Influence of protein food supplement on development of honey bee colonies when no pollen available in nature.

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Product Testing : Zeina's Pro20

Introduction

The honeybee feeds on two main types of food: nectar and pollen. While nectar primarily serves as an energy source, pollen serves mainly as a protein source. Nectar is stored in honeycombs in the form of honey and serves bees as an available energy source for nectar deficiency periods. Honey can be preserved in the combs for an extended period. In contrast, pollen is mainly consumed by larvae and contributes to their development and consequently to the development of the colonies (1). Pollen cannot be stored for an extended period within the combs. Therefore, during times when there is no available pollen in the bee's environment, the queen bee ceases to lay eggs, and the development of the bee colony stops (2). In this situation, the bee population significantly decreases.there are two main periods of pollen deficiency in the environment: first one is during the winter, from mid-December to mid-January approximately, and the second one during the summer, from mid-August to mid-September (3). The current experiment was conducted in the northern area of the Sea of Galilee during late summer, considering that during this period, there is a shortage of pollen in this region. The experiment aims to examine the influence of protein supplements on the development of bee colonies during a period when pollen is unavailable in the bees' environment.

Methods

We conducted the experiment in two grazing groups.The experiment took place from the beginning of August 2022 until mid-September 2022.At each site, the bees were divided into three treatments:

1. Control group (no nutritional supplement)
2. Natual Pollen Patty
3. Alternative Natual nutritional supplement called "Ziena Pro20" by the

company "Infinite Green Global" (See Table 1).
The bees received the food supplement throughout the treatment period, so there was a continuous supply of the supplement to the bees. The food supplements, pollen, and artificial supplements were provided in the form of a patty. The pollen patty included an addition of honey and sugar in a 1:1:1 ratio to create the patty. The artificial food supplement is based on protein from a variety of natural products and contains 22% protein (product label attached in the appendix).
Bees on nutritional supplements, pollen, or artificial ones, received one kilogram of "patty" throughout the experiment. We inserted the patties into the hives and placed them on the barrier mesh. If there was no mesh, we placed the food supplement on the hive frames. In the experiment, we examined the bees in terms of population both at the storage level (if present) and at the hive level. Additionally, we checked the number of combs with brood in each hive.
We conducted these tests at the beginning of the treatment (before providing the food supplement), in the middle, and at the end. Additionally, we assessed the bees about a month after receiving the last food supplement. Bees that died during the experiment were excluded from the average calculations

Table 1. The distribution of the hives according to treatments and the experimental area.

	Treatments			
Area	Control group	Flower Pollen	Pro20	Total
A	10	11	12	33
B	5	12	11	28
Total	15	23	23	61

Results:
The experiment was conducted at two grazing sites. The distribution into treatments and the number of bees in each treatment are shown in Table 1. At "A" grazing site, during the experiment, two bee colonies from the

pollen patty treatment and at the "B" grazing site, three bee colonies from the control group and one bee colony from the 'Pro20' nutritional supplement treatment were exterminated.

Grazing Site: "A"

Bee Population:

Regarding the population size on the hive level, the feeding treatment with 'Pro20' nutritional supplement appeared to be the most effective. The number of occupied combs by bees in this treatment remained stable throughout the experiment, and even a month after the treatment, there was no apparent decrease in the population size (Figure 1). In contrast, treatments with pollen patty and the control group showed a trend of declining bee populations in the hive, approaching nearly a decrease of 10 occupied combs.

At the start of the experiment, the level was around 8 occupied combs per hive. At the end of the experiment, about a month after it concluded, this decreased to approximately 7 occupied combs. Hives treated with the nutritional supplement and those in the control group began with an average of 7 occupied combs on the second level, while hives treated with the pollen patty started with an average of 5 occupied combs. By the end of the treatment period, in terms of the number of occupied combs on the second level (storage level), there was a decreasing trend across all treatments throughout the experiment.

The nutritional supplement-treated hives ended the experiment with an average of 4 occupied combs, while those in the pollen patty and control group ended with less than one fewer occupied comb on the second level.

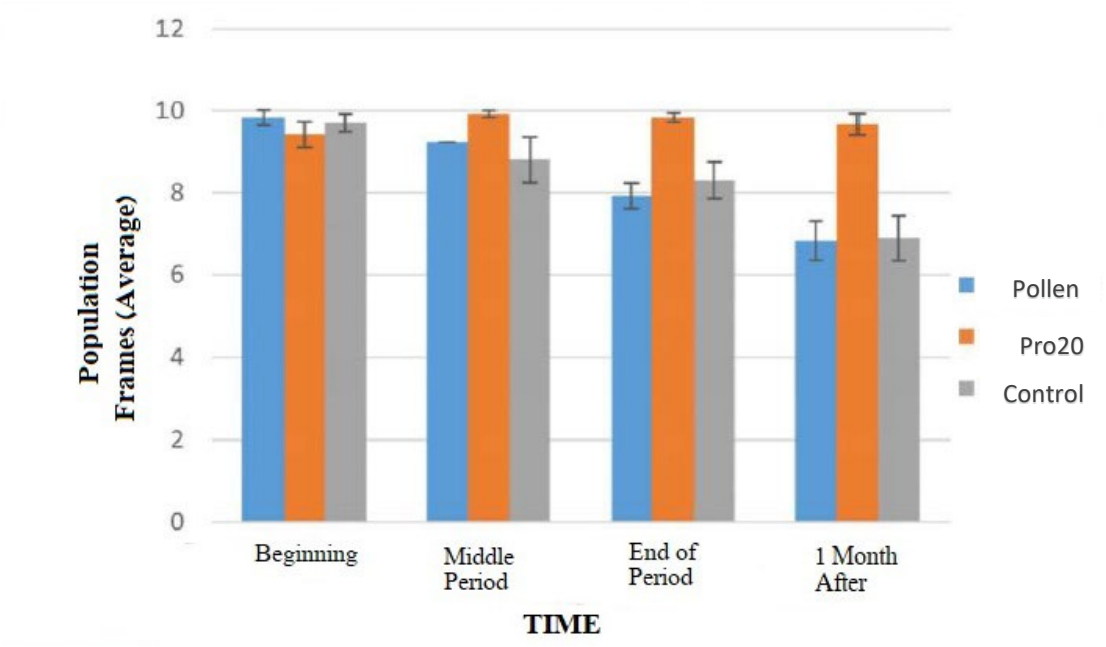


Figure 1 – "A" Site: Average number of occupied combs per hive in each treatment throughout the experiment: start, middle, end, and a month after the end of the experiment. The 'Pro20' nutritional supplement treatment was the only one that maintained, throughout the experiment, an average of occupied combs in the hive. In contrast, the other treatments experienced a decrease in the number of occupied combs. Standard deviation values are highlighted

Brood

Regarding the number of brood cells, at the start of the experiment, there was variability among the different treatments. The brood cell count ranged from an average of 7 brood cells per hive in the control group to 5 brood cells per hive in the pollen patty treatment. In the two treatments where protein supplements were provided to the hives, whether it was pollen patty or 'Pro20' the number of brood cells decreased throughout the experiment, reaching one less brood cell per hive about a month after its conclusion. Conversely, in the control group, the number of brood cells decreased by more than three cells per hive on average, with the most significant decline occurring in the initial phase of the experiment. At the conclusion of the experiment, the highest number of brood cells was observed in the group of hives that received the nutritional supplement.

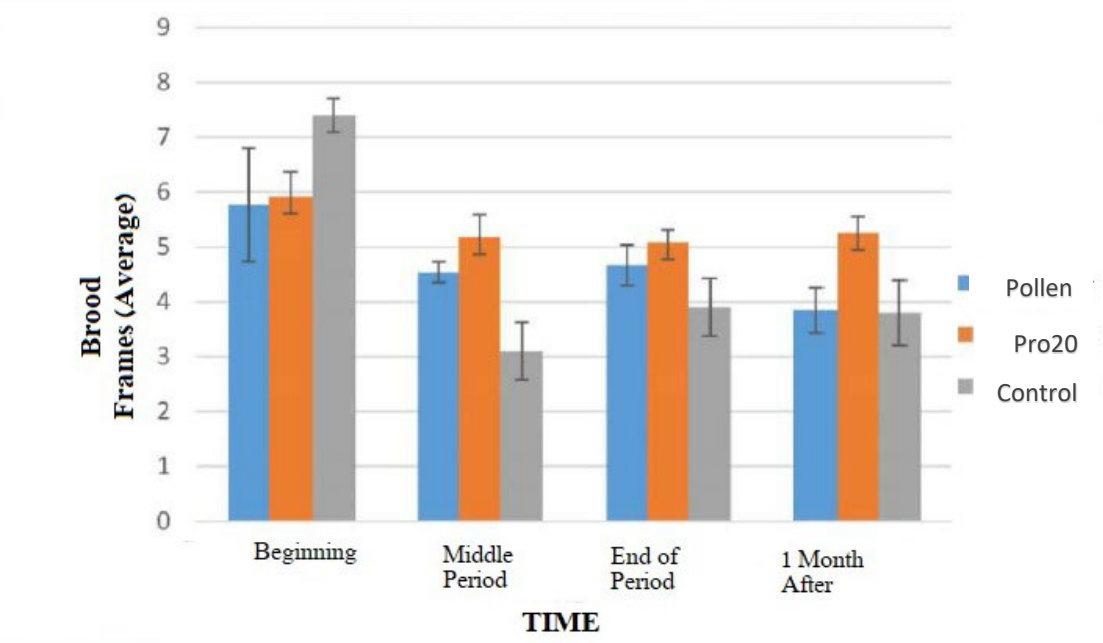


Figure 2. "B" Site - Average number of brood cells with brood in hives across different treatments throughout the experiment. There is a decrease in the number of brood cells observed over the course of the experiment. The most moderate decline is seen in the hives treated with 'Pro20' while the most significant decrease in the average brood cells count is in the control group hives. Standard errors are noted.

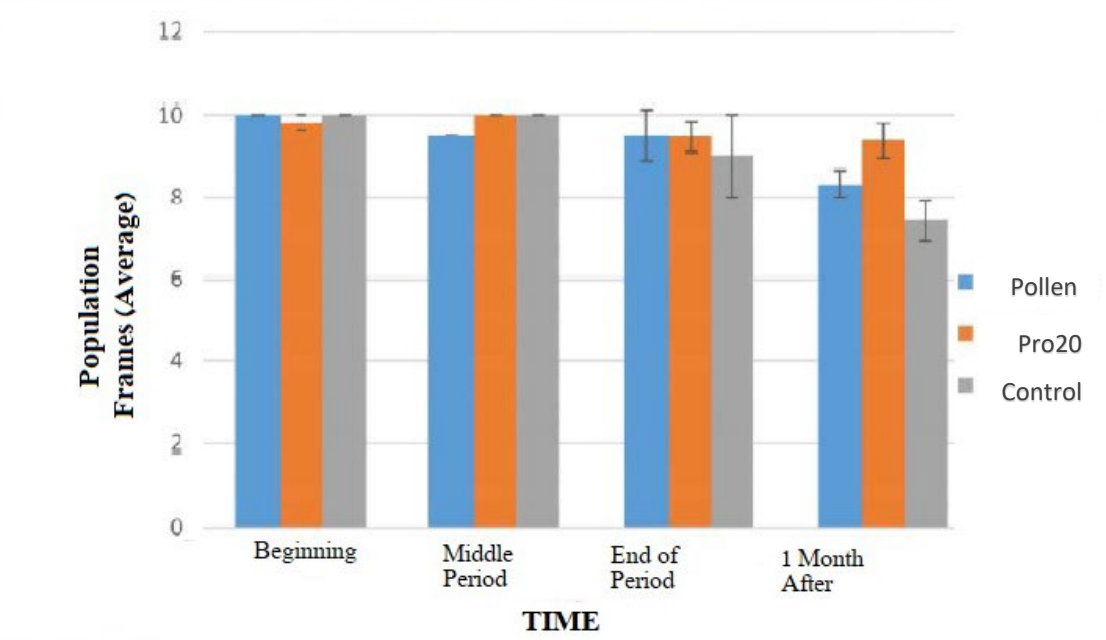
Site: "B"

Bee Population

Regarding the bee population, differences between the treatments throughout the experiment were less noticeable compared to the **"A"** site. The average number of occupied cells at the beginning of the treatment was similar. Throughout the experiment, there was a decrease in the number of cells, reaching approximately half in the bee population across all treatments (Figure 3). Significant differences were observed in a test conducted about a month after the experiment ended, where the colonies that received the food supplement had the highest quantity of cells in the hive, while those in the control group had the lowest number of occupied cells. Moreover, a noticeable decrease in the number of occupied cells in the second level (storage level) was observed across all treatments throughout the experiment. Colonies treated with the food supplement and those in the control group started with an average of 9 occupied cells on the second floor, while those treated with flower pollen started with an average of 8 occupied cells. At the end of the treatment, the colonies

treated with the food supplement finished the experiment with an average of 2 occupied cells, while the colonies in the treatments of flower pollen and the control group ended with less than one occupied cell on the second level.

Figure 3. **"B"** Site - Average number of occupied cells on the hive floor for each



treatment throughout the experiment: beginning, middle, end, and a month after the end of the experiment. The most significant difference between treatments was observed in the examination conducted a month after the last feeding, where in the colonies treated with the nutritional supplement, 'Pro20' the average number of occupied cells was the highest. Standard error values are indicated

Brood

In terms of the number of brood cells per colony, initially, there was a similar count across the different treatments. Throughout the treatment, the number of brood cells decreased by about 2 to 3 brood cells per colony on average in all treatments. However, at the end of the treatment, the group receiving the highest food supplement had the highest number of brood cells (see Figure 4). Nevertheless, in the examination conducted a month after the last feeding, a similar number of brood cells was observed across all treatments

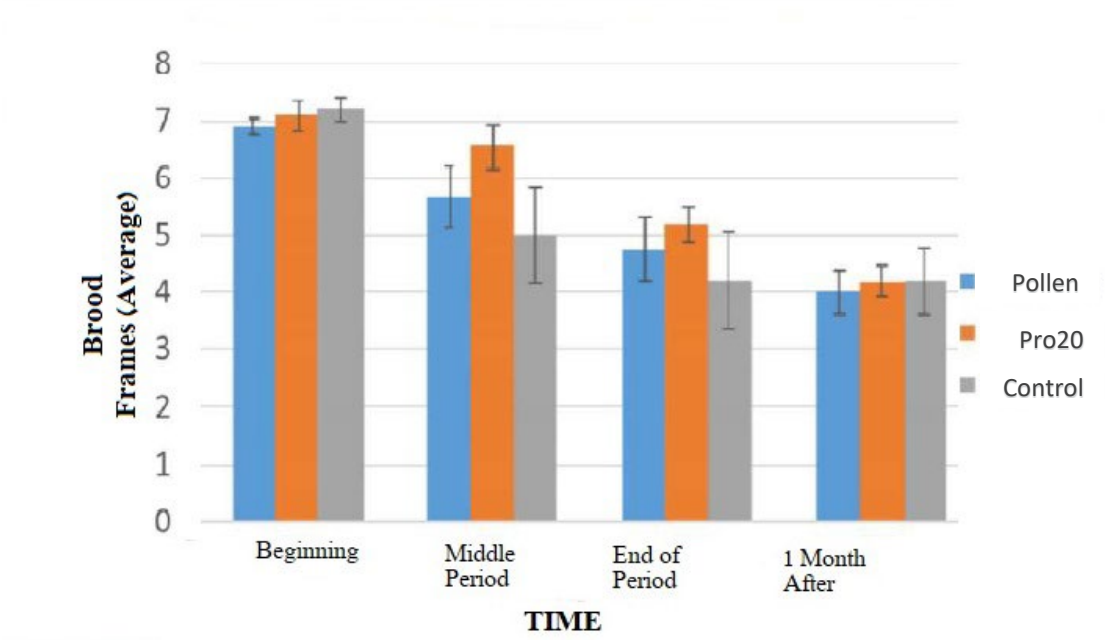


Figure 4: **"B"** Site - Average number of brood cells per colony in different treatments over the experiment. There is a decrease in the average number of brood cells throughout the experiment. The most moderate decline until the end of the experiment is observed in colonies treated with the supplement 'Pro20'. However, a month after the end of the experiment, it appears that the average number of brood cells among the treatments is similar. Standard error values are indicated

Discussion and Conclusions

The flower pollen is one of two types of food collected by honeybees. This component constitutes the protein in the bees' diet and is primarily used in feeding the larvae. When there is a shortage of flower pollen in the bees' habitat, the bee population will decrease, and there won't be proper development within the colony. there are two main periods during the year when there is a shortage of flower pollen in some areas: one is in the winter, and the other is at the end of summer (3). In this experiment, we conducted the test at the end of summer in the northern area of the Sea of Galilee. In this area, there is a shortage of flower pollen during this period, when simultaneously there is a blooming of jujube trees providing abundant nectar, there is potential for honey storage. Therefore, we chose to focus on that. The experiment took place at two feeding stations, one near the **"A"** and the other near the **"B"**. At both feeding stations, the colonies were divided into three groups: control (without a protein supplement), treatment with flower pollen patty, and treatment with a protein patty from Infinite Green Global Company. Throughout the

experiment, we assessed the condition of the colonies at the beginning, during, and at the end, comparing bee populations and the quantity of brood between them. The objective of the experiment was to examine the impact of the protein supplement on the development of colonies in an area where there is a shortage of flower pollen.

The comparison of the number of occupied cells on the honeycomb indicates a gap in population strength that increased throughout the experiment between the colonies treated with the food supplement and the control colonies. The difference between the treatments peaked in the final assessment, about a month after the experiment ended and feeding ceased, standing at about two additional occupied cells in the treated colonies. However, there was a slight variation between the two sites examined (Figures 2, 3). Moreover, the population in the storage cells was higher in the food supplement groups compared to the control colonies. Nevertheless, this measure of counting occupied cells in the honeycomb is problematic for estimation compared to counting occupied cells in the brood area.

Also, concerning the number of brood cells per colony, a similar pattern emerged favoring the treatment with the nutrition supplement over the control colonies. However, contrary to the population difference, which was larger about a month after the experiment ended, the difference in the number of brood cells between treatments peaked midway through the experiment, averaging about one to two more brood cells per colony, depending on the site. Subsequently, the gap in the number of brood cells between groups narrowed (Figures 2, 4), and in the assessment a month after the experiment, it appeared that at the **"B"** feeding station, the quantity of brood cells was similar in both treatment groups and control groups. These results may suggest that the impact of the protein supplement, or conversely, the lack of protein, on brood is rapid, while the effect on population size is slower and becomes evident at a later stage when the brood emerges.

Furthermore, it is interesting to note that despite the relatively small distance between the feeding stations (about 3.5 km), the two sites behaved differently, and the differences between the treatments were distinct. At the feeding station near **"A"**, larger differences between the

treatments were observed compared to the **"B"** site. It is possible that at the **"B"** feeding station, there was vegetation supporting the bees in terms of protein sources.

The comparison between the results of the nutrition supplement treatment and the control treatment versus the treatment of feeding with flower pollen showed a general trend of an advantage for the supplement over the flower pollen, which was mostly somewhat similar to the control. In previous experiments comparing the development of bees with different nutrition treatments, it was found that a protein supplement for bees based on flower pollen generally yielded better results than supplements based on proteins from other sources (4, 6). The current situation, where the effectiveness of feeding with flower pollen supplement is lower than the effectiveness of the artificial supplement, raises questions regarding the quality of the pollen used.

It should be noted that the flower pollen patty used in the experiment did not undergo analysis to determine the percentage of protein, whereas it is known that there is variability in the percentage of protein in flower pollen from different plants, ranging around 8% protein content to 40% protein (5). Therefore, we don't know the amount of protein in the patty and its significance compared to the nutrition supplement. Another experiment, structured similarly, was conducted last winter (2022-2023) as a continuation of this experiment, aiming to examine the same objectives with the same treatments but under different flowering conditions and weather. The experiment took place in the Jordan Valley near the Old Bridge. Unfortunately, the bees in the winter experiment were too weak, possibly affected by Varroa, making it challenging to derive insights from the experiment's results. We aim to repeat the experiment next winter.

Literature

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