



Evaluation of food regime on hygienic behavior of honeybee colonies

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Abstract

Nine colonies of honeybee , *Apis mellifera* L. (Hymenoptera: Apidae) headed with newly open mated local Carnica queens, *A. mellifera carnica*, and placed at the Plant Protection Research Institute, Beekeeping Research Dept., Dokki, Egypt, Agriculture Research Center, were used to study the effect of food regime on hygienic behavior in honeybee colony by determining the time rate at which the bee removed dead brood. The data revealed that the removal of dead brood after 6,12 and 24 hrs. from treatments in case of pollen cake diet was significantly higher than that yeast cake diet, also in spring than autumn seasons. Lastly, the colonies feeding sugar syrup only had the lowest significant differences.

Introduction

The nutritional needs of honeybees are among the factors that have a direct impact on the life of the bee colony in terms of the activity of bees and their ability to shed and resist diseases and encourage the queen to lay eggs. Brood rearing is a major factor in apiary production and is affected by colony feeding in nature on nectar and pollen. Honeybees, *Apis mellifera* L. (Hymenoptera: Apidae) like other living organisms, need carbohydrates, proteins, fats, vitamins, minerals, and water for brood rearing.

The only protein source in the diet of honeybees is the pollen which contains all the lipids, vitamins, and minerals necessary for the normal growth and development of a bee colony (Herbert and Shimanuki, 1978). However, supplementary feeding was developed by many investigators to increase the number of workers in the colony

whenever needed to improve the productivity of commercial apiaries. During a dearth period, the number of bee colonies may decrease, adversely affecting the production of honey in the following season. A colony of honeybees needs to collect about 17 and 34 kg of pollen yearly to meet their requirements for these nutrients, (Crailsheim *et al.*, 1992 and Keller *et al.*, 2005).

Application of 25% and 50% of yeast culture results in the highest significant amount of worker brood during the floral dearth period (Abd El-Wahab and Gomaa, 2005). The most effective pollen substitutes and supplements are those that are most similar in chemical composition and physical consistency to stored pollen (Schmidt *et al.*, 1987 and Saffari *et al.*, 2010), the significant increase in brood rearing reflects on a significant increase the colony population

and colony production (Ghazala and Nowar, 2013).

Hygienic behavior in honeybees was studied for the mechanism of hygienic behavior by Abdel-Wahab (2001), Abdel-Rahman (2004) and Amro (2009) in Egypt, and for the resistance to American foulbrood disease by Spivak, 1996 and 1997 in Germany and by Spivak and Reuter (2008) in the USA. Approximately, 39% of the hygienic colonies developed clinical symptoms of the disease, but five of these recovered (had no visible symptoms) leaving two colonies (11%) with clinical symptoms.

In contrast, 33% of the hygienic colonies developed clinical symptoms of Chalk Brood after they were challenged with American Foulbrood, but all recovered. The diseased non-hygienic colonies produced significantly less honey than the hygienic colonies. The supplement "BEEWELL Amino Plus" induced a significant and consistent increase in hygienic behavior despite the negative effects of *N. ceranae* and viral infections. *N. ceranae* and viruses significantly and consistently decreased hygienic behavior, but also threatened the survival of bee colonies (Zoran et al., 2022)

The present study aimed to throw light on the effect of protein nutrition on hygienic behavior of honeybee colonies.

Materials and methods

The present study conducted in the apiary situated at the Plant Protection Research Institute, Beekeeping Research Dept., Dokki, Egypt, Agriculture Research Center, for two successive years (2021 and 2022).

1. Experimental honeybee colonies:

Nine honeybees (*A. mellifera*) colonies headed by nearly the same age of newly open mated local Carnica queens were used in this experiment. The colonies were about the same in their strength, each one contained 6 combs covered with adult bees, three of which were brood combs. The

colonies were divided into three groups (3 colonies each). Each group received a different type of diet for two months before recording the data for hygienic behavior. The diets were:

- 1.1. 1st group received 50g pollen cake + 250ml sugar syrup /colony / 3day intervals.
- 1.2. 2nd group received 100g Yeast cake + 250ml sugar syrup /colony / 3day intervals.
- 1.3. 3rd group used as a control and received 250ml sugar syrup (1:1) / colony / 3day intervals.

2. Preparing experimental feeding diets:

Diets were provided as follows:

- 2.1. Pollen cake consists of 1kg fresh bee pollen pellets + 350ml honey.
- 2.2. Yeast cake consists of 300g refined sugar + 300g yeast+ 100g powder milk fat free +300g honey.
- 2.3. Sugar syrup is prepared by dissolving 1kg refined sugar in 1 liter of warm water.

3. Estimating hygienic behavior:

The evaluation of the hygienic behavior in the honeybee colony was done as follows:

- 3.1. The bees in each experimental colony were compressed by reducing the number of combs to 5 instead of 6.
- 3.2. The cape of one hundred sealed worker brood cells was damaged and the immature stages within were killed using a toothpick.
- 3.3. Determining the rate at which the bee removed dead brood and cleaned the cells after 6, 12 and 24 hrs.
- 3.4. The proportion of bees of each treatment, participating in hygienic tasks was calculated by dividing the number of bees uncapping cells or removing brood over the number of bees remaining alive for each treatment during the days of observation.

4. Statistical analysis:

Descriptive, ANOVA and LSD test (at 0.05) analyses were calculated by SAS computer program.

Results and discussion

The effect of offering food regimes for two months on the hygienic behavior of honeybees expressed by removal percentages of artificially killed worker brood recorded in the following tables. Data recorded in Table (1) and illustrated in Figure (1) after 6 hrs. of killing the worker brood after two feeding treatments showed that both treatments exhibited hygienic behavior, but with different rates. Where, the removal rate in case pollen cake treatment was significantly higher than that of yeast cake 56.78% and 47.36% for the spring season, respectively. Also, with different values during the autumn

season 48.14% of removed in pollen cake treatment and 40.39% at supplementary cake. While the % of the removal brood after sugar syrup recorded the lowest rate which was 35.90% in spring and 30.34% in autumn season. In general, offering the pollen cake to the bee colony in spring season appears high tendency to improve it hygienic manner followed by autumn season with significant differences between both. In the case of yeast cake, there are no significant differences between offering it in spring or offering pollen cake in autumn.

Table (1): Hygienic behavior of honeybee colonies after 6 hours.

Rep.	Pollen cake		Yeast cake		Sugar syrup	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
R1	57.41%	45.90%	47.62%	42.02%	33.15%	29.44%
R2	55.94%	49.85%	47.88%	42.28%	39.07%	31.12%
R3	56.99%	48.14%	46.57%	40.39%	35.47%	30.47%
Mean±SD	56.78 ^a % ±0.757	47.96 ^b % ±1.981	47.36 ^b % ±0.693	41.56 ^c % ±1.024	35.90 ^d % ±2.983	30.34 ^e % ±0.847
Spring F	98.9					
LSD	2.889					
Autumn F	125.8					
LSD	2.184					
General F	102.5					
LSD	2.349					

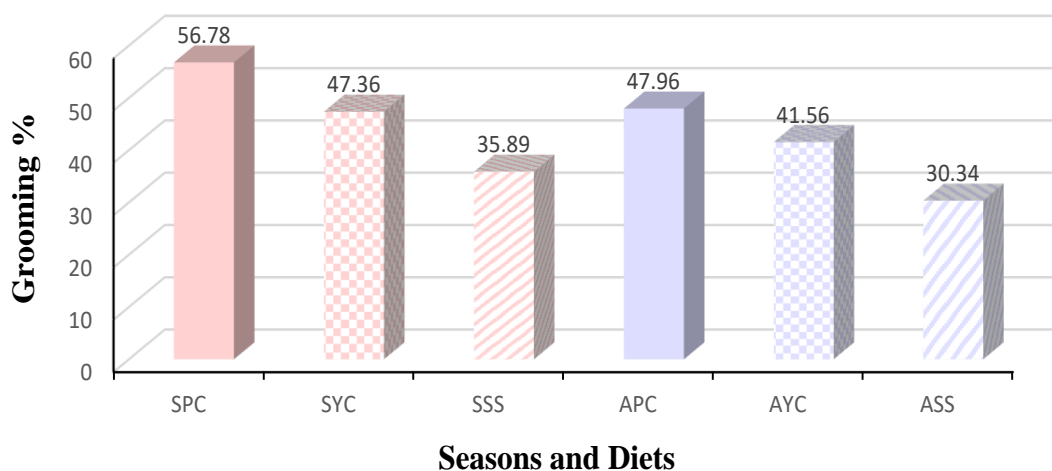


Figure (1): Hygienic Behavior of honeybee colonies after 6 hours.
 SPC: Spring pollen cake; SYC: Spring yeast cake; SSS: Spring sugar syrup.
 APC: Autumn pollen cake; AYC: Autumn yeast cake; ASS: Autumn sugar syrup.

Data recorded in Table (2) and illustrated in Figure (2) after 12 hrs. of killing the worker brood after two feeding treatments showed that both treatments exhibited hygienic behavior, but with different rates. Where, the removal rate in case pollen cake treatment was significantly higher than that of yeast cake being 76.27% and 69.44% for the spring season, respectively. Also, with different values during the autumn season were 73.36% of removal in pollen cake treatment and 65.83% in autumn.

at Supplementary cake. While the % of the removal brood after sugar syrup recorded the lowest rate which was 56.82% in spring and 51.25% in autumn season. In general, offering the pollen cake to the bee colony in the spring season appears high tendency to improve its hygienic manner followed by the autumn season with significant differences between both. In the case of yeast cake, there are no significant differences between offering it in spring and offering pollen cake in autumn.

Table (2): Hygienic Behavior of honeybee colonies after 12 hours.

Rep.	Pollen cake		Yeast cake		Sugar syrup	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
R1	75.71 %	75.68%	68.59%	66.96%	55.84%	49.9
R2	76.44 %	72.09%	70.83%	63.63%	55.64%	52.88
R3	76.67 %	72.30%	68.90%	66.91%	58.97%	50.98
Mean ±SD	76.27 ^a ±0.501	73.36 ^a ±2.01	69.44 ^b ±1.21	65.83 ^b ±1.90	56.82 ^c ±1.86	51.25 ^d ±1.50
Spring F	168.2					
LSD	2.091					
Autumn F	113.9					
LSD	2.892					
General F	111.82					
LSD	2.315					

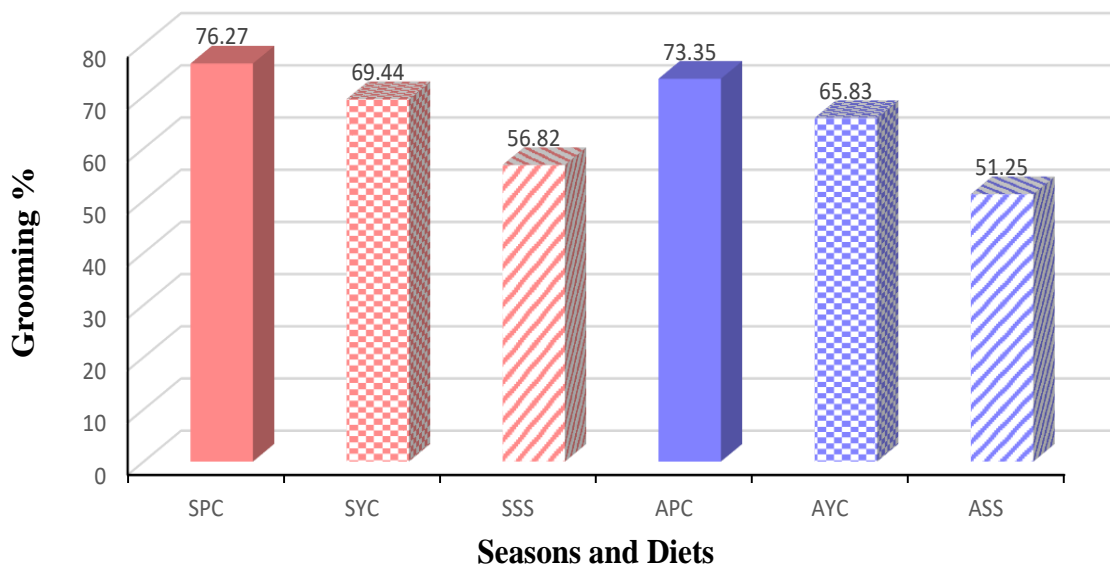


Figure (2): Hygienic Behavior of honeybee colonies after 12 hours. SPC: Spring pollen cake; SYC: Spring yeast cake; SSS: Spring sugar syrup. APC: Autumn pollen cake; AYC: Autumn yeast cake; ASS: Autumn sugar syrup.

Data recorded in Table (3) and illustrated in Figure (3) after 24 hrs. of killing the worker brood after two feeding treatments showed that both treatments exhibited hygienic behavior, but with different rates. Where, the removal rate in the case of pollen cake treatment was significantly higher than that of yeast cake being 90.33% and 88.12% for the spring season, respectively. Also, with different values during the autumn season 87.56% of removed at pollen cake treatment and 82.42%

at the supplementary cake. While the % of the removal brood after sugar syrup recorded the lowest rate which was 80.11% in spring and 76.57 % in autumn season. In general, offering the pollen cake to the bee colony in spring season appears to have a high tendency to improve it hygienic manner followed by the autumn season with significant differences between both. In the case of yeast cake, there are no significant differences between offering it in spring or offering pollen cake in autumn.

Table (3): Hygienic Behavior of honey bee colonies after 24 hours.

Rep.	Pollen cake		Yeast cake		Sugar syrup	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
R1	89.90%	87.22%	88.17%	82.49%	79.11%	76.71%
R2	90.18%	87.53%	87.65%	83.19%	79.47%	76.38%
R3	90.92%	87.94%	88.55%	81.59%	81.74%	76.61%
Mean ±SD	90.33^{ab} ±0.527	87.56^c ±0.361	88.12^{bc} ±0.452	82.42^d ±0.802	80.11^e ±1.426	76.57^f ±0.169
Spring F LSD			103.6 1.452			
Autumn F LSD			339.6 0.82			
General F LSD			154.6 1.082			

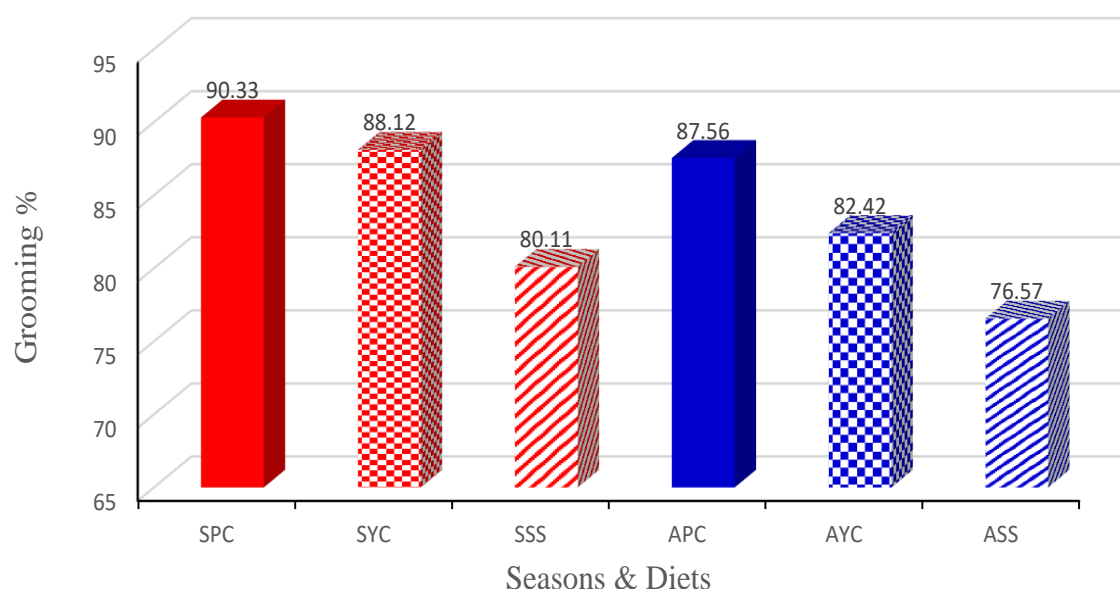


Figure (3): Hygienic Behavior of honeybee colonies after 24 hours.
SPC: Spring pollen cake; SYC: Spring yeast cake; SSS: Spring sugar syrup.
APC: Autumn pollen cake; AYC: Autumn yeast cake; ASS: Autumn sugar syrup.

In this study, we investigated two different feeding treatments to show that both treatments exhibited hygienic behavior, for two months on hygienic behavior of honeybees expressed by removal percentages of artificially killed worker brood after (6, 12 and 24 hrs.) recorded. At a removal rate in case pollen cake treatment was significantly higher than that of yeast cake being. And spring season significantly higher than autumn season and the general LSD at 6,12 and 24 was followed 102.5, 111.82 and 154.6 respectively. Hygienic behavior in honeybees is a complex, disease-resistant, polygenic trait (Guarna et al., 2017, Lapidge et al., 2002 and Oxley et al., 2010). Offering the supplement Beewell Amino Plus significantly stimulates hygienic behavior compared with not supplemented one. Moreover, such behavior increases with time progress (Zoran et al., 2022).

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