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INFLUENCE OF FROZEN AND DRIED POLLEN FEEDING ON FAT BODY DEVELOPMENT IN WORKER BEES (*Apis mellifera* L.)

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Abstract

The study presents the influence of frozen and dried pollen feeding on the fat body development (FBD) of worker bees (Apis mellifera L.). The degree of FBD was determined before and after feeding during the Autumn and Spring periods. During Autumn, the highest percentage of bees (42.9%) with a second degree of FBD was found after feeding with dried pollen. During the same period, 38.1% of bees fed in this way were observed to have reached the third degree of FBD, and 19.0% of bees had reached the fourth degree of FBD. After completion of spring feeding with dried pollen, the highest percentage of bees (58.6%) with the fourth degree of FBD was reported. This percentage was significantly higher than the percentage before feeding (30.8%), with a difference of 27.8%. A significant increase in the percentage of bees (11.1%) fed with frozen pollen with the fifth degree of FBD was recorded for this period. The high degree of FBD of the worker bees indicates the greater effect of frozen pollen feeding compared to dried pollen feeding during the spring period.

Key words: *Apis mellifera L., fat body, feeding, frozen and dried pollen, honey bees.*

INTRODUCTION

The presence of pollen and honey in nature is very important for bee specimens and has a direct impact on the development and functioning of their fat body. Worker bees accumulate fat deposits, carbohydrates, and proteins in it after consuming pollen and honey. Pollen is a major source of amino acids, proteins, fats, vitamins, and minerals for honey bees (Stanley & Linskens, 1974; Roulston & Buchmann, 2000). It is a major factor determining the life span of specimens (Haydak, 1970).
According to some authors, insufficient quantity and poor quality of pollen in nature lead to delayed development of bee brood (Smart et al., 2016), suppression of bee reproduction, and the emergence of worker bees with shorter lifespans (Zheng et al., 2014). The increase and decrease in brood areas depend on the egg-laying activity of queen bees, as well as the availability of pollen in the environment during different seasons of the year (Liolios et al., 2015; Di Pasquale et al., 2016; Filipiak et al., 2017). The pollen collected by bees during autumn is crucial for bee colonies as it is rich in nutrients necessary for bees during wintering (De Grandi-Hoffman & Chen, 2015). There is evidence that "forager" bees exhibit preferences for specific micronutrients in pollen, which vary depending on the season (Bonoan et al., 2018). The rich nutrient content of pollen contributes to the development of worker bee fat bodies (Alaux et al., 2010). Most of the fatty acids obtained from food are transported to it (Skowronek et al., 2021). An unbalanced diet rich in fatty acids disrupts the ability of worker bees to recognize diseased broods, which can negatively affect bee colony hygiene (Bennett et al., 2022). According to Maurizio (1961), the degree of development of the fat body in bees is an important indicator of the physiological state of the insects. Research by several authors shows that it actively participates in the processes of metabolism of other organs as well (Hoshizaki, 2013; Aljedani, 2018). In periods of stress for bee colonies, such as a shortage of food in nature, a long flight of bees, a sudden change in environmental temperature, etc., honey bees maintain homeostasis in their body by mobilizing nutrients from their fat body with the participation of various hormones - octopamine, adipokinetic hormone, and juvenile hormone (Beenakkers et al., 1985; Gruntenko et al., 2000; Hirashima et al., 2000; Arrese & Soulages, 2010; Kodrik et al., 2015;

Tao et al., 2016). The degree of development of the fat body is also of particular importance in the production of royal jelly and the feeding of brood by young worker bees (Crailsheim, 1992). Some authors have reported that malnourished bees have less developed fat bodies (Keller et al., 2005; Toth & Robinson, 2005). Others have found that during the last 1-2 weeks of the life of worker bees, their fat body stores decrease (Toth & Robinson, 2005). Differences in the degree of development of the fat body have been observed in summer and winter bees (Lotmar, 1939). Fluri & Bogdanov (1987) proved that the fat body is larger in winter bees. The food reserves accumulated in it during the autumn are used by the bees during wintering (Maurizio, 1961). Shumkova et al. (2019) found a positive influence of stimulating feeding of bee colonies in the autumn period on the degree of fat body development of worker bees. According to several authors, the floral origin of pollen consumed by bees is also of great importance for the development of the fat body of bees (Di Pasquale et al., 2013). In beekeeping practice, the feeding of bee colonies takes place mostly in the spring and autumn periods. When there is a shortage of pollen in nature and bee nests, bee colonies are provided with protein substitutes for pollen or foods with added pollen. After collecting pollen from the bee colonies, it is stored by drying (Regulation No. 9, 2005; Dominguez-Valhondo et al., 2011) or freezing (Nath & Anderson, 1975). In this regard, it is appropriate to investigate whether there are differences regarding the development of the fat body of worker bees from colonies additionally fed with frozen and dried pollen.

The study's objective is to investigate the influence of feeding bee colonies with frozen and dried pollen on the development of the fat body in worker bees (*Apis mellifera* L.).

MATERIALS AND METHODS

The survey was conducted in the fall of 2022 and the spring of 2023 at the Educational Experimental Base of the Beekeeping section at the Faculty of Agriculture of Trakia University - Stara Zagora, Bulgaria. Feeding of bee colonies of the local honey bee (*Apis mellifera* L.) previously equalized in terms of strength, number of brood, and food reserves was carried out. Two experimental and one control groups were formed with two bee colonies each, as follows:

- **1st Experimental group** – six feedings at seven days' intervals with 160 g polyfloral bee honey mixed with 40g of dried pollen;
- **2nd Experimental group** – six feedings at seven days' intervals with 160 g polyfloral bee honey mixed with 40g of frozen fresh pollen;
- **Control** – six feedings at seven days' intervals with 200 g polyfloral bee honey.

Worker bees were sampled from all bee colonies to determine the degree of fat body development before and after feeding in the autumn and spring periods. The degree of development of the fat body of 530 bees was determined according to the method of Maurizio (1954) - a 5-point scale.

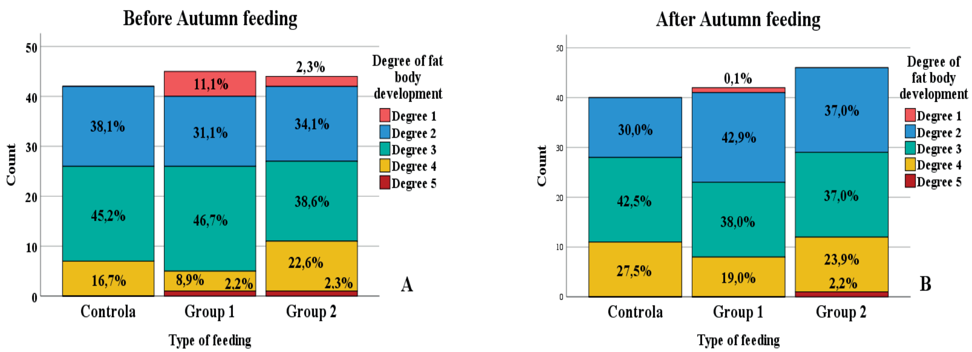
Statistical software IBM® SPSS® Statistics 26.0 (NY, USA) was used for data processing, applying the non-parametric method "x^2 – analysis" (chi-square).

RESULTS AND DISCUSSIONS

1. Autumn period

Figure 1 illustrates the results of the x^2-analysis regarding the degrees of development of worker bees' fat bodies for the periods before and after autumn feeding. Before autumn feeding, the highest percentages of bees whose fat bodies reached the third degree of development have been recorded, both for the experimental groups (46.7%; 38.6%) and for the control group (45.2%). The highest percentage of bees with fat bodies that reached the fourth degree of development has been reported for Group 2 (22.6%), followed by the control group (16.7%). A probable reason for this relatively high rate of fat body development in bees is the supply of bee colonies with pollen from the summer period. The presence of bee pollen in bee colonies during this time of the year is a crucial condition for raising brood from which worker bees will hatch for the successful wintering of the colony (Dorea et al., 2010). The percentage of bees with fat bodies that reached the fifth degree of development is the same in both experimental groups. In the control group, no

bees have been found whose fat bodies have reached the fifth degree of development.
The results for the autumn period after feeding the bee colonies show that when feeding with dried pollen, the highest percentage of bees whose fat bodies have reached the second degree of development (42.9%). The third degree of development has been reached in 38% of the bees also fed with dried pollen. Interestingly, the fourth degree of development of the fat body has been observed in 19.0% of the bees from Group 1, and before feeding this percentage was only 8.9%.
As for bees fed frozen pollen, a higher percentage of fat body development (fourth degree) has also been recorded after feeding (23.9%) compared to the percentage before feeding (22.7%). An equal percentage of bees (37.0%) whose fat bodies have reached the second and third degrees of development has been reported in the group fed with frozen fresh pollen. The percentage of bees whose fat bodies have reached the fifth degree of development was small and remained relatively constant before and after autumn feeding with frozen pollen. In the control group, a high percentage of bees with the third degree of fat body development has also been reported (42.5%), followed by those with the second degree of development (30.0%). A relative increase of bees from the control group whose fat bodies have reached the fourth degree of development (27.5%) has been observed, which is most likely due to the pollen obtained from nature.



Cramer's V = 0.204; Sig. (p) = 0.026

Cramer's V = 0.117; Sig. (p) = 0.471

Figure 1. Influence of feeding on the degrees of fat body development of worker bees before and after Autumn feeding
* $p < 0.05$, Group 1 – feeding with dried pollen, Group 2 – feeding with frozen pollen

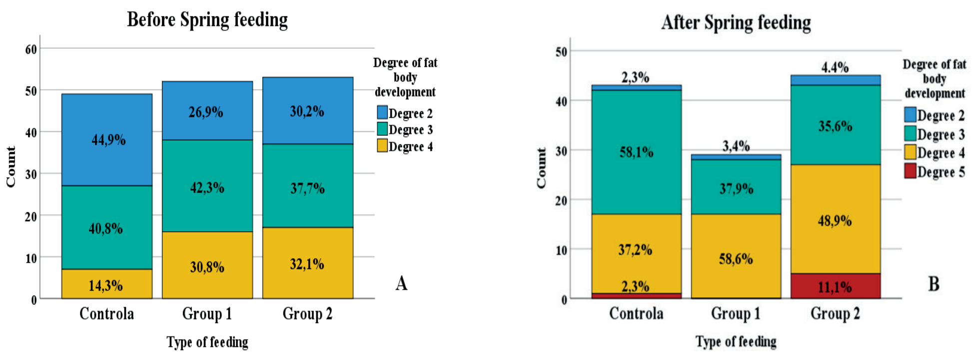
Considering the fact that all the bee colonies included in the study were under the same climatic conditions and bee pasture, it can be assumed that the differences in the development of the fat bodies of the bees from the analysed samples are to a certain extent due to the feeding as well. A support for this statement is Cramer's V coefficients (0.204 and 0.117), which show that the influence of the type of feeding on the development of fat bodies of worker bees is weak, but statistically significant.

2. Spring period

The provision of pollen in bee nests during the Spring period is essential for the proper development of bee colonies. The lack of food, especially the lack of pollen, leads to the weakening of bee colonies (Mattila & Otis, 2006). The nutrients in pollen (proteins, lipids, vitamins, and minerals) are essential for the survival of the colonies (Brodschneider & Crailsheim, 2010). According to some authors, complete deprivation of pollen may reduce the lifespan of bees (Wang et al., 2014). Some authors have reported that malnourished bees have less developed fat bodies (Keller et al., 2005; Toth & Robinson, 2005).
Figure 2 illustrates the results of the x^2-analysis regarding the degrees of development of worker bees' fat bodies for the periods before and after spring feeding. Before the spring feeding, the established trend of development of the fat bodies was preserved, as before the

autumn feeding. The highest percentages of bees whose fat bodies had reached the third degree of development have been found in the experimental groups (42.3%; 37.7%). In the control group, the percentage of bees whose fat bodies had reached the second degree of development was the highest (44.9%), followed by those with the third degree of development (40.8%). The highest percentage of bees whose fat bodies had reached the fourth degree of development has been reported for Group 2 (32.1%), followed by Group 1 (30.8%). No bees have been found the fat bodies of which had reached the fifth degree of development.
After the end of the spring feeding, it has been found that when feeding with dried pollen, the highest has been the percentage of bees the fat bodies of which had reached the fourth degree of development (58.6%). For comparison before feeding, the percentage in this group had been 30.8%. A difference of the order of 27.8% is observed, which is indicative of the effect of additional feeding. Other authors have reached similar conclusions (Shumkova et al., 2019). In the present experiment, it has been found that no bees whose fat bodies had reached the fifth degree of development when feeding with dried pollen.

In the bee colonies fed with frozen pollen, there is a tendency to increase the number of bees whose fat bodies have reached the fourth degree of development (48.9%). An even higher percentage of difference has been found compared to the percentage for this group before feeding (32.1%). The stated difference is 16.8%. In addition, a significant increase in the number of bees with fat bodies developed to the fifth degree (11.1%) has been reported. This shows the greater effect of feeding bee colonies with frozen pollen compared to dried pollen during the spring period. Although there is an influx of pollen from the flowering plant species in the environment during the spring period in the beehives, it turns out that the addition of extra amounts of pollen has a positive effect on the development of the fat bodies of worker bees. In the control group, the percentage of bees whose fat bodies had reached the third degree of development was the highest (58.1%), followed by those with the fourth degree of development (37.2%). In this group, 2.3% of bees with the fifth degree of development of their fat bodies have been found.



Cramer's V = 0,147; Sig. (p) = 0,050

Cramer's V = 0,209; Sig. (p) = 0,016

Figure 2. Influence of feeding on the degrees of fat body development of worker bees before and after Spring feeding
* $p < 0.05$, Group 1 – feeding with dried pollen, Group 2 – feeding with frozen pollen

As with autumn feeding, Cramer's coefficients (0.147; 0.209) show a weak but statistically significant relationship between the type of feeding and the development of worker bees' fat bodies. Based on the results of the spring feeding of the bee colonies with frozen and dried pollen, it can also be assumed that the differences in the development of the fat bodies of the bees from the analyzed samples are to some extent also due to the feeding. Similar conclusions have been reached by other authors (Shumkova et al., (2019).

CONCLUSIONS

In the autumn period after feeding the bee colonies, the highest percentage (42.9%) of development of fat bodies reaching the second degree was found in the bees fed with dried pollen.

During the same period, the third degree of development of the fat body was reached in 38% of the bees fed with dried pollen, and the fourth degree of development was observed in 19.0% of the bees from this group, while before feeding this percentage was only 8.9%.

After completion of spring feeding with dried bee pollen, the highest percentage of bees in which fat bodies reached the fourth degree of development (58.6%) was reported compared to the percentage before feeding (30.8%), with a significant difference in the order of 27.8%.

For this period of the year, when feeding with frozen pollen a significant increase in the percentage of bees whose fat bodies are developed to the fifth degree (11.1%) was recorded. This shows the greater effect of feeding bee colonies with frozen pollen compared to dried pollen during the spring period.

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