of venom was obtained compared to the nectar collection periods. After additional feeding, the highest amount of venom was obtained compared to the nectar collection period. The batch where Colobiotic, essential oil of oregano and Laciium were used had the highest amounts of venom on all 4 harvests. Studies are still needed in which to analyze environmental factors, their way of influencing bee families, but also to analyze, from an economic point of view, the profitability of venom collection in certain seasons (harvests).

References

- Pătruică, S., Hutu, I., Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies, *Turkish Journal of Veterinary and Animal Sciences*, 2013, 37.
- Mishukovskaya, G., Giniyatullin, M., Shelekhov, D., Khabirov, A., Smolnikova, E., Naurazbaeva, A., The use of probiotics in spring supplementary feeding of bee colonies, *Bulgarian Journal of Agricultural Science*, 2023, 29(1), 131–137.
- Romero, S., Nastasa, A., Chapman, A., Kwong, W. K., Foster, L. J., The honey bee gut microbiota: strategies for study and characterization. *Insect Molecular Biology*, 2019, 28(4), 455-472.
- Emery, O., Schmidt, K., Engel, P., Immune system stimulation by the gut symbiont *Friscella perrara* in the honey bee (*Apis mellifera*). *Molecular Ecology*, 2017, 26(9), 2576-2590.
- Raymann, K., Moran, N. A., The role of the gut microbiome in health and disease of adult honey bee workers, *Curr Opin Insect Sci*, 2018, 26, 97-104.
- Maes, P. W., Rodrigues, P. A., Oliver, R., Mott, B. M., Anderson, K. E., Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and *Nosema* disease in the honeybee (*Apis mellifera*), *Mol Ecol*, 2016, 25(21), 5439-5450.
- Raymann, K., Shaffer, Z., Moran, N. A., Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees, *PLOS Biology*, 2017, 15(3), e2001861.
- Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R., Williams, M. A., Honey Bee Gut Microbiome Is Altered by In-Hive Pesticide Exposures, *Frontiers in Microbiology*, 2016, 7.
- Lazăr, R. N., Alexa, E., Obiștioiu, D., Cocan, I., Pătruică, S., The Effect of the Use of Essential Oils in the Feed of Bee Families on Honey Chemical Composition and Antimicrobial Activity, *Applied Sciences*, 2022, 12(3), 1094. Retrieved from <https://www.mdpi.com/2076-3417/12/3/1094>
- Ferreira, C. L. d. L. F., Salminen, S., Grzes'kowiak, L. M., Brizuela, M. A., Sánchez, L., Carneiro, H., Bonnet, M., Terminology concepts of probiotic and prebiotic and their role in human and animal health, 2011

11. Pătruică, S., Dumitrescu, G., Popescu, R., Marioara N.F., The effect of prebiotic and probiotic products used in feed to stimulate the bee colony (*Apis mellifera*) on intestines of working bees, 2013, 1111, 2461-2464.
12. Chmiel, J. A., Daisley, B. A., Pitek, A. P., Thompson, G. J., Reid, G., Understanding the Effects of Sublethal Pesticide Exposure on Honey Bees: A Role for Probiotics as Mediators of Environmental Stress, *Frontiers in Ecology and Evolution*, 2020, 8.
13. Devi, A. B., Sangeeta, Kumar, N. R., Kaur, J., Honey BEE Venom and its Composition: Focusing on Different *Apis* Species -A Review, *Journal of Basic and Applied Engineering Research*, 2016, 3(1), 2350-77
14. Wehbe, R., Frangieh, J., Rima, M., El Obeid, D., Sabatier, J. M., Fajloun, Z., Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. *Molecules* (Basel, Switzerland), 2019, 24(16), 2997
15. Carpena, M., Nunez-Estevez, B., Soria-Lopez, A., Simal-Gandara, J., An Updating Review of Its Bioactive Molecules and Its Health Applications, *Nutrients*, 2020, 2(11), 3360.
16. Pătruică S., *Biotehnologii în apicultură și sericicultură*, Ed. Eurobit, 2021, pp.58
17. Pătruică, S., Moț, D., Bura, M., Boarță, R., Influence of Essential Oils on Queen Prolificacy and Bee Colony Health, *Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii*, 2018, 51(1), 139.
18. Pătruică, S., Lazăr, R. N., Buzamăt, G., Boldea, M., Economic Benefits of Using Essential Oils in Food Stimulation Administrated to Bee Colonies, *Agriculture*, 2023, 13(3), 594.
19. Pătruică, S., Bogdan, A. T., Bura M., Bănățean-Dunea, I., Popovici D., Research on the influence of probiotic products on bee families development in spring, *Bulletin UASVM Animal Science and Biotechnologies*, 2011, 68(1-2).