THE USE OF SOME SUPPLEMENTARY FEEDS AND THEIR INFLUENCE ON THE LONGEVITY AND WAX PRODUCTION OF CAGED HONEYBEES

Dan-Iulian EŞANU, Cristina Gabriela RADU-RUSU, Ioan Mircea POP

University of Agricultural Sciences and Veterinary Medicine Iași, 8, Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: popmirceais@yahoo.com

Abstract

Replacing natural honeybee nutrients sources (pollen, nectar) with different types of alternative feed (sugar syrup, corn syrup, protein flour) has different consequences on bee health and their production, either maintained in colonies or isolated from them. In this context, this study analyzed the influence of using of some energy and energy-protein resources on longevity and wax production of caged honeybees, isolated from the colony. Thus, the introducing of protein substances into alternative feed, especially pollen substitutes (beer yeast, powdered milk), has led to a decrease in the life of bees. The increase in the amount of bee wax was favoured by the introduction of protein sources into the bee's diets, with the best results generated by pollen; instead, the pollen substitutes determined the lowest values of this parameter. The bee's energy-protein mixtures consumption also affected the overall health of those.

Key words: caged honeybees, longevity, pollen, wax.

INTRODUCTION

Natural bee nourishment contains energy components (mana, nectar, honey) and protein components (pollen, bee bread), all of which are quantitatively determined by the climatic conditions of the apicultural season (Moraru, 2006). As with other animals, bee nutrition is also studying how energy and nutrients are used in the body or entire colony, as well as the yield of their use in different functions or their productions (Pop, 2006).

When there are not enough natural food sources for bees, beekeepers administer them different food recipes to provide the nutrients needed for bee colonies based on energy syrups or pollen substitutes(beer yeast, milk powder) and whose consequences on bee development are still study subjects of research papers.

The bee family is an autonomous biological unit, the development of which is based on the continuous interaction between individuals (Rinderer, 2012). The isolation of bees from colony also involves the aggravation of nutritional relationships between individuals, which negatively influences some morphoproductive characteristics of the solitary bee, particularly its longevity.

The isolated bee has a shorter lifespan than the one inside of a colony, where division of labor implies, among other activities, food exchange (trofalaxia) from one individual to another (Sammataro, 2013); the lifespan is also determined by presence of brood and adult bee workers (Eyer, 2016). Some studies are demonstrating that even among bees introduced into cages creates a type of nutritional relationship dependent on the number of individuals, which is reflected in their consumption of food (Brodschneider, 2017). This method of bee nourishment research which involves introducing them into cages of different dimensions was also necessary for elucidating problems related to bee psychology, toxicology or parasitology (Glavinic, 2017), as well as problems related to the activity of colony castles and the analysis of some productive parameters (Williams et al., 2013). The method of feeding bees isolated from colonies allows several experiments to be carried out over a short period of time compared to feeding bees in the field, and this is particularly relevant in studying the impact

on bee lifespan of chemicals substances used in agriculture, like pesticides, fungicides, neonicotinoids (Fries, 2013). Concerning of some parameters of appreciation

of their quality, the bees within a colony react differently in so-called food diets compared to isolated bees (Altaye, 2010); the presence of pollen in nature increases the vitality of bees from field, while the introduction of pollen into diets of caged honeybees decrease their longevity (Pirk, 2010).

Differences between field results and those obtained from caged honeybees have also occurred in the case of feeding with energy diets. For example, the use of honey in diets has determined bigger longevity of field bees than the values obtained from caged bees, while the use of sugar syrup in diets determined smaller longevity of field bees than the one of caged honeybees (Abou-Shaara, 2017).

Another problem related to the research of additional feeding of caged honeybees and the consistency of the obtained results is represented by the cage constructive model and the feeding regimen adopted. Some studies show significant differences between morphological parameters obtained on bees fed with same recipes but kept on different cages or fed with different regimens; even the feeders dimensions used for syrup can influence the results of an experiment (Huang, 2014).

The determination of the characteristics of supplementary feed recipes still remains the subject of more researchers. In the context of attempting to determine the impact of the various ingredients used on morpho-productive characteristics, this study aims to establish the influence of 8 types of feed recipe on longevity and wax production of caged honeybees.

MATERIALS AND METHODS

This study used as biological material bees of *Apis mellifera* (Carpathian ecotype), maintained in 3 experimental series, June 2017-September 2017, each series containing 8 experimental groups of 3 cages/each group (100 bees /cage). All groups were provided with specific food recipes and *ad libitum* water, so a total of 8 food recipes.

Determination of the target indicators (longevity and wax production) involved daily count of dead bees and weighing the wax combs from cages at the beginning and end of the experiment; we had also counted the quantities of each food recipe.

The experiment implied running 3 experimental series, with a 51 days period/series. Each series was formed from 8 experimental groups,

consisting of 3 cages/group, 100 bees/each; we ensured one feed recipe for each group of research (total of 8 recipes).

The scheme of experimental research is presented on Table 1.

Table 1 Experimental	research scheme
----------------------	-----------------

Specification (bees and conditions)	Apis mellifera carpatica - caged honeybee(0-24 h age)- 3 experimental series x 8 groups/series x 3 cages/group(100 bees/cage) -30 days in the dark, 34°C, 55% U	
Feed recipes for each group (B1B8)	B1 B2 B3 B4 B5 B6 B7 B8	Honey Sugar syrup 2:1 Corn hydrolyzate Enzymatic invert sugar syrup Sugar syrup 2:1 + pollen Sugar syrup 2:1 + beer yeast Sugar syrup 2:1 + milk powder Energy-protein cake
Measured parameters	bee longevity the amount of wax deposited	

The newly emerged adult worker bees (0-24 h age) were obtained from 3 different bee colonies in Deleni (Vaslui, Romania) using a specific method for obtaining them:

- Day 1: the queens of those3 bee families were isolated on 2 frames with the Haneman wall boxes and placed back in hives for 24 h;

- Day 2: the queens were removed from the Haneman boxes and placed back to hive;

- Day 18: the brood frames were collected from the hives and placed in the incubator (34°C);

- Day 21: the newly emerged bees were collected from the frames and placed in boxes. Each box was populated with individuals from a single bee family (each group was formed with bees from those 3 different colonies).

The boxes (210x60x120 mm) were made of wood, with two transparent plastic moveable walls (for better observation) and at the bottom had holes covered with wire mesh to allow ventilation. Inside each box was placed a wax frame foundation (about 3 g) on which the bees later deposited the wax. On bottom we placed 2 water feeders (10 ml each), one for water and one for feed recipe; these were plastic

After introducing of bees inside, all cages of a series (24 units) were kept for 30 days in an

incubator (dark, 34°C, 55% U), for reproducing the physical conditions from anormal beehive. Specifications of feed recipes:

B1-honey (collected from our apiary);

B2- 2:1 sugar syrup (2 parts of sugar mixed with 40°C water);

B3-corn hydrolyzate (from the market);

B4-enzyme invert sugar syrup (from the market);

B5-sugar syrup 2:1 + bee-collected pollen (the pollen was grinded and then mixed with sugar syrup 2:1, pollen: syrup = 1:3 based on weight) B6 - sugar syrup 2: 1 + beer yeast (inactivated beer yeast, yeast: syrup = 1:3)

B7-sugar syrup 2:1 + milk powder (skimmed milk powder, milk powder: syrup = 1:3)

B8- energy-protein cake (from the market for bees, mixed with water, 6 g cake/3 ml water).

After introducing bees into the cages, each bee feeder was filled with the specific feed recipe, and the water feeders were also filled.

Every day the feed recipes and water were refreshed, the quantities were determined and recorded and the dead bees were removed and counted as well.

In the end of last day of incubation we released the bees who had been still living, we counted them and also we weighed the wax frames from each cage of experiment.

In the same way we went through hall 3 experimental series of this research study.

The data was recorded and then statistically analyzed for discussions and conclusions.

RESULTS AND DISCUSSIONS

The results of this study relate to the bee longevity and the amount of beeswax deposited by bees in each cage.

Knowing the number of bees at the beginning of the experiment and the number of dead bees collected daily from cages, we have established the evolution of the number of individuals in each group during of the 30 days (incubating period) of each experimental series.

The cumulated data of those 3 series are presented in figure 1, where we tried comparing groups fed with energy syrups (Figure 1, a) as well as those fed with energy-protein mixtures (Figure 1, b); we also tried to establish the influence of introducing of some protein sources in bees feed recipes (Figure 1, c). Analyzing Figure 1, we observe that bee fed with energy recipes had a longer lifetime than those who received energy-protein feed. This can be seen by analyzing the results obtained from group B2 (sugar syrup) and those obtained from lots that were fed with a mixture of sugar syrup and different protein ingredients (B5, B6, B7).



Figure 1. Evolution of bees numbers

Analyzing the result of groups fed with energy recipes (B1, B2, B3, B4) we can see that the least influence on the bee longevity produced the enzyme invert sugar, while honey determined the highest losses bees on experiment days. The low bee longevity caused by the introduction of proteins into food can be seen in the B5, B6, B7 and B8 values that are inferior to those obtained from the first four groups. Taking into account the results obtained in the last four lots we notice that the group fed with sugar syrup and milk powder lost the earliest all bees in cages (day 16). A similar involution of the number of bees was registered also in the group fed with sugar syrup and beer yeast (lost all bees on the day 18). Among the fed energy-protein groups, the one who received the energy-protein cake (B8) showed the longest life of the bees in the cages. The wax frame foundations from cages were weighed before introducing and also when we finished the experimental series. By the difference of those two values we determined the amount of wax (mg) deposited by bees of each group; the average values of this parameter are presented in figure 2.



Figure 2. Amount of beeswax (mg)

Group 1- honey; Group 2: sugar syrup; Group 3 - corn syrup; Group 4 - enzymatic invert sugar; Group 5 - sugar syrup 2:1 + pollen; Group 6 - sugar syrup 2:1 + beer yeast; Group 7 - sugar syrup 2:1 + milk powder; Group 8 - Energo-Protein Cake

In the Figure 2 it can see that this indicator had the highest values at the group fed with sugar syrup and pollen (608.67 mg), and the lowest values in the corn syrup group (267.67 mg). Analyzing this indicator we can state that pollen was much more valuable than the pollen substitutes used.

The importance of pollen in increasing the amount of wax can be seen by comparing the results of the group fed with sugar syrup (302 mg) and that fed with mixture of pollen and sugar syrup (608.67 mg).

Although proteins are important in the amount of beeswax, we can see that the lots fed with sugar syrup mixed with yeast (B6) or powdered milk (B7) recorded low values (348.67 mg and 288.33 mg); the main cause was the negative influence of recipes used on bee longevity and general health status (all the cages of these groups presented at the end of the control period traces of diarrhea).

A ranking, shown in descending order, of the values of this indicator obtained in groups fed with energy recipes would reveal the B1 group (399.67 mg), then B4 (377 mg), B2 (311.33 mg) and finally B3 (267.67 mg). Honey is therefore the best source of energy for bees kept in cages (isolated from the colony) to deposit a higher amount of wax.

CONCLUSIONS

The results obtained from this study show that caged honeybees bees react differently at some feed recipes to bees maintained in the field regarding the life span and the amount of wax deposited on frames.

After analyzing the results of the energy-fed groups, we noticed that honey (the natural source of bee's energy) determined the values of bee longevity lower than the values obtained from feeding with enzymatic inverted sugar syrup, which proved to be the best source of energy for caged bees. The importance of inverting of sugar in the bees nourishment quoted in many studies was also highlighted by this research.

Analyzing the results obtained from groups that received energy-protein recipes, we noticed that processing of proteins by bees has the consequence of decreasing their longevity. There are also another experiments related to feeding caged honeybees claim that protein processing by bees has a negative impact on their longevity.

At the same time, we should also note the lifetime of bees that received mixtures of sugar syrup with pollen substitutes (beer yeast, milk powder), which was lower than that of bees that received recipes with pollen; we can state that pollen was the best source of protein from this point of view.

The highest amount of wax was recorded in the group fed with sugar syrup and pollen (608.67 mg), and the smallest in the group fed with corn-hydrolysed syrup (267.67 mg).

Comparing the results obtained in the group fed with sugar syrup (311.33 mg) and the one fed with a mixture of sugar syrup and pollen, we noticed the influence of proteins on this production indicator.

Groups fed with mixture of sugar syrup and pollen substitutes recorded low values of wax amount (348.67 mg at mixture with beer yeast and 288.33 mg at mixture with milk powder), due to the general health status of bees that have consumed those recipes.

The results of this study contribute to the assessment of the impact of different feed recipes on some quality parameters of caged honeybees, like wax production and longevity. The characterization of the ingredients used in bees nourishment continues to be an interesting subject for researchers around the world.

REFERENCES

- Abou-Shaara H., 2017. Effects of various sugar feeding choices on survival and tolerance of honey bee workers to low temperatures. Journal of Entomological and Acarological Research, 49, 6-12. 10.4081/6200.
- Altaye S.Z., Pirk C.W., Crewe R.M., Nicolson S.W., 2010. Convergence of carbohydrate-biased intake targets in caged worker honeybees fed different protein sources.Journal of Experimental Biology, 213, 3311-3318; doi:10.1242/jeb.046953.
- BrodschneiderR., Libor A., Kupelwieser V., Crailsheim K., 2017. Food consumption and food exchange of caged honey bees using a radioactive labelled sugar solution. doi.org/10.1371/journal.pone. 0174684
- Eyer M., Dainat B., Neumann P., Dietemann V., 2016. Social regulation of ageing by young workers in the honey bee, *Apis mellifera*. Experimental Gerentology, 87(A), 84-91.
- Fries I., Chauzat M.P., Chen Y.P., Doublet V., Genersch E., Gisder S., 2013. Standard methods for *Nosema*

research. Journal of Apicultural Research, 52(2), 1-28.

- Glavinic U., Stankovic B., Draskovic V., Stevanovic J., Petrovic T., Lakic N., Stanimirovic Z., 2017. Dietary amino acid and vitamin complex protects honey bee from immunosuppression caused by Nosemna Ceranae. PloS One, 12(11), doi 10.1371/journale.pone 0187726.
- Huang S.K., Csaki T., Doublet V., Dussaubat C., Evans J.D., 2014. Evaluation of cage designs and feeding regimes for honey bee (Hymenoptera:Apidae) laboratory experiments. J. Econ. Entomol., 107(1):54-62.
- Moraru P., 2006. Nutritia și alimentatia albinelor.Ed. Coral Sanivet, București, România.
- Pirk C.W.W., Boodhoo C., Human H., Nicolson S.W., 2010. The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation

of caged honeybees (*Apis mellifera scutellata*). Apidologie, 41, 62-72.

- Pop I.M., Halga P., Avarvarei T., 2006. Nutriția şi alimentația animalelor. Ed. Tipo Moldova, Iași, România
- Rinderer T.E., Danka R.G., Stelzer A., 2012. Seasonal inconsistencies in the relationship between honey bee longevity in field colonies and laboratory cages. Journal of Apicultural Research, 51(2), 218-219.
- Sammataro D., Weiss M., 2013. Comparison of productivity of colonies of honey bees, *Apis mellifera*, supplemented with sucrose or high fructose corn syrup. J. Insect Sci., 13(19), 1–13.
- Williams G.R., Alaux C., Costa C., Csáki T., Doublet V., Eisenhardt D., 2013. Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. J.Apic. Res., 52(1), 1–36.