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Assessing the Impact of Supplemental Feeding during Dearth Period on Brood Development in *Apis mellifera*

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Abstract

The dearth period, characterized by the scarcity of natural nectar and pollen, poses a significant threat to the health and productivity of *Apis mellifera* colonies. This study was conducted at the BSRI Apiary, Ishurdi, Pabna, during the 2019-2020 cropping season to assess brood development and resource storage. Two wooden honeybee rearing boxes, along with 7 (seven) frames, were selected. This study investigated the efficacy of two supplemental feeding regimens – a carbohydrate-only diet (Sugar syrup, T1) and a carbohydrate-protein diet (Pulse + Sugar diet, T2) – compared with a non-fed control (T3) across various parameters. The experiment tracked changes in Brood Cell (Egg, Larvae), Sealed Brood, Pollen Cell, Honey Cell, and Empty Cell counts from a primary to a final data point. The results demonstrated that the Pulse + Sugar diet (T_2) significantly outperformed the other treatments, resulting in the highest final counts for Brood Cell (926.14a) and Honey Cell (784.57a). T_2 also showed the largest percentage increase in Brood Cell (39.77%) and Sealed Brood (35.37%). Statistical analysis (LSD at 5%) confirmed that T_2's final Brood Cell and Honey Cell counts were significantly higher than T_1 and T_3. The control group (T_3) experienced a severe decline, 71.52% change in Brood Cell, highlighting the nutritional stress of the dearth period. These findings confirm that a protein-rich supplement is critical for stimulating robust brood development and resource accumulation in honey bee colonies during periods of nutritional scarcity.

Introduction

The western honey bee, *Apis mellifera*, is a crucial global pollinator and a vital component of agricultural systems and the honey industry. The health and productivity of a honey bee colony are inextricably linked to the availability and quality of floral resources (Sultana et. al. 2024).

In Bangladesh, beekeeping has traditionally relied on flowering events, which provide ample pollen and nectar to sustain colonies and boost worker bee populations. Nonetheless, maintaining the ideal colony number amid a flora shortage is a significant challenge. The lack of available pollen causes colonies to rear fewer offspring, eventually weakening them.

The lack of available pollen causes colonies to rear fewer offspring, which eventually weakens them. These colonies are also susceptible to bee predators and other natural pests. Seasonal honeybee colony management requires extra caution.

However, beekeeping is often challenged by periods of nutritional dearth, typically caused by seasonal fluctuations or environmental factors, during which natural nectar and pollen become scarce. This scarcity leads to a decline in colony population, reduced immune function, and increased susceptibility to pests and diseases (Gemeda, 2014).

The first option is to feed the bee colonies a protein-rich artificial diet so that brood rearing activity can be enhanced and colony strength is maintained during dearth periods (Mattila & Otis, 2006; Marghitaş et al., 2010; Morais et al., 2013). The other option is migrating honeybee colonies from resource-poor to resource-rich; however, it requires lots of labor, time, and money (Kumar et al., 2013).

To mitigate the negative effects of the dearth period, supplemental feeding has become a standard management practice. Supplemental diets are generally categorized into carbohydrate sources (e.g., sugar syrup) to provide energy and protein sources (e.g., pollen substitutes) to support the development of nurse bees and the production of royal jelly, which is essential for larval growth (Topal et. al., 2022). Previous studies have established that while carbohydrate feeding can stimulate foraging and some brood-rearing activity, a lack of protein often limits overall population growth (Ullah et al., 2021). Conversely, protein-rich diets have been shown to significantly boost colony growth and brood area, making them a cornerstone of effective colony management during periods of stress (Bhandari et al., 2025).

By pollinating a wide range of crops, honeybees (*Apis mellifera*) contribute significantly to global agriculture by ensuring food production and environmental stability. These diligent pollinators do, however, frequently encounter difficult times known as "dearth periods," when natural sources of nectar and pollen become limited (Prakash et. al., 2007). A bee's more important function than collecting honey is to assist in pollination (Reza et. al., 2018).

Seasonal variations, climatic shifts, habitat loss, or agricultural practices that restrict the availability of floral resources can all result in periods of scarcity. When this happens, honey bee colonies may face nutritional stress, which can impair their defense mechanisms, slow down the growth of their young, and cause the colony as a whole to deteriorate. According to Funari et al. (2003), honey bees need protein, carbohydrates, minerals, lipids, vitamins, and water to complete their development and growth, which they obtain by collecting pollen, nectar, and water. Honey bees collect three substances - water, nectar, and pollen to satisfy their nutritional requirements. Bees receive carbohydrates from nectar and proteins from pollen (Javaheri et al., 2000).

An optimum population of honeybee colonies during dearth seasons ensures early buildup and more foraging bees during subsequent honey-flow periods, producing more honey (Somerville & Collins, 2007; Sihag & Gupta, 2013). The use of sugar syrup or pollen supplement or both is useful for dearth period feeding in order to build larger foragers for maximum honey crop (Somerville & Collins, 2007).

This study was designed to quantitatively assess and compare the effects of a carbohydrate-only diet (Sugar syrup, T_1) and a combined carbohydrate-protein diet (Pulse + Sugar diet, T_2) against a non-fed control (T_3) on the key indicators of colony health and development in *Apis mellifera* during a dearth period. The primary objective was to determine which supplemental feeding strategy has the greatest positive impact on brood development and resource storage.

Materials and Methods

Study site

The study was conducted at the BSRI Apiary in Ishurdi, Pabna, Bangladesh (24.115700201877218, 89.0816 7337156924) from September 2019 to June 2020. During external feeding, three colonies with distinct markings and a consistent environment were used to reduce the likelihood of honeybee. Standard wooden hives were used to establish three treatments (including control-unfed), each with 7 frames, in each colony. The colonies were equalized to ensure equal strength.

Experimental Design and Treatments

The experiment utilized a completely randomized design with three treatment groups:

- **T_1: Sugar syrup** (Carbohydrate-only diet)
- **T_2: Pulse + Sugar diet** (Carbohydrate and Protein substitute diet)
- **T_3: Control** (Non-fed, relying solely on natural resources)

Each treatment was applied to one beehive, which had seven replicates, comprised of a brood cell frame. The treatments were applied over a defined period corresponding to a known nutritional dearth.

Preparation of the supplement

There are two supplements that were used in the colonies. Sugar syrup, a commonly used feeding technique by beekeepers in Bangladesh, and the Pulse diet, which is very common in other countries. Sugar syrup was prepared by dissolving one kilogram of crystal sugar in one Liter of fresh water (1:1). Pulse diet was prepared by mixing flour (pea, chick pea, and bean) with skimmed milk powder, Brewer's yeast, and honey. The ratio is 10:1:1:6.

Supplementary feeding time

Supplementary feeding is provided during periods when flowering plants are not in bloom, specifically from April to May, the dry season, and from June to August, the rainy season (Gemeda, 2014). The study colonies were subjected to uniform seasonal management practices. During the dry season, pulse diet and sugar syrup were supplied for seven consecutive days, with the same feeding schedule repeated during the rainy season. The feeding quantities were carefully calculated to meet the colonies' needs.

Brood and honeybee cell estimation

Seven days after supplementary feeding was provided, the colonies were evaluated using parameters including brood cells (eggs and larvae), sealed brood, pollen cells, honey cells, drone cells, empty cells, and queen cells. Frames were removed from each hive and shaken thoroughly to ensure no bees remained on them. High-resolution photos were then taken using a Sony Alpha 6400 camera with a 16-50mm lens. These images were processed to count cells using the specified parameters in Adobe Photoshop CC 2019. Data collection was carried out both before the treatment (primary) and after the treatment (final). Similar parameters were recorded for the control colonies that did not receive supplementary feeding.

Data Collection

Data were collected at two time points: Primary Data (initial measurement) and Final Data (measurement after the feeding period). The parameters measured included the number of cells dedicated to the following: Brood Cell (Egg, Larvae), Sealed Brood, Pollen Cell, Honey Cell, Drone Cell, Queen Cell, and Empty Cell.

Statistical analysis

The collected data were analyzed using Statistix 10 software (Analytical Software, Tallahassee, FL, USA). Descriptive statistics, along with appropriate statistical tests such as ANOVA, t-tests, and linear regressions, were applied to evaluate the effects of supplementary feeding on the measured parameters. Statistical significance was determined at a 5% level ($p < 0.05$).

Results

Data were collected from each colony on both sides of the seven frames. The frame measured an average of 43.64 x 19.57 cm. Every side of the frame has 2952.14 cells, according to the count. Various parameters, including the supplement consumption rate, number of brood cells (eggs and larvae), sealed brood, pollen cells, honey cells, drone cells, empty cells, and queen cells, were recorded and interpreted with the total number of cells presented below.

Supplement consumption status

During the experimental period, the honeybee colonies showed distinct variations in diet consumption across seasons and feed types. In the dry season, colonies fed with sugar syrup exhibited consistently higher consumption (640.92 g on Day 4) than those provided with a pulse-based diet (maximum of 604.51 g). Both diets followed a rising trend through the fourth day, then showed a gradual decline thereafter. In contrast, during the rainy season, total feed consumption was notably lower (average 250–350 g day^{-1}) and more irregular, likely due to the availability of natural nectar and pollen sources and reduced foraging activity under humid conditions. These results demonstrate that sugar syrup is more palatable and provides immediate energy, particularly when nectar resources are scarce, whereas pulse diets provide protein but are consumed less readily. Similar patterns were reported by Mattila and Otis (2006), who found that honeybee colonies prefer carbohydrate-based diets over protein substitutes during resource scarcity.

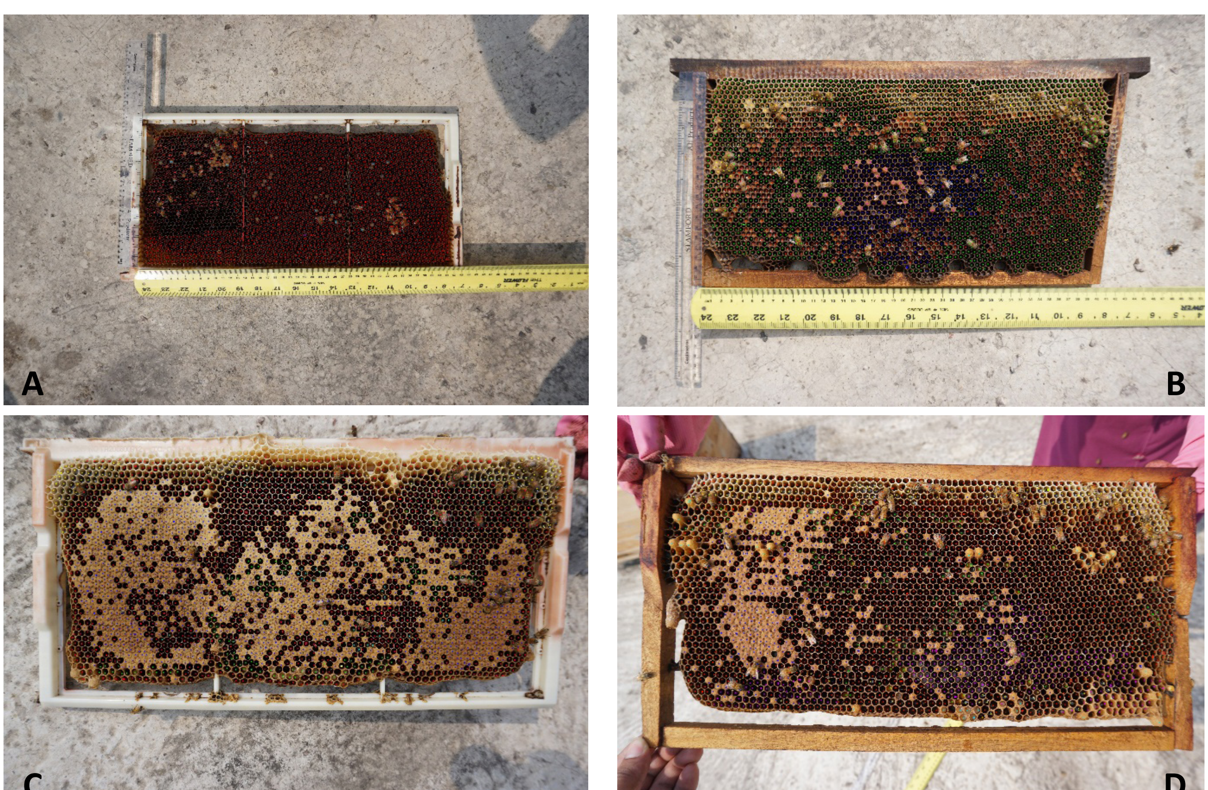

Fig 1 (A-D). Data collection and different honeybee cells counting process.

The overall seasonal comparison indicates that feed intake was nearly twice as high during the dry season as in the rainy season, confirming that supplemental feeding is most essential when natural floral resources are limited. The lower feed intake during the rainy season suggests that colonies may meet much of their nutritional demand from environmental forage. This observation supports findings from Alqarni et al. (2011) and Cervancia & Dy (1992), who reported reduced supplement consumption during periods of natural bloom and higher uptake during dearth periods. Furthermore, a recent comparative study emphasized that colonies fed sugar syrup with protein supplementation showed improved brood rearing and population strength during dearth periods. Therefore, it can be concluded that sugar syrup should be prioritized for energy supply during dry months, whereas protein-rich pulse diets should be strategically provided during brood rearing or when pollen availability declines, ensuring balanced nutrition and colony sustainability throughout the year.

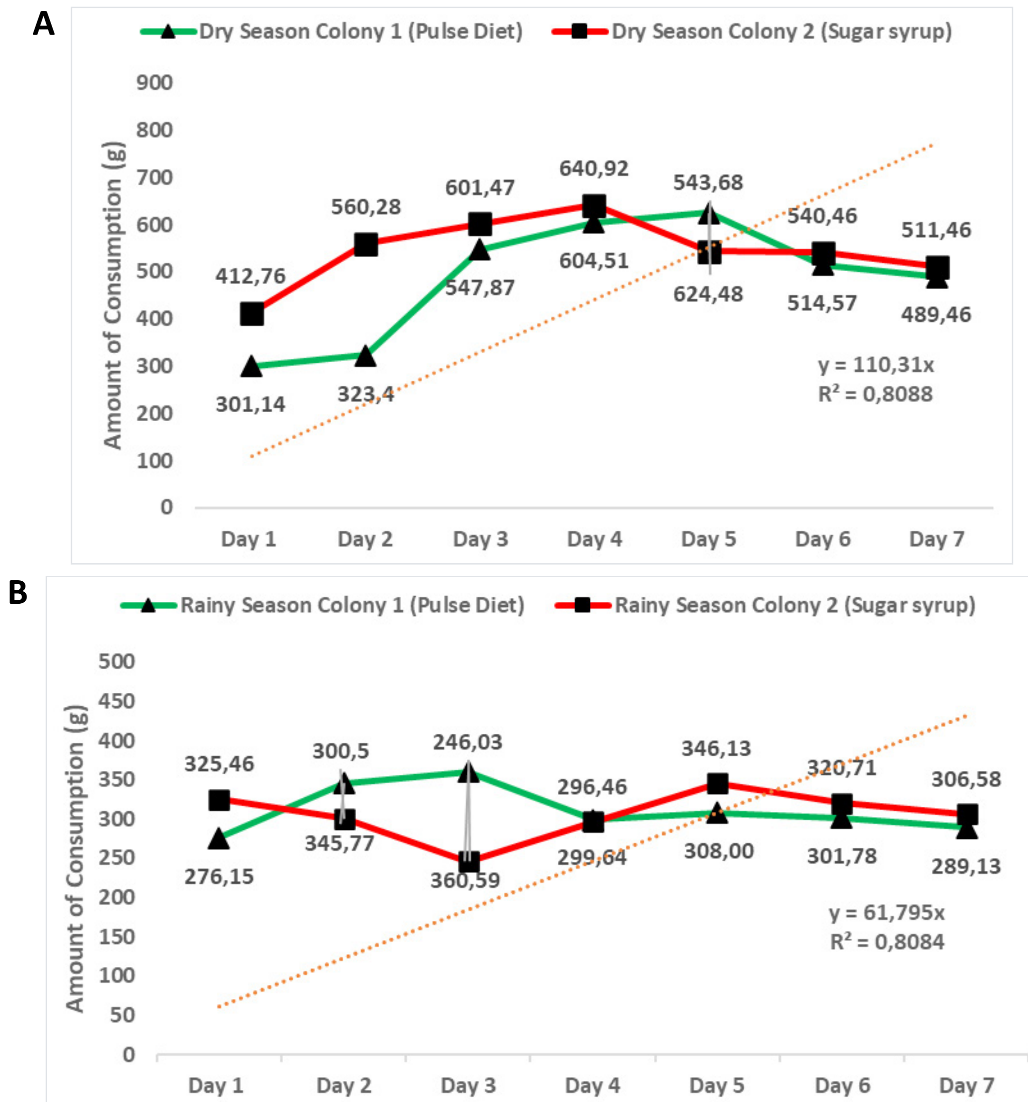

Fig 2 (A, B). Legend?

Impact on Brood Development

The final data on brood parameters showed significant differences among the treatments, confirming the positive impact of supplemental feeding (Table 1).

For Brood Cell (Egg, Larvae), T_2 (Pulse + Sugar diet) recorded the highest final count (926.14a), which was statistically significant ($P \leq 0.05$) compared to T_1 (377.43b) and T_3 (134.43b). T_1 and T_3 were statistically similar. For Sealed Brood, both T_1 (954.4a) and T_2 (1240.9a) were significantly higher than the control T_3 (110.9b), but T_1 and T_2 were not significantly different from each other. The ranking of means for Brood Cell was $T_2 > T_1 = T_3$, while for Sealed Brood it was $T_2 = T_1 > T_3$.

These results align with the findings of Mattila and Otis (2006), who observed greater brood expansion when colonies received pollen or protein supplements during periods of natural forage scarcity. Similarly, Ahmad et al. (2021) reported that colonies fed a mixture of carbohydrate and protein sources exhibited higher sealed brood numbers and better colony strength than those fed sugar syrup alone.

Table 1. Effect of Supplemental Feeding on Brood Development (Mean Cell Counts).

Treatments	Primary Data (Brood Cell (Egg, Larvae))	Final Data (Brood Cell (Egg, Larvae))	Primary Data (Sealed Brood)	Final Data (Sealed Brood)
T_1: Sugar syrup	237.14	377.43b	834.29	954.4a
T_2: Pulse + Sugar diet	557.86	**926.14a**	802.00	**1240.9a**
T_3: Control	230.57	134.43b	126.86	110.9b
LSD (5%)	NS	**464.63**	NS	**640.98**

Furthermore, Alqarni et al. (2011) demonstrated that balanced supplementation enhances the productivity of *Apis mellifera jemenitica* colonies under arid conditions. Collectively, these findings suggest that integrated diets containing both energy and protein components are important for maximizing brood rearing, especially during periods of nutritional stress, such as dry seasons or floral dearth.

Impact on Colony Resources

The storage of colony resources, specifically honey, was also significantly affected by the treatments (Table 2).

Honey Cell counts were significantly highest in T_2 (784.57a), which was statistically different from T_1 (362.29b) and T_3 (189.29b). T_1 and T_3 showed no significant difference in final Honey Cell counts. No significant difference (NS) was found among the treatments for Pollen Cell counts in the final data.

Numerous studies have demonstrated that during periods of natural pollen scarcity (dearth), protein becomes the limiting factor for colony growth. Researchers have observed that while sugar syrup provides the energy necessary for maintenance and basic activities, it "does not sustain brood rearing" to the same extent as a complete diet (Irandoust & Ebadi, 2013).

Impact on Other Cell Types

The treatments also influenced the number of Empty Cells, a key indicator of colony space utilization and resource depletion (Table 3).

Table 2. Effect of Supplemental Feeding on Colony Resource Storage (Mean Cell Counts).

Treatments	Primary Data (Pollen Cell)	Final Data (Pollen Cell)	Primary Data (Honey Cell)	Final Data (Honey Cell)
T_1: Sugar syrup	413.86	364.29	264.86b	362.29b
T_2: Pulse + Sugar diet	306.86	609.14	683.29a	**784.57a**
T_3: Control	220.29	207.57	186.57b	189.29b
LSD (5%)	NS	**NS**	**301.25**	**332.89**

Table 3.: Effect of Supplemental Feeding on Other Cell Types (Mean Cell Counts).

Treatments	Primary Data (Drone cell)	Final Data (Drone cell)	Primary Data (Queen Cell)	Final Data (Queen Cell)	Primary Data (Empty Cell)	Final Data (Empty Cell)
T_1: Sugar syrup	13.00	12.571	0.1429	0.4286	4159.4b	3744.30b
T_2: Pulse + Sugar diet	23.714	22.429	0.2857	0.8571	3423.3b	**2320.30c**
T_3: Control	23.714	37.857	0.00	0.00	5286.3a	**5394.30a**
LSD (5%)	NS	NS	NS	NS	**1046.80**	**842.40**

T_3 (Control) had the highest number of Empty Cells (5394.30a), which was significantly higher than those of T_1 (3744.30b) and T_2 (2320.30c). T_2 had the fewest Empty Cells, indicating the most efficient use of comb space. No significant differences were observed for Drone Cell or Queen Cell counts among the treatments.

Percentage Change Analysis

The percentage change from primary to final data clearly illustrates the relative performance of the feeding strategies (Table 4).

T_2 (Pulse + Sugar diet) showed the highest percentage increase in Brood Cell (39.77%), Sealed Brood (35.37%), and

Table 4. Percent Change in Cell Counts from Primary to Final Data.

Cell Type	T_1: Sugar syrup (%)	T_2: Pulse + Sugar diet (%)	T_3: Control (%)
Brood Cell (Egg, Larvae)	37.17	**39.77**	-71.52
Sealed Brood	12.59	**35.37**	-14.43
Pollen Cell	15.76	**32.06**	-6.13
Honey Cell	**26.89**	12.91	1.43
Drone cell	-3.41	-5.73	37.36
Queen Cell	66.67	66.67	0.00
Empty Cell	-11.09	**-47.54**	2.00

Pollen Cell (32.06%). In stark contrast, the control group (T_3) exhibited a dramatic decline of -71.52% in Brood Cell and -14.43% in Sealed Brood, confirming the detrimental effect of the dearth period without intervention. T_2 also showed the largest decrease in Empty Cells (-47.54%), indicating rapid consumption of the supplement and utilization of comb space for resources and brood.

Cluster Heatmap Analysis of Cell Type Composition

A cluster heatmap was generated using standardized cell count data to provide a holistic visualization of the treatment effects (Figure 3). The analysis revealed a clear separation between the treatments and cell types, strongly supporting the quantitative results.

The hierarchical clustering of cell types grouped the essential resource and developmental cells (Sealed Brood, Pollen Cell, Brood/Larva, and Honey Cell) into a single cluster, indicating their co-variation in response to the feeding. The clustering of treatments showed that the Pulse_Final state was compositionally distinct from the other fed groups and clustered with the Control states.

The color intensity, representing standardized cell counts, confirmed the superior performance of the protein-supplemented diet. The Pulse_Final state showed the highest standardized values (dark red) for both Brood/Larva and Honey Cell, demonstrating a successful physiological response to the complete diet. Conversely, the Empty Cell column showed the most striking contrast: Control_Final exhibited the highest standardized value (red), indicating a large amount of unused comb space due to the cessation of brood rearing, while Pulse_Final showed the lowest standardized value (dark blue), reflecting the most efficient utilization of comb space for brood expansion and resource storage. This visual evidence reinforces that the Pulse + Sugar diet resulted in the most robust and resource-rich colony state.

Discussion

The results of this study unequivocally demonstrate the critical role of supplemental feeding, particularly a protein-rich diet, in maintaining and enhancing *Apis mellifera* colony strength during a period of nutritional dearth.

The superior performance of the Pulse + Sugar diet (T_2) is the most significant finding. The significantly higher final counts and percentage increase in Brood Cell (Egg, Larvae) and Sealed Brood in T_2, compared to T_1 and T_3, directly support the hypothesis that protein is the limiting nutritional factor during dearth (Bhandari et. al., 2025). Protein, supplied by the "Pulse" component of the diet, is essential for the hypopharyngeal glands of nurse bees to produce royal jelly, the primary food for young larvae and the queen. The robust brood development in T2 translates directly into a larger, healthier colony population, which is crucial for survival and subsequent productivity.

The Sugar syrup (T_1), while stimulating, showed a mixed effect. It resulted in a final Sealed Brood count statistically similar to T_2 and significantly higher than T_3. This suggests

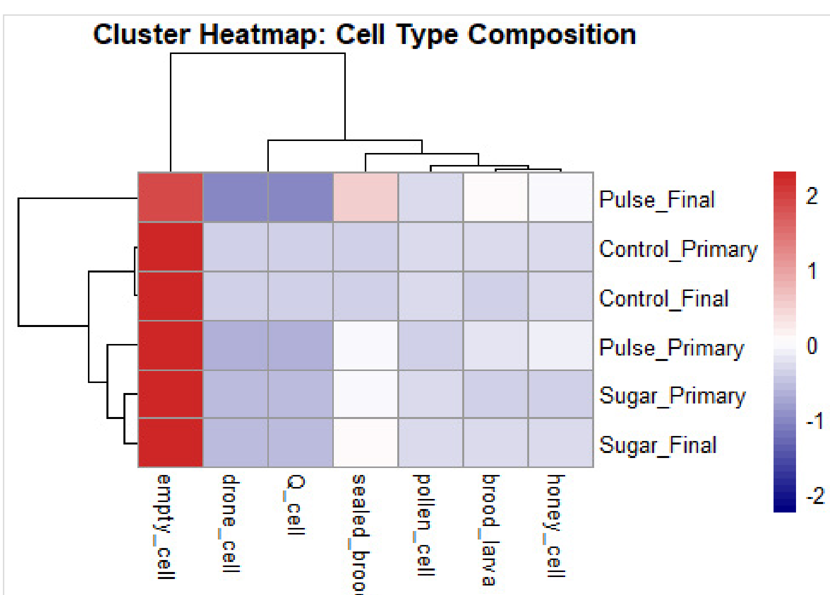

Fig 3. Cluster Heatmap of Cell Type Composition.

that the carbohydrate provided the energy the colony needed to maintain some level of wax production and sealing activity. However, T1's final Brood Cell (Egg, Larvae) count was statistically similar to the Control (T_3), indicating that the lack of adequate protein in T_1 ultimately restricted the queen's laying rate and the nurse bees' ability to rear new larvae. This finding aligns with the understanding that carbohydrate-only feeding can stimulate the colony but cannot sustain a high rate of brood production without a protein source (Ullah et. al., 2021).

The dramatic decline of the Control group (T_3), evidenced by the -71.52% change in Brood Cell and the highest final Empty Cell count, underscores the severity of the dearth period. Without supplemental feeding, the colony was forced to consume its limited internal protein stores, leading to a sharp reduction in brood rearing – a natural survival mechanism to conserve resources, but one that severely weakens the colony.

Regarding resource storage, T_2's significantly higher final Honey Cell count suggests that a healthy, protein-supported colony is more effective at converting the provided sugar into stored honey, either through more efficient processing by a larger workforce or by stimulating greater foraging activity. The significant reduction in Empty Cells in T_2 further confirms that the colony was actively utilizing the comb space for the expansion of brood and the storage of resources.

Conclusion

In conclusion, the Pulse + Sugar diet represents a highly effective and balanced nutritional strategy for *Apis mellifera* management during the dearth period. It provides both the energy (sugar) and the critical protein (pulse) necessary to overcome nutritional stress, leading to superior brood development and resource accumulation compared to carbohydrate-only feeding or no intervention. This study confirms that supplemental feeding is essential for the survival and growth of *Apis mellifera* colonies during the dearth period. Specifically, the Pulse + Sugar diet (T_2), a carbohydrate-protein combination, proved to be the most effective intervention, resulting in significantly higher final counts of Brood Cell (Egg, Larvae) and Honey Cell compared to the Sugar syrup (T_1) and Control (T_3) treatments. The findings emphasize that protein supplementation is the key driver for robust brood development, which is vital for maintaining colony strength and population during periods of nutritional scarcity. Beekeepers operating in areas with pronounced dearth periods should prioritize feeding a balanced diet that includes a high-quality protein substitute.

Authors' Contribution

MER: Methodology, investigation, Formal analysis, writing-review.
NS: Investigation, writing-review & editing.
AA: Formal analysis, writing-review.
MSI: Methodology, investigation.
MNAS: Methodology, investigation.
MNA: Methodology, investigation.
MAR: writing-review & editing.

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