



RESEARCH ARTICLE - BEES

Artificial Diet Supplementation and Its Role in Enhancing Winter Survival and Productivity of Honey Bee Colonies

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Abstract

Artificial diets are crucial for the survival of honey bee colonies during the winter season. A pollen-rich diet provides protein to the colonies and enhances their performance. To investigate the effect of various artificial diets on the performance of honeybee colonies (*Apis mellifera*) during winter, a study was conducted at the Agriculture Research Institute, Tarnab, Peshawar, from November 2023 to January 2024. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications, involving ten different artificial diets. The diets (D1–D10) were formulated using powdered sugar, powdered pollen, and soybean flour. Diets 1, 2, and 3 consisted of only one ingredient each at 100%, while Diets 4, 5, and 6 combined powdered sugar and soybean flour in three different ratios: 75:25, 50:50, and 25:75. Diets 7, 8, and 9 mixed powdered pollen and soybean flour in the same respective ratios. Diet 10 was a mixture of all three ingredients in roughly equal proportions. Significant differences in diet consumption were observed, with Diet 10 showing the highest intake, followed by Diets 2, 4, and 5. The foraging behaviour, measured by pollen collection, was significantly higher in Diet 1 (67 pollen balls per 5 minutes). However, Diet 10 demonstrated superior results in queen fecundity (98 eggs per 24 hours), brood area (285.8 cm²), and colony weight gain (8.7 kg) compared to the other diets. Additionally, Diet 10 resulted in the highest honey production (9 kg per colony per season) and frame coverage (8 frames per colony). In contrast, Diet 1 showed the poorest performance across all parameters. These findings highlight the importance of diet composition in promoting honeybee colony health and productivity during periods of dearth. It is recommended to use Diet-10 with a balanced formulation to enhance colony performance, as it significantly improves queen fecundity, brood rearing, honeybee population growth, weight gains, and honey yield. It is also suitable for winter feeding. The Diet-10 composition should be tested in other seasons, and its effects on productivity, colony performance, and worker bee physiology should also be evaluated.

Introduction

The European honeybee (*Apis mellifera*) not only produces valuable products such as honey, wax, royal jelly,

and propolis but also plays a crucial role in pollinating various crops and is recognized as a vital insect pollinator globally (Garibaldi et al., 2013). Around 75% of global crop species depend on pollinators, including honeybees (*A. mellifera*)



and various wild bees, to facilitate the growth of fruits and seeds. Pakistan has four species of honeybees used for honey production. Three species, *Apis dorsata*, *Apis florea*, and *Apis cerana*, are indigenous, while *Apis mellifera* is exotic (Ali et al., 2021; Khan et al., 2014).

Honeybees actively gather pollen and nectar from various flowers. The colony requires a balanced intake of protein, carbohydrates, minerals, lipids, vitamins, and water to fulfill its developmental and growth needs, all of which are acquired through the collection of pollen, nectar, and water (Funari et al., 2003). Inadequate protein levels in the bee diet pose challenges, affecting the development of the hypopharyngeal glands, which are responsible for provisioning food to larvae. Consequently, this deficiency influences the overall colony production and reproduction (Pereira et al., 2006; Shakeel et al., 2020).

Apiculture in Pakistan is predominantly concentrated in Khyber Pakhtunkhwa, central and northern Punjab, and is rapidly expanding into other regions. An estimated 10,000 beekeepers actively manage around 600,000 colonies of *A. mellifera*, resulting in an annual honey yield surpassing 12,000 Tons. This honey is utilized for both self-consumption and local sales. The honey produced in Pakistan has garnered a positive reputation in Gulf markets and is exported annually at a rate of 4,000 tons, amounting to 23 million USD (Khan et al., 2022).

The lack of sufficient natural forage during winter often leads to a shortage of essential resources, such as nectar and pollen, which can result in colony losses and significant challenges in maintaining vitality and survival. The role of artificial diets in helping honeybees endure the winter months is a subject of increasing importance and interest (Zahra & Talal, 2008). Artificial feeding is believed to play a crucial role in enhancing the effectiveness of honeybee colonies, as it stimulates their biological activities, increases production rates, and strengthens their immunity (Skubida et al., 2008).

Different formulations of diets have been studied earlier, with different compositions. Some commercial diets, which are available at a high cost, can also be found in markets. Numerous researchers have investigated the effectiveness of various diets on multiple levels of honeybee colony performance and production (Khan et al., 2022; Standifer et al., 1960; Sihag & Gupta, 2011).

Various diets were formulated and utilized to enhance the colony's performance during periods of dearth. Colonies fed with artificial diets showed improvements in foraging activity, honey production, and brood rearing (Islam et al., 2024). Additionally, Ricigliano et al. (2022) reported that artificial diets not only boost colony performance but also support gut health. The current studies were conducted to develop artificial diets that are affordable, easy to prepare, and readily accepted by honeybees. Also, the research aimed to analyze the impact of these diets on several variables, including brood rates, honey yield, colony strength, and overall bee health and productivity.

Materials and Methods

Experimental Site and Colonies

The experiment was conducted in the Entomology section of the Agriculture Research Institute, Tarnab, Peshawar, from November 2023 to April 2024. There were 10 diet combinations (treatments) replicated using three colonies per diet, leading to a total of 30 experimental units. Colonies of similar strength, each comprising six frames of bees, were selected for the experiment. All colonies had newly introduced queens, which were introduced in March 2023. In the months leading up to the experiment, all colonies received uniform management practices, including identical treatments for mite control and other standard health measures, to ensure consistency across experimental groups. Each colony was assigned a specific diet, and the respective diet was provided to the assigned colony at weekly intervals. The following diets were used in the experiment.

Diets	Compositions
D1	Powdered Sugar (100%)
D2	Powdered Pollen (100%)
D3	Soya bean flour (100%)
D4	Powdered Sugar (75%) + Soya bean flour (25%)
D5	Powdered Sugar (50%) + Soya bean flour (50%)
D6	Powdered Sugar (25%) + Soya bean flour (75%)
D7	Powdered Pollen (75%) + Soya bean flour (25%)
D8	Powdered Pollen (50%) + Soya bean flour (50%)
D9	Powdered Pollen (25%) + Soya bean flour (75%)
D10	Powdered Sugar (33.3%) + Powdered Pollen (33.4%) + Soya bean flour (33.3%)

Diet preference

A 150g diet was placed directly on top of the frames in Petri dishes. The control colonies were fed sugar syrup. After 24 hours, the weight of the petri dishes was subtracted from the total weight to determine the amount of diet consumed by the animals.

Foraging activity

The foraging activity of *A. mellifera* was observed for five minutes at different intervals, and the average was calculated. Each colony was visually inspected for 5 minutes, and the honeybees returning to the colonies with orbicular pollen balls were counted as pollen foragers.

Queen Fecundity

An empty frame was placed in the box with an exclusion board and the queen for 24 hours. Then, the eggs were counted on the frame using a magnifying glass, following the methods described by Khan et al. (2022).

Colony brood rearing

The evaluation of before- and after-diet provision data for sealed and unsealed brood involves using a specialized measuring frame with a wire grid, where each division corresponds to one square inch. This data was converted to square centimeters by multiplying it by 6.4516 (Abd El-Wahab et al., 2016). The adult bee population was assessed by determining the number of combs covered with bees.

Colony weight gain

The experimental colonies were weighed with a standard scale before the initiation of feeding the diets and extraction of the honey. The data were recorded twice during the experiment to evaluate the weight gains according to the methods used (Mattila & Otis, 2006).

Strength of honeybee colonies

The overall strength of honeybee colonies was assessed by documenting the total number of frames entirely populated by bees, following the methodology by Burgett et al. (1984).

Honey production

The honey production data, measured in kilograms per colony, was recorded to analyse and compare the honey yield between colonies nourished with supplemental diets. The objective was to assess the impact of the supplemental diets used in this investigation, as described by Aziz et al. (2015).

Statistical analysis

The recorded data was subjected to statistical analysis using the computer program Statistix 8.1. Means were compared with 5% level of significance, followed by an LSD test.

Results

Diets preferences/consumption

Figure 1 shows the consumption of different diets during the experimental period. Significant differences were observed in diet consumption. The highest consumption was recorded in Diet-10, with an average of 96.3 g, followed by Diet-2, with 92.3 g after 24 hours. The lowest consumption was observed on Diet-1, with 58.04 g.

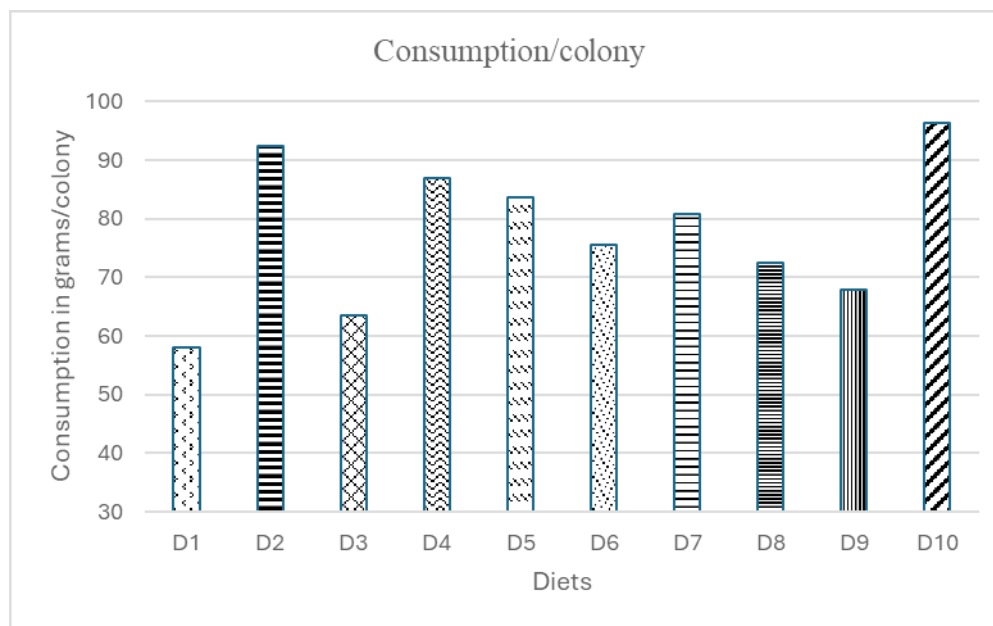


Fig 1. Diet preferences/consumption of *Apis mellifera*.

Foraging activity

The impact of various diets on honeybee foraging/pollen collection activity is presented in Figure 2. The results showed that colonies fed Diet-1 had the highest pollen collection, with 67 pollen balls, followed by those fed Diet-4, which had 66 pollen balls. In contrast, colonies fed with Diet-2 and Diet-10 collected the fewest pollen balls, with 42 and 40, respectively.

Queen Fecundity

The fecundity of the queen significantly varied with various feed diets. Diet-10 resulted in the highest egg production with 98 eggs/queen. In comparison, Diet-1 produced the fewest 39 eggs/queen after 24 hours (Figure 3). This suggests that the composition of the diet has a significant influence on the reproductive success of queens, with some diets promoting higher egg production than others.

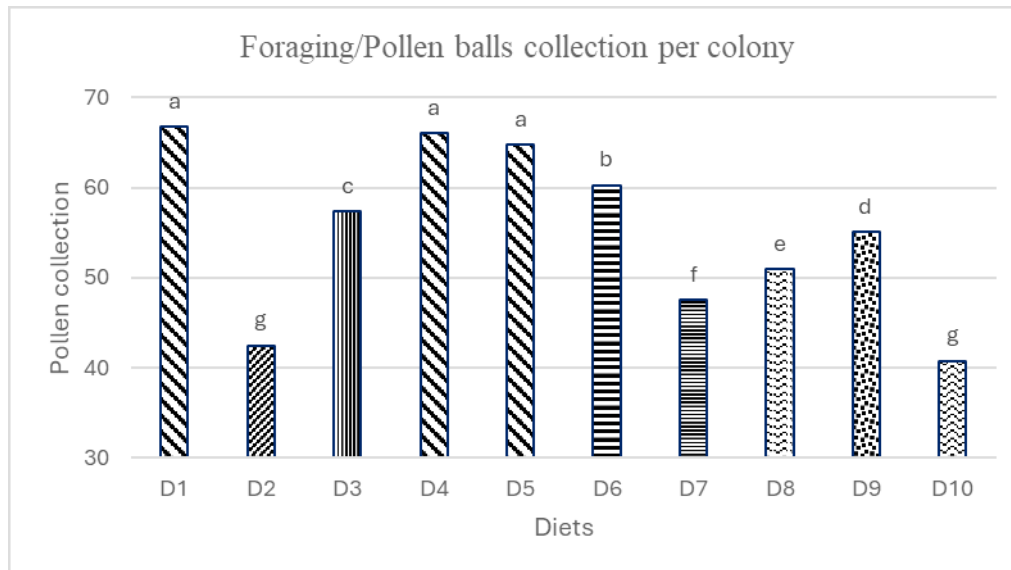


Fig 2. Effect of diets on the foraging/Pollen collection activity of *A. mellifera*.

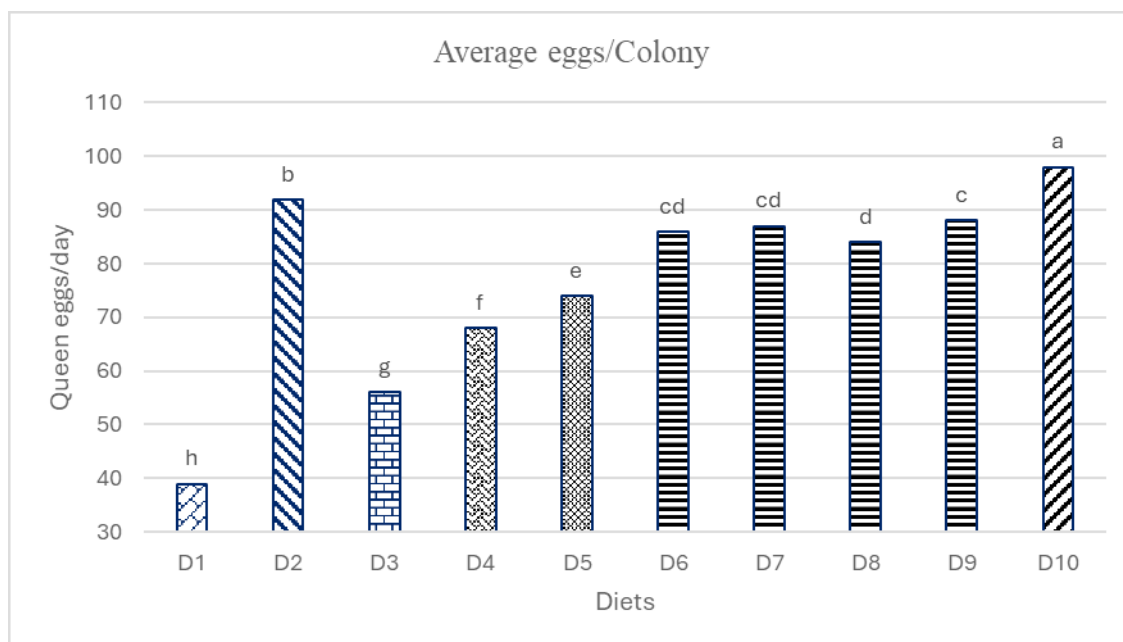


Fig 3. Effect of diets on Fecundity/egg laying of *A. mellifera* Queen.

Colony brood rearing

The effect of various diets on colony brood rearing for *A. mellifera* is shown in Figure 4. The data was recorded before the tested diets on 26 October 2023, with a 14-day interval till the end of the experiment. The highest brood-rearing area was observed in colonies fed Diet-10, averaging 285.8 cm², followed by Diet-2 at 282 cm². The lowest brood-rearing area was recorded in colonies fed with Diet-1, with an average of 248.4 cm².

Effect on colony weight

Figure 5 shows the weight difference among the different experimental colonies fed the diets. The maximum weight gain was observed in the colonies fed Diet-10 (8.7kg),

followed by Diet-2 (8.2 kg). The lowest weight gain was recorded in Diet-1 (1 kg).

Effect of diets on the colony strength of *A. mellifera*

Figure 6 illustrates the effect of various diets on the strength of honey bee populations, measured by the number of frames covered by bees during November, December, and January before honey extraction. The data reveal that colonies fed Diet-10 had the most outstanding coverage, with 8.3 frames per colony, followed by Diet-2 with eight frames per colony. The lowest coverage was observed in colonies fed with Diet-1, which consisted only of powdered sugar, showing four frames per colony. All tested diets showed significant differences from one another, and all were found to be more effective than the control group.

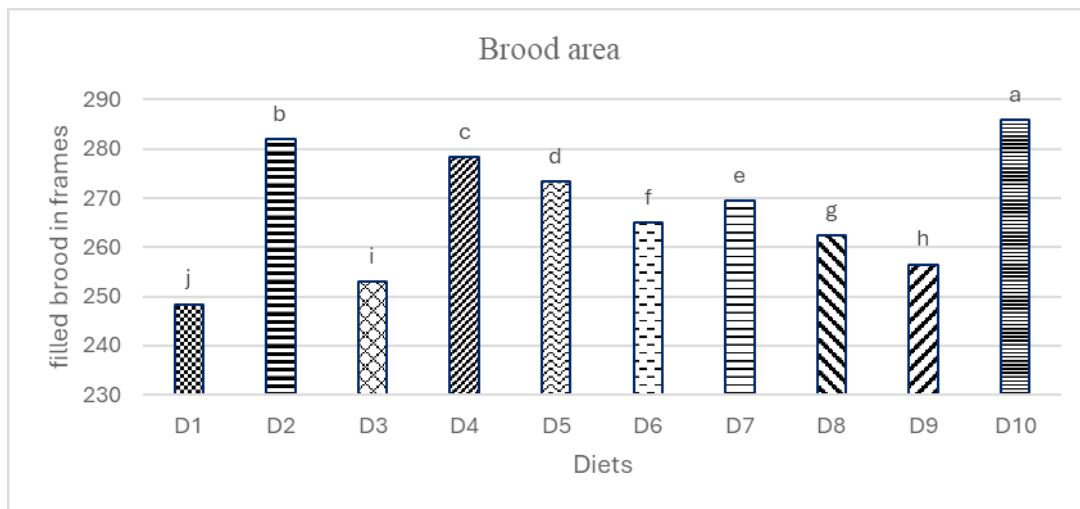


Fig 4. Effect of diets on colony brood rearing (Brood area cm²).

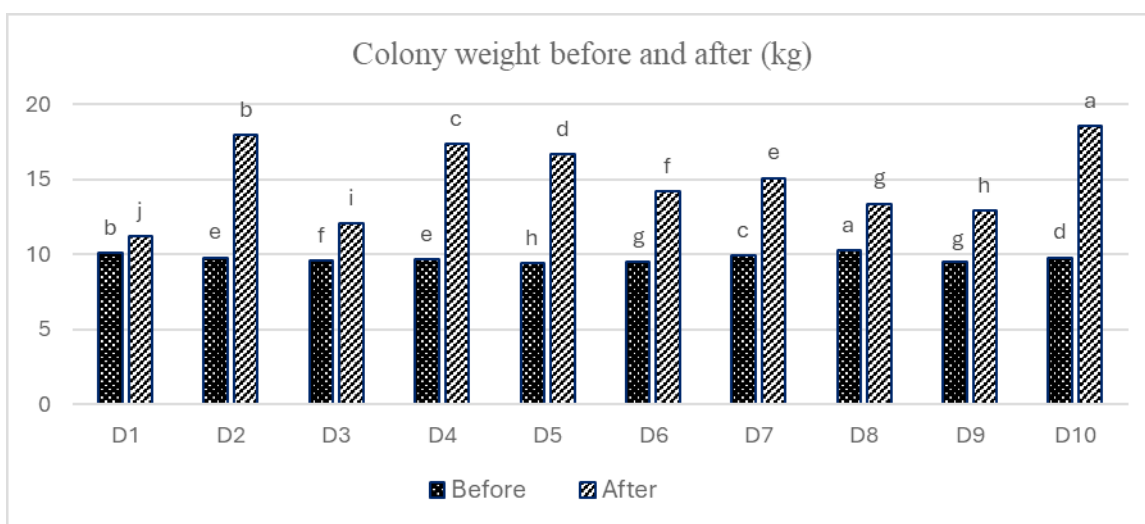


Fig 5. Effect of diets on colony weight of *Apis mellifera*.

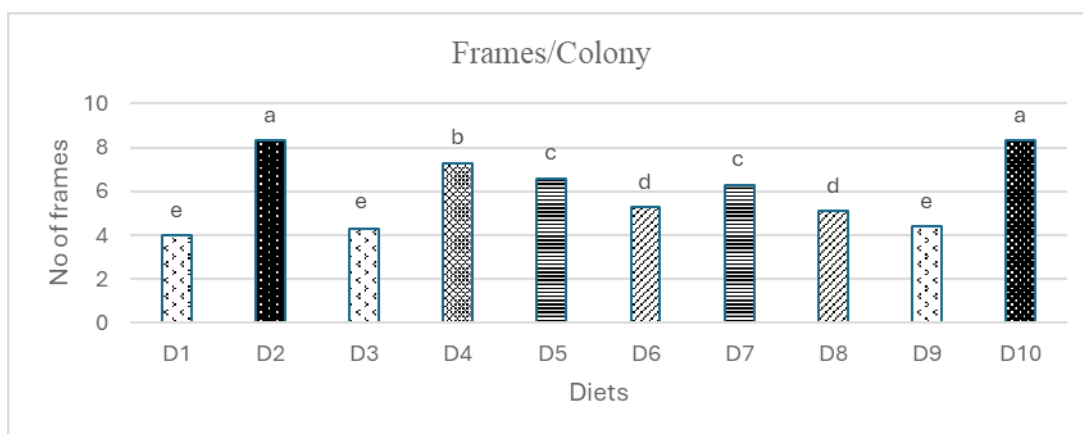


Fig 6. Effect of diets on colony strength of *A. Mellifera*.

Honey production/colony

The supplemental diets provided to the colonies exhibited notable variances in their impact. Diet 10 displayed

the most excellent frame coverage and honey yield (9.06kg/colony), trailed by Diet 2 with a honey yield of 8.04 kg. The lowest honey production (3.34kg/colony) was recorded in Diet 1-fed colonies (Figure 7).

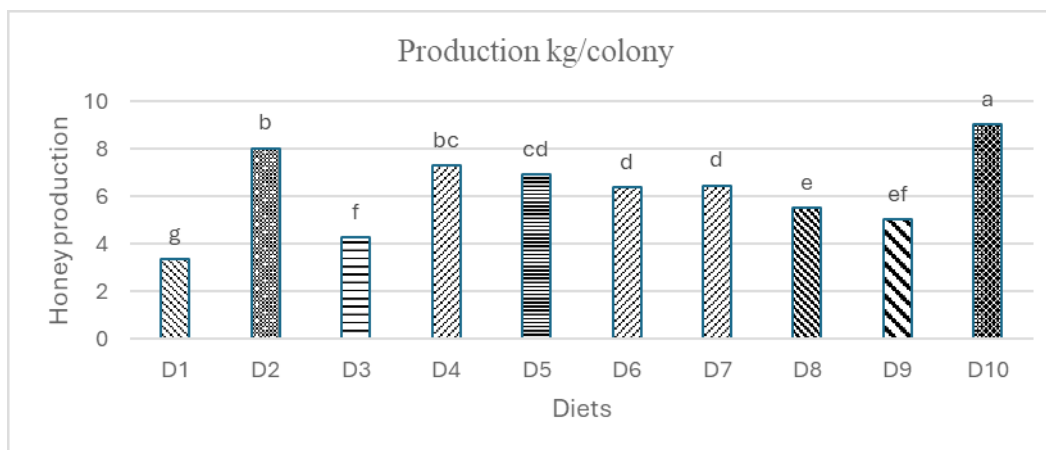


Fig 7. Honey production in kg/colony.

Discussion

In the current study, it was observed that Diet-10, which comprised 33.3% powdered sugar, 33.4% powdered pollen, and 33.3% soybean flour, was highly preferred and consumed by the *A. mellifera* colonies, followed by Diet-2, consisting of 100% pollen. The lowest consumption was observed with Diet-1. These findings align with Abd El-Wahab et al. (2016), who also reported that artificial diets with different ingredients, such as soybean flour mixed with sugar and pollen, resulted in higher consumption. DeGrandi-Hoffman et al. (2008) also reported that a diet with equal amounts of sugar and pollen is the most preferred, with high consumption rates. Islam et al. (2024) prepared different diets using various flours and reported that the colonies preferred the flour-based diets.

Significantly high foraging behavior and pollen collection were reported in colonies fed only a sugar diet (Diet-1), indicating that pollen is crucial for the colony's survival during winter, as it fulfills the colony's protein requirements. Diet-2 and Diet-10, which had sufficient pollen content, showed the lowest foraging for pollen collection. An earlier study by Topal et al. (2022) also reported that higher consumption of supplemental diets correlates with reduced foraging activity. Pernal and Currie (2001) also reported that the decision-making process regarding nectar and pollen collection in colonies is strongly influenced by the type of artificial diets provided. Specifically, when diets rich in pollen are offered, there is a significant reduction in the colony's pollen collection activity.

Queen fecundity was high in Diet-10 and lowest in Diet-1. Queen fecundity is positively correlated with the amount of pollen stored in the frames within the colony. Queen egg-laying capacity also varies with the amount of pollen and nectar collected. This finding aligns with Fèvre and Dearden (2024), who reported that diets with higher nutritional value improve queen reproductive performance, while less optimal diets decrease fecundity, highlighting the significant impact

of diet composition on queen success. Khan et al. (2022) also reported high fecundity in colonies fed artificial diets.

This study found that different diets affected brood rearing in honeybee colonies. Diet 10 produces the most brood area (285.8 cm²) and Diet 1 the least (248.4 cm²). Other diets fell between these extremes. These results suggest that diet quality affects the effectiveness of brood rearing, aligning with the findings of Ullah et al. (2021), who discovered that improved nutritional supplements lead to more efficient brood development and healthier colonies. DeGrandi-Hoffman et al. (2008) reported high brood rearing in a diet with balanced pollen and sugars. Kumar et al. (2021) also reported high brood rearing in colonies fed artificial diets during periods of dearth.

Colonies fed Diet-10 exhibited the highest weight gain (8.7 kg), followed by Diet 2 (8.2 kg), and the lowest weight gain was observed in colonies fed Diet-1 (1 kg). This finding indicates that egg laying is significantly associated with protein diets, and diets rich in pollen resulted in higher weight gain compared to sugar-fed colonies. These results are similar to those of Ashley et al. (2019), who observed that supplemental diets influence honeybee colony weight and health. Colonies gain weight if they are fed diets with pollen as an ingredient. Sultana et al. (2024) also reported high weight gain in colonies fed with pollen-rich diets. Ricigliano et al. (2022) previously studied how protein-based diets improve the gut health of colonies, which in turn leads to increased colony strength.

The results showed that colonies fed Diet-10 had the highest coverage, with eight frames per colony. In contrast, colonies fed Diet-1, consisting only of powdered sugar, had the lowest coverage, at four frames covered with bees per colony. These results align with the studies conducted by DeGrandi-Hoffman et al. (2008) and Sultana et al. (2024), who reported that high consumption rates by colonies affect the brood and adult populations.

The colonies with greater frame coverage produced higher honey yields during this study. These findings align with those of Lamontagne-Drolet et al. (2011), who investigated

the effects of different nutritional supplements on honey production and frame coverage in honeybee colonies. They observed that colonies receiving more nutritionally balanced diets had higher honey yields and better frame coverage. Mahmood et al. (2013) also reported high honey production in colonies fed with pollen diets during winter compared to only sugar-fed colonies.

Conclusion and recommendations

The performance of colonies is significantly influenced by the supplemental diets provided. Among the tested diets, Diet-10 (Powdered Sugar 33.3% + Powdered Pollen 33.4% + Soybean Flour 33.3%) boosted queen fertility, increased brood rearing, enhanced the honeybee population, and contributed to more significant weight gains and honey yield. Other diets also showed some good results. Beekeepers can use this diet composition to maintain healthy colonies during periods of dearth. Further research should also explore alternative diet formulations and their effects across different seasons.

Authors' Contribution

NUS: Methodology, investigation.

HA: Methodology, investigation.

AU: Methodology, investigation.

MS: Methodology, investigation.

KAK: Formal analysis, writing-review & editing.

HAG: Formal analysis, writing-review & editing.

MMFO: Formal analysis, writing-review & editing.

EMA: Formal analysis, writing-review & editing.

MS: Formal analysis, writing-review & editing.

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