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Impact of winter feeding with protein diets on wintering ability and performance of honeybee (*Apis mellifera*) colony

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Abstract

Feeding honeybees with pollen substitutes is essential for maintaining the health of honeybee colony, especially during dearth periods. This study aimed to evaluate the use of five pollen substitutes as winter feed for honeybee during the pollen-scarcity period from October 2024 until April 2025, and to compare their effects on wintering ability, colony growth, and citrus honey yield. Cumulatively, honeybees consumed the largest amounts of diet4 (2073.34 g), followed by diet3, diet1, and diet5 (1785.47, 1773.87 and 1767.44g, respectively), and the lowest amounts of diet 2 (1404.89g). The results showed increase wintering ability in colonies fed pollen substitutes compared to control colonies. Also, colonies fed on diet1 showed the highest growth rate in terms of sealed brood area, bee bread area, and bee population size, followed by colonies fed on diet2 then diet5, while the lowest growth rate was observed in the unfed colonies (control). Similarly, citrus honey production increased in colonies supplemented with pollen substitutes compared to the control colonies. The diet1, diet2, and diet5, are based on spirulina and chlorella as primary ingredients, outperformed the others. These results bode well for the development of more effective diets, which could contribute to improving the performance of honeybee colony.

Key words: *Apis mellifera*, pollen substitutes, brood production, population density, bee bread, honey yield.

Introduction

Honeybees (*Apis mellifera*) live across a wide geographic range worldwide and inhabit in diverse climatic zones, facing different challenges throughout the year **(Rottner, 1988)**. The biggest challenge to honeybee colonies in temperate regions is winter, which often results in significant mortality rates (sometimes 30–40% in Western countries) because of the cold, limited foraging, and scarcity of feed **(Neumann and Carreck, 2010; Doeke *et al.*, 2015; Minaud *et al.*, 2024; Mainardi *et al.*, 2025)**. Winter losses have recently reached as high as 60% in America from June 2024 and March 2025 **(Nearman *et al.*, 2025)**. Honeybees are essential for pollination in both natural and agricultural habitats world-wide **(Khalifa *et al.*, 2021; Kaya *et al.*, 2023; Kumar *et al.*, 2024)**. Therefore, the successful overwintering of honeybee colonies is crucial for meeting the pollination needs of early spring-flowering crops.

Naturally, bees' diet consists of nectar and pollen, which they collect during their visits to flowers. Pollen provides essential nutrients for bee growth and development, containing proteins, amino acids, fatty acids, sterols, minerals, and vitamins **(Brodschneider & Crailsheim, 2010; Taha et al., 2019; Al-Kahtani & Taha, 2020; Al-Kahtani *et al*., 2020, 2021)**, while the nectar is the primary energy source for bees, consisting mainly of sugars and water **(Nicolson, 2011)**. During the winter, weather conditions are not suitable for bees, and the availability of natural feed resources such as nectar and pollen is very low **(Kumar & Agrawal, 2014; Tawfik *et al.,* 2020; Vincze *et al.,* 2025)**.

High mortality rates of honeybee colonies have been recorded during the winter in many countries, attributed to low temperatures during the winter season **(Al-Ghamdi *et al.,* 2016; Gray *et al.,* 2019; Mainardi *et al.,* 2025)**. Colony losses are also frequently attributed to malnutrition and starvation **(Brodschneider *et al.,* 2010)**. Ensuring the survival of bee colonies requires substantial feed supplies during scarcity periods.

During periods of pollen scarcity or complete absence, or when only poor-quality pollen is available, beekeepers often feed honeybee colonies with pollen substitutes or supplements **(Saffari *et al.,* 2006, 2010a, 2010b, Taha, 2015; Elwakeil *et al*., 2025; Taha *et al*., 2025, 2026)**. Some of the improved pollen substitutes have been shown to be better accepted and nutritionally valuable than pollen by honeybees **(De Grandi-Hoffman *et al.,* 2008; De Jong *et al.,* 2009; Saffari *et al.,* 2010a, 2010b).** However, to provide suitable protein feed for bees, 23-30% is considered the optimal ratio of protein in feed for honeybees **(Herbert *et al.,* 1977)**. In addition to total protein, **De Groot (1952)** identified the requirements of essential amino acids for honeybee growth and development. The fundamental principle of any pollen substitute is that it should contain all the components, textures, and compositions that are acceptable to honeybees and that meet their essential amino acid requirements.

Microalgae such as spirulina and chlorella **(Jehlík *et al.,* 2019; Nichols and Ricigliano, 2022)** as well as moringa *Moringa oleifera* **(Ali and Khalil, 2017; El-Bahnasy *et al.,* 2017)** have emerged as alternative feed additives to pollen in bee feed. These supplements provide essential nutrients, particularly during pollen shortages, to boost bee immunity, increase brood production, and improve colony productivity.

At the site of this experiment, weather conditions are unfavorable for bees during winter, and feed resources are extremely limited. Also, the bee colonies suffer greatly from feed shortages, in addition to the unsuitable conditions for foraging during the winter. Therefore, this study was conducted to examine the role of winter feeding with pollen substitutes on honeybee colonies throughout the winter season. This work aimed to evaluate the impact of winter-feeding on the wintering ability of colonies, eggs and sealed brood area of workers, colony population density, bee bread area, and citrus honey yield.

Materials and Methods

The experiments were conducted from October 2024 to April 2025 in the apiary of the Faculty of Agriculture, Benha University at Moshtohor, Qalyoubia Governorate, Egypt (Elev. 52 ft., 30.3527N and 31.2209E).

1. **Bees*:***

A hybrid of Carniolan honeybees (*Apis mellifera carnica* Pollmann × *A. m. lamarkii* Cockerell) was used in this experiment. Honeybee colonies were housed in Langstroth wooden hives, and headed by young sister open mated queens of the same age. These colonies were of equal strength (five combs, three sealed-brood, two honey, and pollen, covered with bees per colony).

2. **Diet preparation**

Pollen substitutes were prepared in tablet form according to the ingredients listed in Table **(1)** and were administered in cake form to the experimental colonies.

Table 1, Raw ingredients and their ratio in the various protein diets.

Diets	Spirulina (g)	Chlorella (g)	Moringa (g)	Pollen (g)	Corn flour (g)	Prowers yeast (g)	Wheat germ (g)	Sugar powdered (g)	Honey (ml)	Skimmed milk (ml)	Glycerin (ml)
Diet 1	30	--	--	--	20	20	25	30	25	15	10
Diet 2	--	30	--	--	20	20	25	30	25	15	10
Diet 3	--	--	30	--	20	20	25	30	25	15	10
Diet 4	--	--	--	30	20	20	25	30	25	15	10
Diet 5	10	10	10	10	20	20	25	30	25	15	10

3. **Experimental design**

24 colonies with the specifications mentioned above were used for the experiment of this study. Four colonies were allocated to each of the six treatment groups. Every week, each colony was provided with 200 g of the diets described previously in a perforated polyethylene bag above the combs on top of the brood nest + one liter of sugar syrup 50%.

Group 1: colonies fed on diet 1 based on spirulina, group 2: colonies fed on diet 2 based on chlorella, group 3: colonies fed on diet 3 based on moringa, group 4: colonies fed on diet 4 based on pollen, group 5: colonies fed on diet 5 based on a mix of all tested ingredients, and group 6: unfed colonies (control group). Each colony was provided with one liter of sugar syrup every week.

4. **Measurement of colony parameters**

4.1. Feed consumption

Throughout the experiment, feed consumption (g/colony/week) was recorded by calculating the difference in diet weight before and after feeding. Unconsumed diets were collected and weighed to determine the consumption rate for each diet per week.

4.2. Wintering ability

Wintering ability was calculated by using the following formula described by **Genç (1990).**

$$Wintering\ Ability\ (\%) = \frac{No.\ combs\ covered\ with\ bees\ until\ spring}{No.\ combs\ covered\ with\ bees\ in\ autumn} \times 100$$

4.3. Estimate of colony development (workers sealed brood area, bee bread area, and number of combs)

- ***Workers sealed brood and bee bread areas***

By using a wired hive frame divided into square inches, put over each side of a brood comb, the worker sealed brood area (square inches) was measured every 13 days. The measurement of sealed brood was performed 42 days after beginning the experiment. Also, the bee bread area was measured monthly using the same method.

- ***Colony population density (colony strength)***

The number of combs entirely covered with bees on both sides was monthly counted. A visual assessment of these combs was then performed on each side, and the number of bees was estimated by multiplying the total values (This was previously estimated at 2000 bees per fully covered both sides of the comb **(Guzman-Novoa et al. 2024, Kim et al. 2024)** by the number of combs fully covered by adult workers bees.

4.4. Honey yield

During citrus season 2025. The honeycombs were pulled from experimental colonies and then weighed before and after honey extraction, and the honey yield was estimated and recorded as kg per colony **(Ghazala and Nowar, 2013)**.

5. **Statistical analysis**

Data were analyzed using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to **Steel et al. (1997)**. In the experiment, a two-way analysis of variation (ANOVA) with a balanced, fixed-effect, independent design, which was performed to compare the effects of pollen substitutes consumption on colonies' bee bread area, brood area and population density of workers. Also, one-way ANOVA was used to compare the effects on wintering ability and citrus honey yield. The significance level of the tests was set at < 0.05.

Results:

1. **Feed consumption**

Regarding monthly consumption for diets ($P = 0.00$, **Fig. 1_A**). Colonies consumed an average of 178.56±4.68, 259.66±7.74, 256.05±7.25, 262.21±7.85, 270.44±8.01, 269.42±7.64 and 264.66±7.49g per month in October, November, December, January, February, March and April, respectively.

On the other hand, significant ($p < 0.05$) differences were observed in the consumption of different pollen substitutes from October to April. Cumulatively, honeybees consumed large amounts of diet 4 (2073.34g), followed by diet 3, diet 1, and diet 5 (1785.47, 1773.87 and 1767.44g, respectively), and lowest consumed feed was from diet 2 (1404.89g) **(Fig. 1_B and Table 2)**.

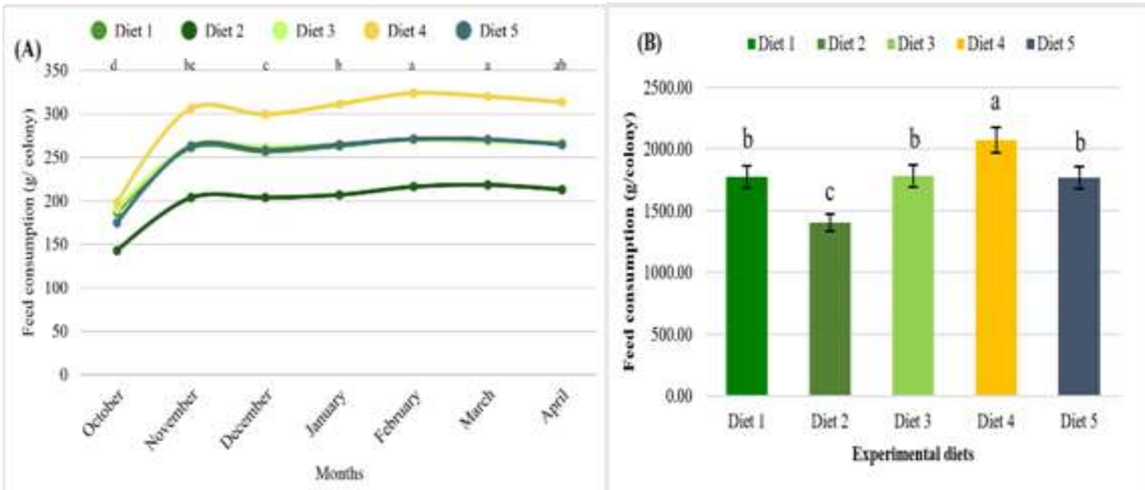

Fig 1 Feed consumption by honeybee colonies fed different pollen substitutes. **A)** Pattern of change in feed consumption in fed colonies during months of the experiment. **B)** Total feed consumption during the experiment. Different letters above the bars and patterns indicate statistically significant differences according to Duncan's multiple range comparisons (p < 0.05) (mean ± SE, n = 4).

2. Impact of winter feeding with pollen substitutes on wintering ability

Wintering ability was highest among honeybee colonies fed pollen substitutes compared to control colonies (70±5.77%). It was higher 170±5.77, 165±9.57, 140±11.55, 135±5.00 and 145±9.57 % in those fed diet1, diet2, diet3, diet4, and diet5, respectively, (*P* = 0.00, **Fig. 2)**.

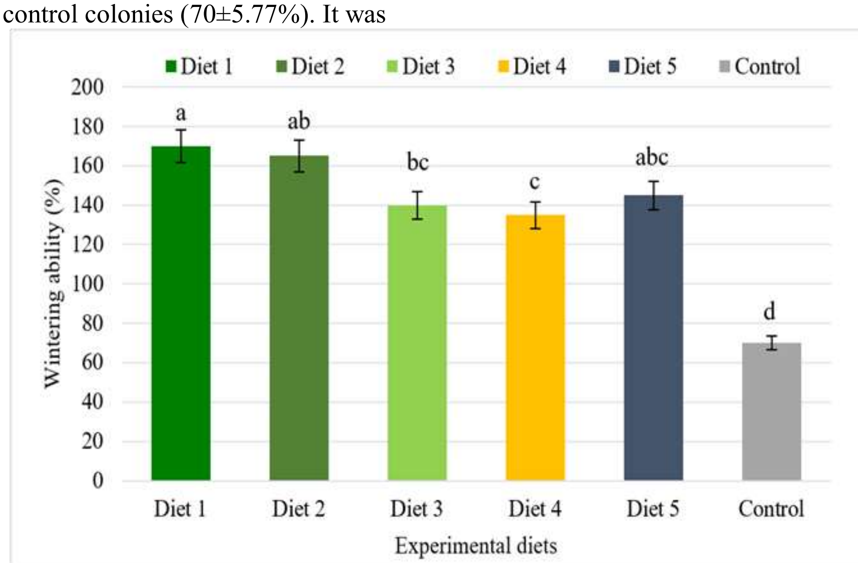

Fig 2 Mean wintering ability for honeybee colonies fed different pollen substitutes. Different letters above the bars indicate statistically significant differences according to Duncan's multiple range comparisons (p < 0.05) (mean ± SE, n = 4).

3. Impact of winter feeding with pollen substitutes on growth of colonies

To measure the rate of increase in the growth of honeybee colonies in the tested groups, the areas of the bee bread and workers sealed brood were measured, in addition to the population density of honeybee workers, from the beginning of the experiment to its end **(Figs. 3, 4, and 5)**. The values for brood area, bee bread area, and population density were fairly similar at the beginning of the experiment in October 2024, and there were no significant differences (p>0.05) among treatments. However, by April 2024, the average bee bread area, sealed brood area, and overall population density of workers had increased significantly **(Table 2)**.

Table 2, Total feed consumption, worker sealed brood area, number of bees, and bee bread area of honeybee colonies fed different pollen substitutes at the experiment's beginning from October 2024 through April 2025.

Diets	Total feed consumption (g/colony)	Worker sealed brood area ($inch^2$/colony)	Develop. (%) compared with control	Number of bees (1000bees)	Develop. (%)	Bee bread area ($inch^2$/colony)	Develop. (%) compared with control
Diet 1	1773.87±19.11^{b}	3712±33.24^{a}	112.59±4.58^{a}	40.00±0.00^{a}	100.00±0.00^{a}	432±7.55^{a}	135.23±32.07^{a}
Diet 2	1404.89±13.2^{c}	3615±77.77^{ab}	106.79±1.59^{a}	39.00±1.00^{a}	95.00±5.00^{a}	426±3.46^{a}	131.11±28.51^{a}
Diet 3	1785.47±26.36^{b}	3374±125.79^{bc}	93.03±6.07^{b}	35.00±1.91^{b}	75.00±9.57^{b}	397±14.53^{a}	118.40±36.25^{a}
Diet 4	2073.34±35.31^{a}	3311±85.14^{c}	89.58±5.26^{b}	35.00±1.00^{b}	75.00±5.00^{b}	404±11.25^{a}	119.72±29.28^{a}
Diet 5	1767.44±6.57^{b}	3508±80.75^{abc}	100.68±1.54^{ab}	37.00±1.91^{ab}	85.00±9.57^{ab}	417±5.17^{a}	125.66±25.42^{a}
Control	--	1749±50.77^{d}	--	18.00±1.15^{c}	0±0	192±19.52^{b}	--
P-value	**0.000**	**0.000**	**0.00**	**0.000**	**0.00**	**0.000**	0.024
LSD	60.94	240.54	11.53	3.96	18.53	34.62	82.80

a, b & c: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

The overall average area of sealed brood was highest, particularly in colonies fed the diet 1 (309.29±11.62inch^2/colony), followed by diet 2, diet 5, diet 3 and diet 4 by means of 301.21±10.77, 292.33±10.47, 281.17±9.94 and 275.94±9.01inch^2/colony, respectively. While the lowest mean of sealed brood area in control colonies (145.75±6.95inch^2/colony) (*P* = 0.00, **Fig. 3$_B$**). Furthermore, the area of sealed brood began to increase at the beginning of March (320.96±17.61inch^2/colony) and reached its peak by April (361.25±12.96inch^2/colony) (*P* = 0.00, **Fig. 3$_A$**).

Similarly, the highest overall average population density of workers were observed in colonies fed the diet 1 (25714.29±1475.42bee/colony), followed by diet 2, diet 5, diet 3 and diet 4 for 25000±1478.24, 24000±1284.83, 22857.14±1265.87 and 22714.29±1182.83bee/colony, respectively, and the lowest population in control colonies by the mean of 18000±1155 bee/colony (*P* = 0.00, **Fig. 4$_B$**). Monthly, the overall average worker bee population decreased during winter (November, December, and January for 19166.67±338.72, 18166.67±537.26 and 17166.67±612.87 bee/colony, respectively) and then increased again, reaching its peak in April by the mean of 34000±1615.15bee/colony (*P* = 0.00, **Fig. 4$_A$**).

Furthermore, the largest area of bee bread was in the colonies fed diet 1 by the mean of 61.68±4.87inch^2/colony, followed by diet 2 by 60.86±4.85inch^2/colony, while the lowest area of bee bread was in the control colonies by 28.37±2.61inch^2/colony (*P* = 0.00, **Fig. 5$_B$**). The area of bee bread decreased during the winter months of December and January by the means of 35.04±1.54 and 33.70±1.8inch^2/colony, respectively, then reached its peak in April by 89.42±4.24 inch^2/colony (*P* = 0.00, **Fig. 5$_A$**).

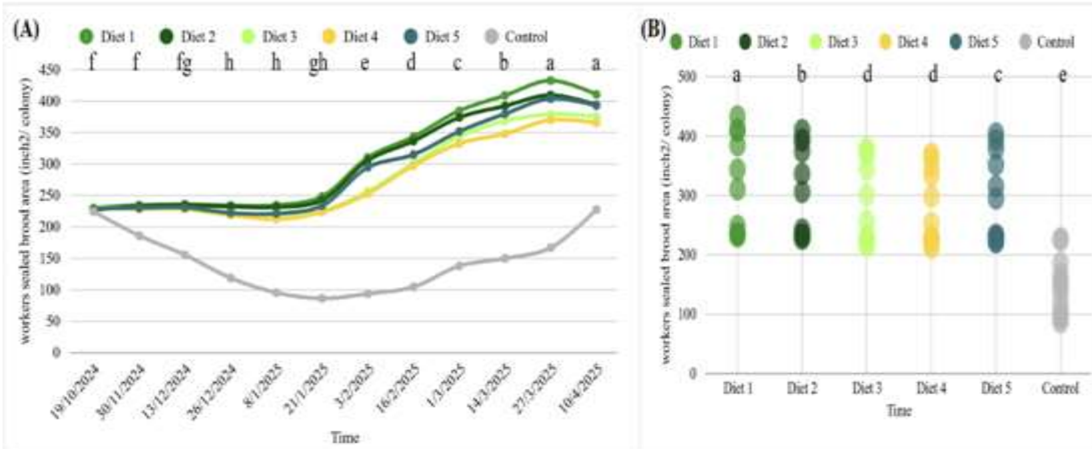

Fig 3 Fluctuation of workers sealed brood area for honeybee colonies fed different pollen substitutes. **A)** Pattern of change in workers sealed brood area in fed colonies during months of the experiment. **B)** Total means of workers sealed brood area. Different letters above the scatter plots and patterns indicate statistically significant differences according to Duncan's multiple range comparisons ($p < 0.05$) (mean ± SE, n = 4).

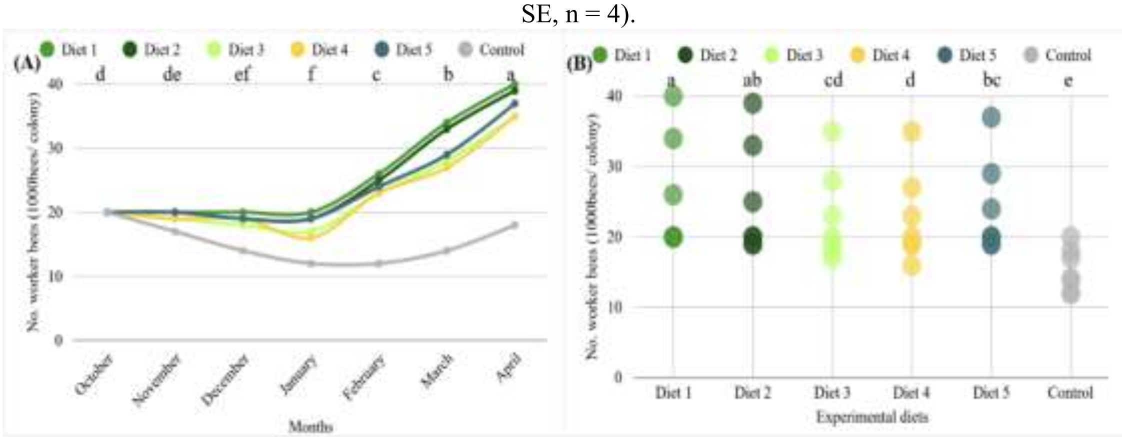

Fig 4 Fluctuation of bee number for honeybee colonies fed different pollen substitutes. **A)** Pattern of change in number of workers in fed colonies during months of the experiment. **B)** Total means of workers' number. Different letters above the scatter plots and patterns indicate statistically significant differences according to Duncan's multiple range comparisons ($p < 0.05$) (mean ± SE, n = 4).

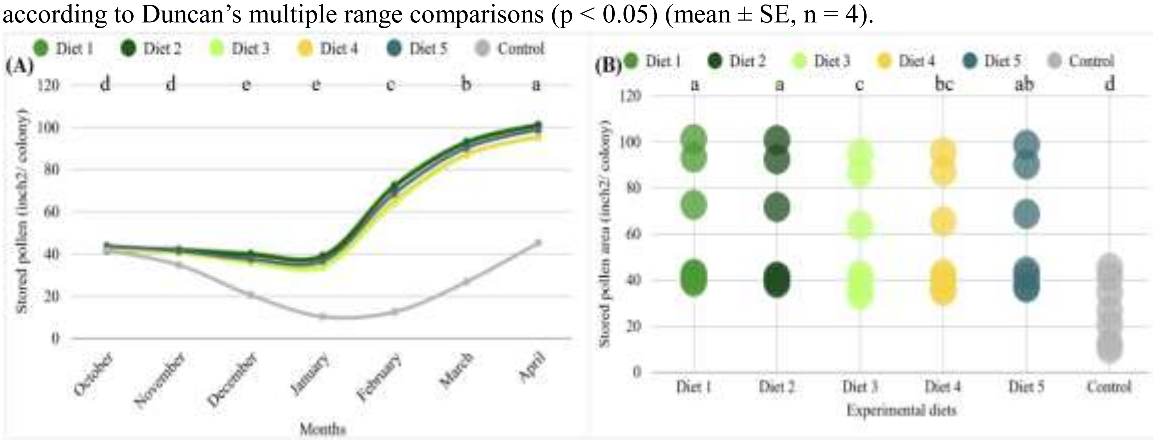

Fig 5 Fluctuation of bee bread area for honeybee colonies fed different pollen substitutes. **A)** Pattern of change in bee bread area in fed colonies during months of the experiment. **B)** Total means of stored pollen area. Different letters above the scatter plots and patterns indicate statistically significant differences according to Duncan's multiple range comparisons ($p < 0.05$) (mean ± SE, n = 4).

4. Impact of winter feeding with pollen substitutes on honey production

Colonies fed pollen substitutes, particularly diet 1, had the highest mean in citrus honey yield per colony (5.61±0.16kg/colony), followed by diet 5, diet 2, diet 4 and diet 3 by means of 5.17±0.11, 5.06±0.16, 4.04±0.19 and 3.92±0.17kg/colony, respectively, while the lowest average citrus honey yield was in the control colonies (2.90±0.18kg/colony) (P = 0.00, **Fig 6**.

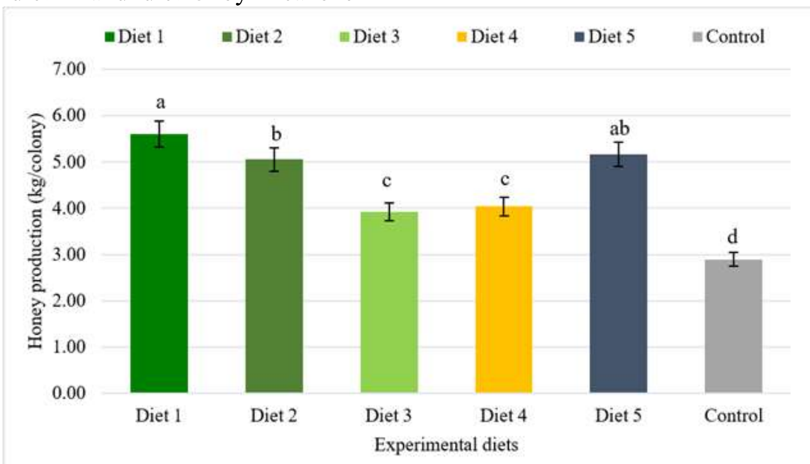

Fig 6 Honey yield from honeybee colonies fed different pollen substitutes. Different letters above the bars indicate statistically significant differences according to Duncan's multiple range comparisons (p < 0.05) (mean ± SE, n = 4).

Discussion

During winter, honeybee colonies are exposed to several unfavorable conditions, such as low temperatures and limited feed availability. Therefore, beekeepers resort to supplementing their honeybee colonies with pollen substitutes to face increasing environmental challenges **(Bocquet and Tosi, 2022; Elwakeil *et al*., 2025; Taha *et al*., 2026)**. This study compared the feed consumption of five pollen substitutes and measured the effects on wintering ability of colonies, colony development, and honey yield.

Regarding feed consumption, the highest cumulative consumption rate was observed with diet 4, which is based on pollen. This is attributed to the bees' preference for consuming protein supplements containing pollen over other protein diets **(Vaudo *et al.,* 2020; El Ghabawy *et al.,* 2022; Ghramh and Khan, 2023)**. Some estimates suggest that pollen consumption may reach 57 kg annually to provide sufficient protein for a colony of 20,000 bees **(Tsuruda *et al.,* 2021)**. No statistically significant differences were found between colonies' consumption of diets 1, 3, and 5, which are based on spirulina, moringa, and a mix of all tested ingredients. This aligns with some studies **(Ricigliano and Simone-Finstrom, 2020; Khan and Ghramh, 2022)**, which indicated that bees consume low amounts of algae-based (spirulina) diets more than other protein diets, due to their high protein content, which can reach up to 60%. Meanwhile, consumption of the second chlorella-based diet has decreased due to its high fat content, potentially reaching 12% **(Jay *et al.,* 2018; Moradi-Kheibari *et al.,* 2022)**, this is because bees prefer low-fat diets than high-fat diets **(Al-Esawy, 2023)**. On a monthly basis, the highest average feed consumption occurred in February, as colonies build up and expand their brood area, which requires a large amount of protein. One gram of the diet containing 20% protein is sufficient to raise five larvae from egg to adulthood, assuming a total protein requirement of 40 mg per reared larva **(Brodschneider and Crailsheim, 2010)**.

Colony wintering ability was fairly similar among colonies that fed the different pollen substitutes compared to the control colonies. This is attributed to the protein-supplying nature of the pollen substitutes, which provide essential nutrients for honeybees that are significantly reduced during winter and periods of natural pollen scarcity. According to studies, feeding honeybees colonies protein in the fall boosts their body fat and protein stores **(Irandoust and Ebadi, 2013; Shumkova et al., 2017)**. Also, a study by **DeGrandi-Hoffman *et al.,* (2016)** indicated that protein-based feeding reduced queen loss during winter, as queen failure is a major cause of colony loss during this season **(VanEngelsdorp *et al.,* 2010, 2013)**, which increases their ability to survive the winter. In another study, **Tawfik *et al.,* (2020)** indicated that supplemental feeding during the winter improved the growth and health of honeybee colonies and reduced their losses during the winter. In a further study supporting our findings, **Topal *et al.,* (2022)** found that honeybee colonies fed only sugar syrup had a reduced survival rate of only 51% during winter.

Overall, in our experiment, the growth rate of colonies supplied with pollen substitutes was higher than that of the control colonies, this is consistent with several studies **(DeGrandi-Hoffman *et al.,* 2008 and 2016; Tawfik *et al.,* 2020; Kim *et al.,* 2024)**. Also, in this study, colonies supplied with algae-based *e.g.* spirulina and chlorella (diets 1, 2, and 5) showed increased development in terms of brood area, bee bread area, and population density of workers, this is consistent with **McMenamin *et al.,* (2023)** who found that pollen substitutes containing 2٥% spirulina improved brood production in the colonies. Furthermore, some studies have indicated increased queen fertility in algae-fed colonies, leading to an increase in the number of sealed brood and brood viability (**Eremia *et al.,* 2013; Kumar *et al.,* 2013; Toderaş *et al.,* 2014 and 2017; Cebotari *et al.,* 2016 and 2017; Jehlík *et al.,* 2019; Dağ *et al.,* 2025**). This positively affects the increase in the number of bees in colonies supplied with pollen substitutes **(DeGrandi-Hoffman *et al.,* 2008; Toderaş *et al.,* 2014 and 2017; Hoover *et al.,* 2022; Dağ *et al.,* 2025)**. The increased bee population, in turn, leads to increased pollen-gathering activity.

Regarding honey yield, the protein diets enhance the produce of colonies from honey, it is correlation with the increasing workers population **(Ghazala and Nowar, 2013,** Taha and Al-Kahtani, 2020**)**. In our case the honey yield of colonies fed pollen substitutes was greater than in the control colonies, especially in colonies fed diets 1, 2, and 5 (based on algae *e.g.* spirulina and chlorella). Overall, several studies reported increasing honey yield in colonies supplied with algae products **(Nichols and Ricigliano, 2022)**. Honeybee colonies supplied with *Chlorella vulgaris* exhibited a 17.2 to 12.1% increase in honey yield than control colonies **(Eremia *et al.,* 2013 and 2021)**. Other study by **Dağ and Koç (2025)** indicated that the colonies fed spirulina patty producing more honey than control colonies.

Conclusion

Finally, pollen substitutes are beneficial for honeybees during periods of natural pollen shortages, especially during winter, as they improve pollen foraging activity and brood rearing, thus increasing the population density of workers within the colonies. This also results in increased honey production and improved better preparation before the active season and winter hardiness. According to our results in this study, diet 1, diet 2, and diet 5, based on spirulina, chlorella, and a mix of all tested ingredients were the most effective diets, improving all targeted parameters in the treated colonies. These results bode well for the development of more effective diets, which could contribute to improving performance of honeybee colonies.

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