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(Original Article)

The Effect of Feeding Fermented Pollen Substitutes on Honeybee Colonies Activities During Pollen Scarcity Under Apiary Conditions

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Abstract

The primary requirement in beekeeping is to provide honeybee colonies with a protein-rich diet during periods of pollen scarcity. The present experiment was conducted using four types of protein diet treatments, classified as follows 1) unfermented diet (control), 2) fermented diet, 3) fermented diet containing 10% *Chlorella* and *Spirulina* (5% each), 4) unfermented diet + 10^7 CFU *Lactobacillus* spp. (probiotic) The treatments were compared to a 50% sugar solution, which served as a negative control. The results indicated that the fermented diet containing 10% *Chlorella* and *Spirulina* (5% each), significantly improved total food consumption, brood-rearing activity, and colony population under apiary conditions. Protein content in nurse bees and bacterial counts (probiotics) in the hindgut showed a significant increase ($p < 0$). 05) when fed different substitute diets compared to the control group. This research suggests that the abundance of microbes in the honeybee gut may play an important role in preventing the loss of bee colonies in winter. Among these diets the fermented diet containing microalgae proved to be the most effective during periods of pollen scarcity. The effectiveness of the diet is generally attributed to its nutrient levels. The current findings suggest the importance of using a fermented pollen substitute as a supplementary diet during periods of pollen scarcity.

Keywords: *Colony performance, Honeybee, Pollen substitute, Pollen scarcity*

Introduction

As landscape compositions change to agriculturally heavy monocultures that may fail to meet bees' nutritional needs, feeding pollen substitutes is now a regular management method (Naug, 2009), as well as intensive beekeeping (Paray *et al.* 2021). Pollen and sugar substitutes are being utilized more frequently by Egyptian beekeepers during fall and winter (Abdella *et al*. 2024). Therefore, improving the effectiveness and sustainability of pollen substitute diets can be considered important to modern beekeeping.

Honeybees are filled with a simple and consistent microbiota with roles in nutrition and immune functions (Kwong and Moran, 2016; Raymann and Moran, 2018). Gram-positive bacteria, such as *Bifidobacterium asteroides* and two *Lactobacillus* taxa (Firm

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5 and Firm 4), predominate in the bee stomach. (Kwong *et al*. 2014; Anderson and Ricigliano, 2017). Brar *et al.* (2025) suggests that the genus Lactobacillus may play a significant role in bee health during winter. It has been demonstrated that the microbiota of non-thriving colonies is deprived of taxa that promote health, especially *Lactobacillus* (Ribière *et al*. 2019).

When suitable pollen from blooming plants is unavailable or has just a limited nutritional value, beekeepers supplement colonies to encourage the development of brood or to cover up for times when forage is scarce (Nabors, 2000; Matilla and Otis, 2006). According to Hussein (1981), pollen collection was most effective from March to September in the Assiut region. By comparison, the least activity was recorded in November, December, and January.

These pollen substitute diets contain raw proteins such as soybeans, yeast, eggs, wheat, or lentils. Early attempts at formulating pollen substitute diets were reported by Standifer *et al.* (1973). Soy products are commonly used as pollen substitutes, with concerns that they may contain anti-nutritional components, including toxic sugars (Barker, 1977) and protease inhibitors (Liener, 1994; Sagili *et al*. 2005). When taken in the right amounts, probiotics benefit the host (Kalam *et al*. 2018). According to earlier research, probiotics release metabolites and digestive enzymes that enhance the host's ability to absorb nutrients and digest food (Jger *et al.,* 2019and Skrypnik *et al*. 2019). Additionally, probiotics can change the composition of intestinal microbial populations (Kim *et al*., 2019), enhance host immunity (Georgieva *et al*., 2015), and safeguard gut health (Oyetayo *et al.* 2003).

Dietary management is crucial for addressing certain nutritional deficiencies. A suitable nutritional supplement should contain key biomolecules involved in bee metabolism. This study aimed to compare the feeding impact of various artificial diets during a pollen scarcity period on certain activities and improve the health of honeybee colonies in Assiut, Upper Egypt.

Materials and Methods

Twenty local hybrid Carniolan honeybee colonies, (*Apis mellifera* L.), equal strength, led by mated sisters' queens, were chosen and kept in the apiary of the Faculty of Agriculture at Assiut University in Egypt. Each colony had five combs covered with bees. Colonies were randomly assigned to 5 groups (4 colonies each as replicates), and each group was provided with one of the five examined treatments. Colonies had no visible honeybee illnesses, adequate bee bread and honey stocks, and comparable strength at the start of the studies. Experiments were applied throughout the scarcity period, which lasted from November to January, as described by Zeinab (2019) in the same area.

1. Types of honeybee diets.

The tested pollen substitute types were administered in five forms as follows:

- SS: Control (0.5 litre of 50% sugar syrup).

- Diet 1: Unfermented pollen substitute.

- Diet 2: Fermented pollen substitute.

- Diet 3: Fermented pollen substitute containing 10% Chlorella and Spirulina (5% each).

- Diet 4: Unfermented pollen substitute, and probiotic (Apiprobiotic) (10^7CFU *Lactobacillus* spp.)

Each colony was given 100 grams of the diet every 6 days until the experiment ended in March. All supplemented diets were formed into patties, placed above the brood frames, and wrapped with a plastic sheet to prevent drying. Control colonies did not receive any pollen substitute diets. During the experiment, each colony (including the control group) was given 0.5 liters of sugar syrup (50:50 w/v) every week. The following parameters were used to assess the effectiveness of pollen substitutes on colony activity.

2. Measurements

During the experimental period, the following parameters were determined.

- Diet consumption

After the colony was provided with the diet, the amount consumed during the six days was calculated as the difference between the diet weight before and after it was introduced to the colonies (g/colony). The consumption was calculated every 12 days during the experimental period.

- Workers' population

Every 12 days, the adult honeybee population was assessed by counting how many frames were completely covered with bees. Based on the prior estimate, a fully covered frame from each side was determined to have two thousand bees. (DeGrandi-Hoffman *et al*. 2008).

- Worker sealed brood and beebread areas determination

A modified grid method was used to measure the sealed worker brood and beebread areas every 12 days. One-inch squares made up the grid (Amir and Peveling, 2004; Mattila and Otis, 2007). The comb area contained by sealed brood or bee bread was measured after the grid was set up on the brood frame area.

- Protein content of adult honeybees

Evaluating honeybee protein content is a widely used approach for evaluating the quality of various protein diets (De Jong *et al*. 2009 and Li *et al*. 2012). Five newly emerging bees from each colony were obtained at the start and end of the experiment to determine the change in total protein content. Only nursing bees were utilized to reduce protein variations caused by age. Nurse bees were determined by checking a brood frame and collecting out bees that care for the larvae. The body's protein content without the alimentary canal was detected using the Kjeldahl method, adapted by Rabie *et al*. (1983). The results were calculated as mg/g.

- Microbiological monitoring of hindguts to body protein content

A small number of newly emerged workers from each treatment were marked with paint on their thoraxes and allowed to age for 7 days in the same colonies. They were recaptured after 7 days of age for hindgut dissection and to determine the change in microbial count. At this age, the microbiota content can be established for nurse bees. Intestinal microbial content was determined at the beginning and after 8 weeks of feeding with the diet using the method of (Kaznowska *et al*. 2015).

- Estimating colony loss percentages

By dividing the average number of dead colonies until March by the average number of live colonies at the beginning of the experiment (November). The loss percentages were calculated.

3. Statistical analysis

Analysis of variance (ANOVA) was performed on all collected data using the CoStat software (Version 6.303, CoHort, USA, 1998-2004). The revised least significant difference (RLSD) approach was applied to compare mean differences at a 5% threshold of significance (Gomez and Gomez, 1984).

Results

1. Diet consumption

Table 1 represents the means of diets consumed from different pollen substitutes by honeybee colonies. Statistically significant differences were observed among the various supplement diet consumption over the time of the experiment. Honeybee colonies significantly consumed a higher amount of fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) (1314.75 ± 5.4 g) in comparison to unfermented pollen substitute (473.0 ± 2.3 g) fermented pollen substitute (921.75 ± 5.4 g) and pollen substitute +10^7CFU *Lactobacillus* spp. (578.75 ± 2.7 g) respectively.

Table 1. Means of consumption amounts by honeybee colonies fed different pollen substitute diets during the scarce period in the Assiut area (2022-2023).

Dates	Means of amount of consumed diet (g/colony)			
	Diet 1 pollen substitute (unfermented)	**Diet 2 fermented pollen substitute**	**Diet 3 Fermented pollen substitute + 10% microalgae.**	**Diet 4 Pollen substitute +10^7CFU *Lactobacillus* spp.**
13/11/2022	30.25±1.1 c	50±1.29 a	53.50±0.96 a	36.75±1.89b
25/11/2022	68.5±1.84 c	92.3±2.28b	143.50±1.84 a	95.00±1.47 b
7/12/2022	51.75± 1.54d	102.25±2.01b	154.00±2.08 a	67.50±1.04 c
19/12/2022	43.75± 1.49d	82.25±1.65b	126.00±1.68 a	53.00±1.41 c
31/12/2022	43.00±1.22d	86.75±1.18 b	127.50±1.32a	54.25±0.75 c
12/1/2023	50.75±0.47 d	91.25±0.75 b	131.50±0.95a	54.25±1.10 c
24/1/2023	40.00±0.81d	105.00±0.81 b	145.75±1.37 a	53.25±1.03c
5/2/2023	57.57±1.25c	116.00±1.29b	157.00±0.81a	55.25±0.62 c
17/2/2023	49.25±0.85d	102.00±1.47 b	154.00±1.29 a	56.75±1.18 c
1/3/2023	38.00±1.08 d	94.00±1.08 b	122.00±1.29a	52.75±1.25c
Total	473.0± 2.3 D	921.75±5.4 B	1314.8 ±5.4 A	578.75± 2.7 C
Deviation %	------	+ 94.87	+ 177.96	+ 22.36

Means followed by the same letter are not significantly different at 0.05 level of probability

2. Sealed brood, beebread areas and adult populations

The average sealed brood, beebread areas, and adult population at the beginning of the study did not differ among all groups of colonies chosen for different diets.

The data on sealed brood area of bee colonies fed on various diets are presented in Table 2. The total worker-sealed brood area differed significantly at the end of the experiment after feeding various diets. Colonies fed fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) had a significantly greater increase in brood areas than the other diets. Irrespective of the month, statistically, the highest average of sealed brood area was recorded for fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) (2108.0 ± 1.8 inch2/colony), followed by consumption of fermented pollen substitute (1268.4 ± 21.1 inch^2/colony). The lowest average brood area was recorded in the control group (471.25 ± 6.3 inch^2/colony) over the twelve-day intervals.

Table 2. Effect of feeding different pollen substitute diets on sealed brood areas during the pollen scarce period in the Assiut area (2022-2023).

Dates	Mean of sealed brood mean areas ± standard error (inch^2/colony)				
	SS Sugar syrup only	**Diet 1 pollen substitute (unfermented)**	**Diet 2 fermented pollen substitute**	**Diet 3 Fermented pollen substitute + 10% microalgae.**	**Diet 4 Pollen substitute +10^{7}CFU *Lactobacillus* spp.**
1/11/2022	165.00±0.57 a	165.00±0.01 a	165.33±0.66a	165.17±0.67a	165.17±0.93a
13/11/2022	137.75±1.10 c	136.75±0.85c	146.50±1.5b	174.25±2.05a	148.00±1.77b
25/11/2022	82.50±2.72 e	129.25±2.17d	164.75±2.86b	230.75±2.78a	144.00±3.10c
7/12/2022	40.75±2.25e	83.50±3.30d	130.50±1.65 b	210.25±1.49 a	105.25±2.92c
19/12/2022	25.00±0.91 e	46.25±2.05d	88.00±4.67 b	170.50±3.96a	57.00±0.91c
31/12/2022	6.50±0.95e	38.00±0.91d	89.5±4.69 b	191.50±3.66 a	56.00±1.87c
12/1/2023	5.25±0.25e	42.25±1.10 d	71.75±3.90b	170.25±2.65a	58.50±2.9c
24/1/2023	3.25±0.25e	34.75±1.18d	79.00±4.10b	179.25±2.95 a	56.25±2.01c
5/2/2023	3.0±0.00e	39.50±2.21d	96.00±2.85b	192.50±3.09a	57.50±2.72c
17/2/2023	1.25±0.25e	43.75±1.49d	111.00±1.82b	201.25±1.37a	69.00±3.80c
1/3/2023	1.00±0.40e	60.75±1.65d	126.00±2.85b	222.25±1.79a	79.75±1.37c
TOTAL	471.25±6.3 E	819.75±10.5 D	124.33±21.1 B	2108.0±11.8 A	996.5±4.1 C
Deviation %	------	73.95	169.16	347.32	111.46

Means followed by the same letter are not significantly different at the 0.05 probability level

The data on a number of adult bee populations of honeybee colonies fed on various pollen substitute diets during the scarcity period are presented in Table 3. The mean number of adult bee populations was significantly higher in the case of fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) diet (5562.5 ± 62.5), followed by those fed fermented pollen substitute diet (4125.0 ± 125), pollen substitute + 10^{7}CFU *Lactobacillus* spp. diet (3500.0 ± 0.0) and finally, unfermented pollen substitute diet (2875 ± 125). The number of adult bee populations was the lowest in the control groups.

Table 3. Effect of feeding different pollen substitute diets on the bee population during the pollen scarce period in the Assiut area (2022-2023).

Dates	Number of the bee population (mean ± SE)				
	SS Sugar syrup only	Diet 1 Pollen substitute (unfermented)	Diet 2 Fermented pollen substitute	Diet 3 Fermented pollen substitute + 10% microalgae.	Diet 4 Pollen substitute +10^7CFU *Lactobacillus* spp.
1/11/2022	4750.00±28.87a	4750.00±16.67a	4750.00±28.8a	4750.00±66.66a	4750.00±42.2a
13/11/2022	4250±144b	4250.0±144.3 b	4750.0±144.3a	4375.0±125.0ab	4187.5±119.7a
25/11/2022	3312.5±119.7c	3187.5±119.7c	412.0±125.0b	4937.5±62.5a	3500.0±102.1c
7/12/2022	2212.5±126.44d	2937.5±62.5 c	4175.0±118.1b	4850.0±119.0a	3062.5±62.5c
19/12/2022	1387.5±42.7e	2125.0±72.16d	3062.5±62.5b	4325.0±59.5a	2562.5±62.5c
31/12/2022	937.5±62.5d	2250.0±144.3c	3625.0±125.0b	4062.5±62.5a	2187.5±119.7c
12/1/2023	1000±0e	2050.0±50.0d	3375.0±72.2b	4375.0±72.2a	2362.5±80.0c
24/1/2023	725.0±14.4e	2000.0±0.0d	3225.0±103.0b	4262.5±102.8a	2362.5±80.0c
5/2/2023	512.5±2.5e	2000.0±0.0d	3500.0±0.0b	4812.5±119.7a	2812.5±119.7 c
17/2/2023	287.5±12.5e	2250.0±144.3d	4075.0±75.0b	5137.5±80.0a	3000.0±0.0c
1/3/2023	187.5±12.5e	2875.0±125.0d	4125.0±125.0b	5562.5±62.5a	3500.0±.0.0c

Means followed by the same letter are not significantly different at 0.05 level of probability

The colony feeding began on November 1st when the stored pollen areas were at their lowest level in honeybee colonies in the Assiut region. Beebread areas gradually decreased from the start of feeding, completely disappeared from mid-December, and started to increase in March for all tested diets under supplemental feeding conditions with fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each), followed by feeding with fermented pollen substitute, then pollen substitute + 10^7 CFU *Lactobacillus* spp, and then unfermented pollen substitute. However, the stored bee bread area had disappeared entirely in control colonies, which depended on feeding with sugar syrup only until the end of the experiment.

Table 4. Effect of feeding different pollen substitute diets on stored bee bread area during the pollen scarce period in the Assiut area (2022-2023).

Dates	Mean of bee bread areas (inch^2/colony)				
	SS Sugar syrup only	Diet 1 Pollen substitute (unfermented)	Diet 2 Fermented pollen substitute	Diet 3 Fermented pollen substitute + 10% microalgae.	Diet 4 Pollen substitute +10^7CFU *Lactobacillus* spp.
1/11/2022	40.33±0.17a	40.33±0.33a	40.5±0.21 a	40.16±0.44a	40.5± 0.29a
13/11/2022	39.50±0.64a	40.25±1.65a	28.75±2.17b	19.50±0.86c	36.25±0.75a
25/11/2022	21.50±0.95a	20.00±1.29a	7.00±0.81b	6.25±0.94b	18.75±1.31a
7/12/2022	3.00±0.81ab	0.75±0.47c	4.75±0.85a	1.25±0.25bc	1.75±0.47bc
19/12/2022 To 5/2/2023	0	0	0	0	0
17/2/2023	0.00	0.75±0.25b	1.00±0.40b	9.25±0.85a	2.00±1.08b
1/3/2023	0.00	9.00±0.81d	20.75±0.85b	26.25±0.75a	14.00±1.29 c

Means followed by the same letter are not significantly different at 0.05 level of probability.

3. Protein content of bees reared under different feeding conditions

The total protein content of newly emerged bees reared under different feeding conditions with pollen substitute diets was determined at the beginning and the end of the experimental work. Data in table (5) showed that the total protein percentages in all colonies fed different types of pollen substitute were positively increased when compared with the treatment fed sugar syrup only. At the end of experiment, the total protein content ranged from 164.8 ± 0.026 to 2270. ± 0.005. There was a significant effect of all pollen substitutes in comparison with colonies fed sugar syrup only; the colonies received sugar syrup produced bees with a significantly lower content of protein, whereas colonies fed fermented pollen substitute + 10% microalgae had the highest protein content, 227. 0± 0.005

Table 5. Effect of feeding different pollen substitute diets on protein content of adult honeybees during the pollen scarce period in the Assiut area (2022-2023).

Treatments		protein content (mg/gm)	Deviation% from control
At the beginning	**SS Sugar syrup only**	172.3 ± 0.075 d	---
At the end of the experiment	**SS Sugar Syrup only**	164.8 ± 0.026 e	- 4.35
	Diet 1 pollen substitute (unfermented)	198.2 ± 0.028 c	+ 15.03
	Diet 2 Fermented pollen substitute	209.3 ± 0.023 b	+ 21.47
	Diet 3 Fermented pollen substitute + 10% microalgae	227.0± 0.005 a	+ 31.74
	Diet 4 Pollen substitute +10^7CFU *Lactobacillus* spp	199.2± 0.053 c	+ 15.61

Means followed by the same letter are not significantly different at 0.05 level of probability

4. Hindgut bacterial counts

The changes in bacterial counts in hindgut were measured in seven-day-old nurse bees. At the beginning of feeding, there were no significant differences in hindgut bacterial numbers between different diets. However, at the end of the experiment fermented diet containing 10% *Chlorella* and *Spirulina* (5% each) had a higher impact on increasing bacterial counts (6.52±0.008) log CFU/ml in bees, followed by fermented pollen substitute (5.92±0.012), then pollen substitute + 10^7 CFU *Lactobacillus* spp, (5.63±0.012) and then pollen substitute (unfermented) (5.46±0.017). In general, hindgut bacterial counts were significantly reduced when bees were fed sugar syrup only.

Table 6. Effect of feeding different pollen substitute diets on hindgut bacterial count during the pollen scarce period in the Assiut area (2022-2023).

Treatments		Bacterial counts in hindgut log CFU/ml Mean ± SE	Deviation% from control
At the beginning	**SS Sugar syrup only**	4.729±0.086 e	---
After 8 weeks from the start of feeding	**SS Sugar Syrup only**	3.98±0.04 f	- 15.84
	Diet 1 pollen substitute (unfermented)	5.46±0.017 d	+ 15.46
	Diet 2 Fermented pollen substitute	5.92±0.012 b	+ 25.19
	Diet 3 Fermented pollen substitute + 10% microalgae	6.52±0.008 a	+ 37.87
	Diet 4 Pollen substitute +10^7CFU *Lactobacillus* spp	5.63±0.012 c	+ 19.05

Means followed by the same letter are not significantly different at 0.05 level of probability.

5. The loss rate of the colonies

The loss rate of the colonies was calculated by the type of diet (Table 7). There was no loss in the colonies fed on the fermented pollen substitute, Fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each), and pollen substitute +10^7CFU *Lactobacillus* spp. but it was (25%) in the colonies that fed on the unfermented pollen substitute, where the highest loss rate appeared in the control colonies fed sugar syrup only (50%).

Table 7. Loss percentage of the colonies in the apiary.

	Loss rate of the colonies (percentage %)				
Number of colonies	**Control (without diet)**	**pollen substitute (unfermented)**	**fermented pollen substitute**	**Fermented pollen substitute + 10% microalgae**	**Pollen substitute +10^7CFU/ l *Lactobacillus* spp.**
At the beginning of the experiment	4	4	4	4	4
At the end of the experiment	2	3	4	4	4
Loss rate %	50	25	0	0	0

Discussion

Nutritional stress and sanitary conditions appear to have a key influence on the significant amount of colony losses observed globally, including in Egypt (Abdel-rahman and Moustafa, 2012; Moustafa, 2013; Zeinab *et al.,* 2018)

Based on the results obtained from the laboratory tests, fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) diet and pollen substitute + 10^7 CFU

Lactobacillus spp were applied in the apiary during the scarcity period. Data on the population size of colonies indicated that the fermented pollen substitute + 10% microalgae diet group had a greater number of workers than that fed on pollen substitute + 10^7 CFU *Lactobacillus* strains. This can be explained by the fact that the addition of microalgae and the fermentation process (probiotics) to the pollen substitute provided nutritional benefits, allowing for increased growth and development.

Honeybee colonies may be encouraged to rear brood using pollen substitute diets (Standifer *et al*., 1973; Nabors, 2000; Mattila and Otis, 2006). However, these diets must be both nutritional and beneficial to bees. The bees in the current study consumed different amounts of each of the tested diets. Not every diet had the same effect on the adult bee population or brood rearing. Colonies fed the fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) consumed significantly more food than colonies fed the other three diets. High consumption can be used as a sign of feed palatability.

Consuming a large quantity of good-quality pollen has shown to lower the risk of the gut parasite *Nosema ceranae*, reduce the levels of harmful pathogens, boost honey bee immunity, and help them survive winter. Additionally, it improves the quality of drone semen and creates healthier honey bees. These healthier bees are better suited to handle stresses from pesticides, transportation, and parasites (Di Pasquale *et al.* 2013).

The results showed a significant increase in brood rearing and adult populations in colonies fed diet fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) compared with those fed the other diets. These findings imply that changes in diet nutritional quality, as well as the digestibility and accessibility of nutrients to worker bees, influence brood production. In addition to dietary considerations, brood rearing and population increase in colonies are influenced by the state of the queen and the number of worker population (Winston, 1987; DeGrandi-Hoffman *et al.,* 1989). Statistical differences were observed, with a similar trend toward greater increases in beebread reserves in supplemented colonies. The present results are similar to the results of Garcia-Vicente *et al.* (2023). The current study aimed to investigate the effect of dietary supplements containing probiotics and postbiotics on the bee population. The findings showed that postbiotics ingestion increased bee strength, egg laying, and bee population. Fortification of pollen substitute with the feed additive 10^7CFU *Lactobacillus* spp. improved pollen substitute intake compared to control group values. According to Han *et al*. (2023), these probiotics can enhance the digestion of nutrients in honeybees by increasing the activity of digestive enzymes, and the results of the laboratory-controlled experimentation show that *Lactobacillus spp*. and *Bacillus subtilis* may be more useful to honeybees than the other two examined strains.

The lack of natural pollen during the experimental period may have affected the colonies' response in terms of brood rearing and adult bee growth, especially in the early spring. The only food source available to bees was the food we fed, and colonies differed in brood production and adult bee growth. Some studies have shown that the application of protein supplements in honeybee colonies in late autumn/early winter increased the amount of brood, and reserves of pollen and honey. However, these benefits were observed after winter, in the following spring after supplementation, when the colony

continued developing and weather conditions improved (Younis, 2019; Topal *et al*., 2022). Perhaps similarly, in our investigation, some of these benefits in spring population growth were observed in treated colonies.

Nutritional supplementation generally resulted in higher protein content in nurse bees compared to control bees. The difference was significant, and we believe it is of biological importance. Given the significant challenges facing modern beekeeping, anything we can gain from better beekeeping practices can contribute. When combined or interacted with other factors, these factors can make a significant difference in colony performance.

The present findings are consistent with those of Srivastava *et al.* (2004), Sihag and Gupta (2011), and Pătruică *et al*. (2013). They found that after giving pollen supplements, bee colonies' pollen hoarding capacity was higher than that of the control.

Honeybees are characterized by a consistent and straightforward microbiota that plays roles in nutrition and immune functions (Kwong and Moran, 2016; Raymann and Moran, 2018)., Nutritional stress impacts the population of certain types of bacteria in honey bees, particularly *Lactobacillus* spp. and *Bifidobacterium* spp. which can impact host immunological function and pathogen sensitivity (Castelli *et al*., 2020). The efficacy of probiotics relies on their capacity to maintain a persistent presence in the bee gut, offering long-term advantages beyond rapid introduction. Diet significantly impacted the abundance of *Lactobacillus* spp. in colonies fed the fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each)-based diets are usually efficient pollen substitutes in laboratory circumstances (Ricigliano and Simone-Finstrom, 2020; Ricigliano *et al*., 2021). Using probiotics to improve the gut health of bees seems to be a promising approach to safeguard these important pollinators.

The loss rate of the colonies was no loss in the colonies fed on the fermented pollen substitute, fermented pollen substitute containing 10% *Chlorella* and *Spirulina* (5% each) and pollen substitute $+10^7$CFU *Lactobacillus* spp. but it was (25%) in the colonies that fed on the unfermented pollen substitute; where the highest loss rate appeared in the control (50%). Probiotics have been shown to increase the lifespan of honey bees. For instance, bees fed with probiotic supplements like SpasiPchel and PcheloNormosil exhibited increased colony strength and brood numbers during wintering, indicating improved health and survival rates (Mishukovskaya *et al*., 2020). Probiotics may affect the brain-gut axis, influencing bee behavior and social interactions. This can lead to improved foraging, recruitment, and disease response behaviors, potentially reducing colony losses by enhancing collective colony resilience (Killam *et al*., 2024).

Conclusion

The findings of this study clearly show the potential benefits of fermented protein supplementation with microalgae for honeybee colonies during pollen scarcity. We found that colonies supplemented with a fermented protein substitute contained more sealed brood than colonies supplemented with a traditional protein substitute after just two weeks. This effect persisted even in adult bee populations, and it also suggests increased winter survival rates through the accumulation of stored food resources and increased bee numbers. Furthermore, the combination of probiotics and protein probably

improved the honeybees' immune system. More research is needed to determine the effectiveness of probiotics in apiculture.

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تأثير تغذية بدائل حبوب اللقاح المخمرة على أنشـــطة طوائف النحل أثناء ندرة حبوب اللقاح تحت ظروف المنحل

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الملخص

من العمليات الأســـاســـية لرعاية طوائف النحل امداها بالغذاء الغني بالبروتين خلال فترات ندرة حبوب اللقاح. أجريت التجربة الحالية باستخدام أربعة أنواع من الأنظمة الغذائية البروتينية، مصنفة على النحو التـالي 1) نظـام غـذائي غير مخمر (الكنترول)، 2) نظـام غـذائي مخمر، 3) نظـام غـذائي مخمر يحتوي على 10% من الكلوريلا والســـبيرولينـا (5% لكل منهمـا)، 4) نظـام غذائي غير مخمر + 10^7 CFU من *Lactobacillus* spp. (بروبيوتيك). تمت مقارنة المعاملات بمحلول ســـكري 50%، والذي كان بمثابة كنترول ســـلبي. أشـــارت النتائج إلى أن النظام الغذائي المخمر الذي يحتوي على 10% من الكلوريلا والسبيرولينا (5% لكل منهما) قد حسن بشكل كبير من إجمالي استهلاك الغذاء ونشاط تربية الحضـنة وتعداد النحل تحت ظروف المنحل. أظهر محتوى البروتين في النحل الحاضـن وتعداد البكتيريا (البروبيوتيك) في المســـتقيم زيادة كبيرة ($p < 0.05$) عند إطعامها الأنظمة الغذائية المختلفة مقارنة بالكنترول السلبي (محلول سكري فقط). يشير هذا البحث إلى أن وفرة البروبيوتك في المعي الخلفي قد تلعب دورًا هامًا في منع فقدان طوائف النحل في الشـــتاء. ومن بين هذه الأنظمة، أثبت النظام الغذائي المُخمّر الذي يحتوي على الطحالب الدقيقة أنه الأكثر فعالية خلال فترات ندرة حبوب اللقاح. وتُعزى فعالية النظام الغذائي عمومًا إلى مستوياته الغذائية. وتشير النتائج الحالية إلى أهمية استخدام بديل حبوب اللقاح المُخمّر كغذاء مُكمّل خلال فترات ندرة حبوب اللقاح.

الكلمات المفتاحية: *أداء الطائفة، بديل حبوب اللقاح، نحل العسل، ندرة حبوب اللقاح.*