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Original Article

Assessment of consumption and digestibility of artificial diets and their effects on few life study parameters of *Apis mellifera* L.

Amir Manzoor[1], Muhammad Anjum Aqueel[*1], Shams Ul Islam[1], Eldessoky S. Dessoky[2], Muhammad Haseeb Ahsan[1], Bashir Ahmad[1,3], Muhammad Usman Yousuf[1], Muhammad Saqib[4], Muhammad Fahim Raza[5], Rashid Iqbal[*4,6]

[1]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan
[2]Department of Biology, College of Science, Taif University, Taif, Saudi Arabia
[3]PARC-Arid Zone Research Institute, Bahawalpur, Pakistan
[4]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan
[5]Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4, Canada
[6]Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

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Abstract

Artificial diets are necessary for managed honeybees (*Apis mellifera* L.) in arid regions when naturally occurring flora becomes scarce. The current study was planned to evaluate different artificial diets in terms of consumability and digestibility of *A. mellifera* and their effects on weight of queen bee, worker bee longevity and number of broods for colony survival and management in dearth period. Different artificial diets were administered separately to 33 colonies in three replications. The best combination was 25 g, each of soya flour, dry apricot powder, date paste and powder sugar, mixed in 8 ml of vegetable oil. Honeybees consumed most of diet-T_8 in all weeks (average 49.53 g), followed by diet-T_7 (average 38.81 g) and diet-T_6 (average 34.55 g); while the minimum consumption was of diet-T_1 (average 6.67 g). In diet digestibility experiment, diet-T_2 showed the highest digestibility (73.9%) followed by diet-T_1 (71.3%) and diet-T_4 (66.7%) with the lowest digestibility in diet-T_9 (59%). The protein present in hemolymph was highest in diet-T_8 (26.9 *ug*/*u*l) followed by diet-T_7 (24.1 *ug*/*u*l) and diet-T_6 (22.9 *ug*/*u*l) with the lowest in diet-T_1 (17.7 *ug*/*u*l). As a response to the feeding on the prepared diets, the queen's bee weight was highest in diet-T_8 (198.65 ± 0.36 mg) followed by diet-T_7 and diet-T_6 while the lowest in diet-T_0 (control group, 182.28 ± 0.55 mg). Similarly, the worker bee longevity was highest in diet-T_8 (53 ± 2.14 days) followed by diet-T_7 and diet-T_6 and lowest in diet-T_0 (control group, 34 ± 1.73 days). In the brood experiment, the number of eggs, larvae and pupae was highest in diet-T_8 (85 ± 2.03 eggs, 66 ± 1.15 larvae and 78 ± 1.15 pupae) while lowest in diet-T_0 (51 ± 1.5 eggs, 26 ± 1.5 larvae, 23 ± 1.5 pupae) after 28 days of diet feeding. Therefore, diet-T_8 can be used as substitute diet for the honeybees during dearth period for the survival and colony management of *A. mellifera*

Keywords: Apis mellifera diet, Substitute diet, Dearth period management, Diet digestibility, Weight of queen, Worker bee longevity, Number of broods

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**Corresponding author email:*
a.aqueel07@alumni.imperial.ac.uk
rashid.iqbal@iub.edu.pk

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Introduction

Honeybees require highly nutritive, diets as being eusocial, they have to perform different tasks in their life (Seeley, 2019; Free and Williams, 1972) e.g., they feed their larvae and build comb for about the first half of their life (Seeley, 2019). They require proteins and royal jelly for the larval growth (Helm et al., 2017). During a certain period of the year, floral scarcity occurs due to seasonal changes and environmental stressors in various parts of the world (Paray et al., 2021). In this period when natural flora is insufficient or unavailable for the colony's need, beekeepers feed artificial diets to ensure survival, strengthen colonies and reduce their susceptibility to diseases (Fleming et al., 2015). In Pakistan, during the dearth eras (June-July), beekeepers frequently feed honeybee colonies with sugar syrup and fruit supplements (Al-Ghamdi et al., 2011; Mahmood et al., 2013). The majority of beekeepers also use powder sugar syrup as an additional nutrition to promote brood growth and fertilization of queen (Usha et al., 2014). But only sugar syrup is not enough as a diet of honeybees for better survival and growth. Low nutrition adversely affects colony performance and health (Paray et al., 2021). It also leads to insect pest and disease attacks, which may result in colony collapse disorder.

Honeybees require a balanced diet of protein, carbohydrates, lipids, vitamins, and minerals to maintain their health and productivity (Brodschneider and Crailsheim, 2010). Black (2006) proposed the specifications for an efficient pollen substitute diet: approximately 25–30% protein, 5% lipids, 10–20% fiber, 40–60% sugar, 1–1.5% minerals and vitamins, along with the appropriate amount of essential amino acids, linoleic acid, and cholesterol. Antioxidants can be included to ensure the long-term preservation of the diet (Weirich et al., 2002). High quality dietary sources are necessary to enhance bee production. Diets containing artificial pollen can be used for honeybee colonies' survival and reproduction in the absence of naturally occurring pollen sources (Mattila and Otis, 2006) rich protein feedings are essential for the reproduction and care of bee colonies, bee brood rearing, and younger bee development (Manning, 2001) and also for honey production (Saffari et al., 2004). Beans and pulses are the important source of proteins and increase the longevity and overall health of the bees. Carbohydrates are most important for the development of honeybee colonies and are found in the plant nectar. However natural sources of nectar are not available throughout the year (Pawlikowski, 2010). The most important energy source for many birds and animals is fruits as they mainly provide sugar, vitamins, and minerals (Howe, 1986). Therefore, the fruits function similarly to nectar. Many insect species including honeybees forage on fruits (Evison and Ratnieks, 2007; Helanterä and Ratnieks, 2008).

Significant research work on substitute diets has been conducted worldwide, utilizing various ingredients to prepare artificial diets (Gemeda, 2014; Usha et al., 2014; Aly et al., 2014; Amro et al., 2020). However, these ingredients or artificial diets cannot be used and implemented everywhere due to vast differences in climatic and environmental conditions, floral variety, presence of raw ingredients, specific needs and economic aspects. Scientists have also explored the influence of pollen and nectar substitute diet formulations on honeybee colonies. A large number of diet formulations have been developed by the scientists combining different ingredients (Paray et al., 2021) but no substitute diet has been formulated so far that can completely replace the nutrition provided by the pollen (Manning, 2018).

The goal of this study was to discover alternative diets which would be most palatable and easily digested by honeybees and how these diets affect life study parameters during food scarcity, in order to enhance colony survival. The results of this study may help in providing guidelines to beekeepers on how to run their businesses and come up with creative solutions to deal with the issue of food scarcity during the dearth period.

Material and Methods

Study site

The study was conducted in the Apiculture research area in the Department of Entomology, The Islamia University of Bahawalpur (29.372800 longitudes, 71.764988 latitudes). Bahawalpur is located in the Punjab province of Pakistan. Bahawalpur has a desert climate characterized by extremely hot summers (41-

43^0C average maximum temperature) and mild winters (9.4-22.8^{0}C) and recorded rainfall in summer is 31 mm to 58 mm, while in winter it is 5 mm to 14 mm. During the winter season, beekeepers from various regions come to Bahawalpur to take advantage of the flowering mustard fields. Mustard blooms provide an abundant source of nectar and pollen for bees, which is essential for the growth and maintenance of bee colonies during the colder months.

Experimental colonies

Thirty three colonies were selected for diet consumability and digestibility experiments and 12 colonies were selected for the life study parameters. Each colony had nine frames, a single fertile queen and approximately twenty thousand bee population. The colonies were homogeneous in population, ensuring consistent and reliable results across the study. Experiments were done during summer from 5th June to 5th October 2023.

Preparation of different combinations of diet ingredients

Economically cheaper ingredients were selected for diet preparation. The main components included soya flour (main source of protein), date paste (Source of protein and carbohydrate), dry apricot powder (source of carbohydrate vitamins A & C and minerals), skimmed milk (source of protein carbohydrate and vitamins) and the powdered sugar.

In the mixing process, the soya flour and dry apricot powder were combined in a large bowl, while the date paste was gradually added and thoroughly mixed to achieve an even distribution. Powdered sugar was then incorporated slowly to achieve a consistent texture. Skimmed milk was also added and thoroughly mixed in treatments where required. Finally, vegetable oil was added as to our specific composition ratio. The detailed ingredients are outlined in Table 1.

Table-1. Different ingredients selected for diet preparation

Sr. No.	Ingredients
1	Soya Flour
2	Date Paste
3	Dry Apricot Powder
4	Skimmed Milk
5	Powder Sugar
6	Vegetable Oil

The specific composition ratio of ingredients is described below:

Diet 1 (T1) = 33.33 g soya flour + 33.33 g skimmed milk + 33.33 g powder sugar

Diet 2 (T2) = 33.33 g soya flour + 33.33 g skimmed milk + 33.33 g powder sugar + 8 ml vegetable oil

Diet 3 (T3) = 33.33 g soya flour + 33.33 g dry apricot powder + 33.33 g powder sugar

Diet 4 (T4) = 33.33 g soya flour + 33.33 g dry apricot powder + 33.33 g powder sugar + 8 ml vegetable oil

Diet 5 (T5) = 33.33 g soya flour + 33.33 g date paste + 33.33 g powder sugar

Diet 6 (T6) = 33.33 g soya flour + 33.33 g date paste + 33.33 g powder sugar+ 8 ml vegetable oil

Diet 7 (T7) = 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar

Diet 8 (T8) = 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil

Diet 9 (T9) = 20 g soya flour + 20 g skimmed milk + 20 g dry apricot powder + 20 g date paste + 20 g powder sugar

Diet 10 (T10) = 20 g soya flour + 20 g skimmed milk + 20 g dry apricot powder + 20 g date paste + 20 g powder sugar + 8 ml vegetable oil

Diet 11 (T_0) = Control (50% sugar solution)

Petty feeding diet

The diet was prepared as to the composition ratio and were filled equally in the petri dishes and were wrapped with polythene paper leaving the space for bees to consume the diet as proposed in the study of Amro et al. (2016). Petri dishes were placed under the bee frames inside the bee colony.

Consumability of diet

The data were recorded after at one week interval up to 8th week. At first, fresh diet was weighed with electronic weight balance (OHAUS Diamond MCT 500) and then weighed it again after 1 week and the data were recorded up to 8th week. The diet was replaced with fresh diet after every week (Islam et al., 2020).

Digestibility of diet

This experiment was conducted during the summer, from August 7th to August 21st, 2023. The diets were fed for two weeks to determine the digestibility of diet, only the nurse bees were selected from the hives. For this purpose, concentration of the protein present in contents of the hindgut of a nurse bee were studied and compared with the concentration present in the provided diet. 3 random nurse bees were collected from the hives of all the colonies (from each treatment and replication) and were dissected, to extract and weighted their hindgut. The hindgut content from of 1 µl was mixed with 99 µl Phosphate-Buffered Saline (PBS) and 1% EDTA (ethylene diamine tetra acetic acid) without HPIC (High-Performance Ion Chromatography) reagents. Then, mixed solutions were added to 900 µl of PBS containing 1% EDTA-free HPIC and kept at -80°C until we used a spectrophotometer (UV-Visible spectrophotometer) to measure the total soluble proteins at 595 nm (Cremonz et al., 1998). Then protein concentrations of samples of diets were to find out. Three random samples were collected from the diets. 1 mg from the diet sample were added to the 999 µl of PBS containing 1 EDTA-free HPIC. The same procedure was adopted to find out the soluble proteins present in the diet. Diet digestibility was determined as to the following formula.

$$\text{Approximate Digestibility} = \frac{\text{Protein conc. in diet} - \text{Protein conc. in hindgut}}{\text{Protein conc. in diet}} \times 100$$

Then, the amount of total protein present in nurse bee hemolymph was measured. For this purpose, three honeybees were randomly selected from each colony from all the treatments and hemolymph was harvested following Borsuk et al. (2017) technique. Briefly, after separating the bee's antenna, the abdomen of the bee was carefully pressed. A drop of hemolymph appeared at the excised antenna's base as a result, and it was scooped up using a pipette. Hemolymph samples were stored at -80°C. Total protein contents were measured with the same procedure as adopted for protein contents in hindgut and diet using 1 µl of hemolymph samples and mixing it with 99 µl of PBS and 1% EDTA-free HPIC.

Weight of queen

This trial consisted of 12 colonies including 3 replications. Colonies were fed on the screened diet (i.e., T_6, T_7, T_8 and T_0) for 4 weeks from 7th August to 4th September 2023. After being fed on the screened diet, the frames in the colonies were observed to find the queen cells. When the queen emerged, it was weighed on the electronic balance (Akyol et al., 2008). The data were calculated based on the weight of the emerged queens (treatments) and the weight of the queen in the unfed colony (control) and were analyzed according to statistical standard procedures.

Worker bee longevity

For this experiment, 12 honeybee colonies were selected along with three replications. Experiments were carried out from 7th August to 5th October 2023. As the adult honeybees emerged from the cells, they were marked with a permanent color marker (Shurjeel et al., 2020). The date and time of the bee emergence was noted. In this manner, 15 honeybees were marked daily from each colony for three days. Longevity was assessed by daily inspection of the bee colony and identification of bees (Martin et al., 2021). The colony was inspected daily in the morning at 10 am. Worker bee longevity data were collected and analyzed according to statistical standard procedures.

Number of broods

For this experiment, 12 honeybee colonies were selected along with three replications. The worker bee broods (eggs, larvae and pupae) were counted after 14 days and 28 days of feeding diet. For this purpose, a region in the brood area of the honeybee hive was selected and counted the newly emerging broods (Amir and Peveling, 2004). The data were compared with the controlled colony (with no fed screened diets) and statistically analyzed.

Statistical analysis

The total amount of diet consumed, and diet digested were compared across the treatments. Analysis of variance (ANOVA) following the Completely Randomized Design (CRD) was performed to test the statistically significant differences among the treatments. Further, the Least Significant Difference (LSD) test was performed for multiple comparisons among the groups at 5% level of significance for the diet consumability, digestibility, weight of queen and worker bee longevity experiments. While two-way ANOVA (factorial design) was used for the number of broods to test the statistically significant differences among the treatments. Further, the Tukey's HSD post-hoc test was performed for multiple comparisons among the groups at the 5% level of significance. Analyses of variance were performed by Statistix (8.1) software, and the graphs were developed in MS Excel (2016).

Results

Diet consumption

In week 1, there was a statistically significant difference among ten diets in terms of mass consumed ($p < 0.000$). Honeybees consumed maximum diet-T_8 (41.29 ± 0.66 g) followed by diet-T_7 and T_6; while minimum diet-T_1 (4.13 ± 0.50 g). In week-2, there were also significant differences among all the treatments ($p < 0.001$). Maximum diet-T_8 (44.40 ± 1.25 g) was consumed by honeybees followed by diet-T_7 and T_6, while the diet-T_1 was consumed minimum (4.59 ± 0.59 g). In week-3, there were also significant differences among the treatments ($p < 0.001$). Honeybees consumed maximum diet-T_8 (45.64 ± 0.85 g) followed by diet-T_7 and T_6; while minimum diet-T_1 (8.4 ± 0.55 g). In week-3, there was also significant difference among all the diets ($p < 0.001$). Maximum diet consumption was recorded in diet-T_8 (45.10 ± 1.06 g) followed by diet-T_7 and T_6; while minimum in diet-T_1 (8.4 ± 0.55 g).

In week-4, there were statistically significant differences among all the treatments ($p<0.001$). Honeybees consumed maximum diet-T_8 (45.10 ± 1.06 g) followed by diet-T_7 and T_6; while minimum diet-T_1 (8.73 ± 0.68 g). In week 5, there were also significant differences among all the treatments ($p < 0.001$). Maximum diet-T_8 (52.02 ±1.21 g) was consumed by honeybees followed by diet-T_7 and T_6, while the diet-T_1 was consumed minimum (7.39 ± 1.27 g). In week-6, there were also significant differences among the treatments ($p < 0.001$). Honeybees consumed maximum diet-T_8 (52.57 ± 0.36 g) followed by diet diet-T_7 and T_6; while minimum diet-T_1 (7.50 ± 0.47). In week 7, there were also significant differences among all diets ($p < 0.001$). The maximum diet consumption was recorded in diet-T_8 (58.53 ± 1.41 g) followed by diet-T_7 and T_6; while minimum diet-T1 (6.38 ± 1.17 g.). In week-8, there were also statistically significant differences among all the treatments ($p < 0.001$). Honeybees consumed maximum diet-T_8 (56.74 ± 0.58 g) followed by diet-T_7 and T_6; while minimum diet-T_1 (6.28 ± 0.17 g).

Table-2. **Diet consumed by honeybees (*Apis mellifera*) in 8 weeks**

Diet		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Diet 1		4.13 ± 0.50 h	4.59 ± 0.59 i	8.4 ± 0.55 i	8.73 ± 0.68 h	7.39 ± 1.27 h	7.50 ± 0.47 i	6.38 ± 1.17 i	6.28 ± 0.17 j
Diet 2		7.95 ± 0.41 g	7.51 ± 0.35 h	11.56 ± 1.20 h	11.7 ± 0.66 g	14.42 ± 0.63 g	11.5 ± 0.51 h	12.8 ± 0.64 h	12.51 ± 0.50 t
Diet 3		13.69 ± 0.38 f	13.07 ± 0.67 g	12.96 ± 0.78 gh	16.38 ± 0.94 f	15.48 ± 0.93 g	16.07 ± 0.52 g	17.98 ± 0.71 g	17.19 ± 0.37 f
Diet 4		20.83 + 0.92 d	23.04 ± 0.82 e	22.77 ± 0.96 e	24.96 ± 0.97 de	25.21 ± 1.14 e	27.04 ± 1.47 d	27.73 ± 0.82 e	26.84 ± 0.41 e
Diet 5		24.01 ± 1.31 c	26.29 ± 0.42 d	27.02 ± 0.46 d	27.83 ± 0.46 d	29.30 ± 0.32 d	29.86 ± 2.24 d	33.74 ± 0.70 d	32.56 ± 0.37 d
Diet 6		30.44 ± 0.89 b	30.94 ± 1.20 c	32.56 ± 1.22 c	35.37 ± 1.19 c	35.11 ± 1.31 c	33.71 ± 0.52 c	39.7 ± 1.00 c	38.62 ± 0.77 c
Diet 7		32.94 ± 1.37 b	36.93 ± 1.13 b	36.20 ± 0.82 b	38.57 ± 1.36 b	40.67 ± 0.60 b	39.59 ± 0.78 b	43.44 ± 0.58 b	42.19 ± 0.73 b
Diet 8		41.29 ± 0.66 a	44.40 ± 1.25 a	45.64 ± 0.85 a	45.10 ± 1.06 a	52.02 ±1.21 a	52.57 ± 0.36 a	58.53 ± 1.41 a	56.74 ± 0.58 a
Diet 9		16.77 ± 0.93 e	18.10 ± 0.43 f	15.38 ± 1.08 g	19.05 ± 1.37 f	19.36 ± 1.04 f	20.35 ± 0.94 f	20.18 ± 1.41 g	19.52 ± 0.71 g
Diet 10		19.08 ± 0.77 de	21.33 +1.07 e	19.82 ± 1.56 f	22.37 ± 0.72 e	21.6 ±1.36 f	23.71 ± 0.68 e	23.63 ± 0.39 f	23.07 ± 0.78 f
ANOVA	d.f	9	9	9	9	9	9	9	9
Results	f	170	215	146	144	170	178	300	704
	p	0	0	0	0	0	0	0	0

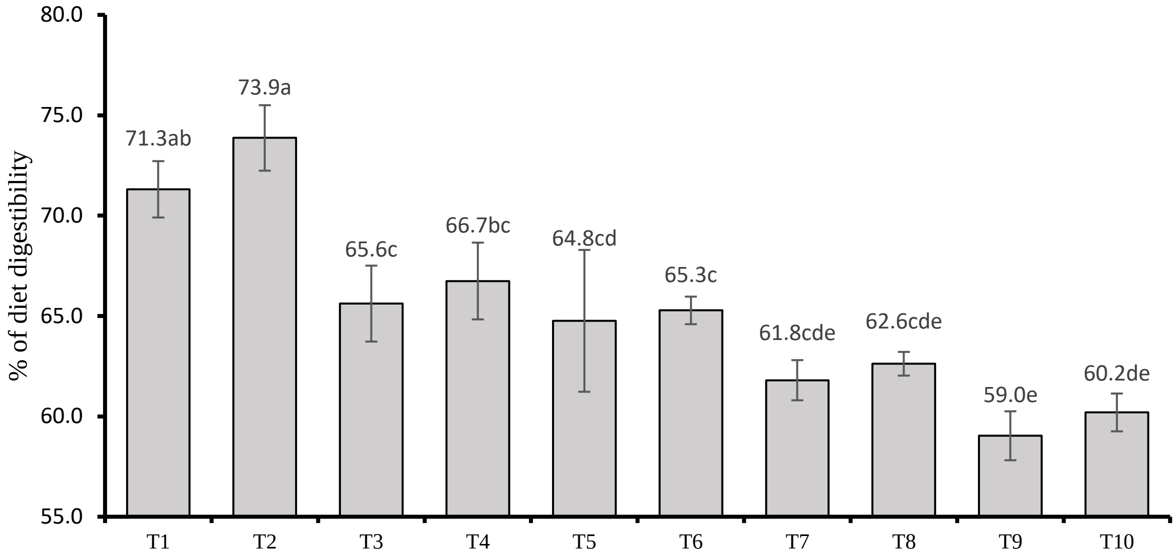

Figure-1. Percentage diet digestibility of all the treatments

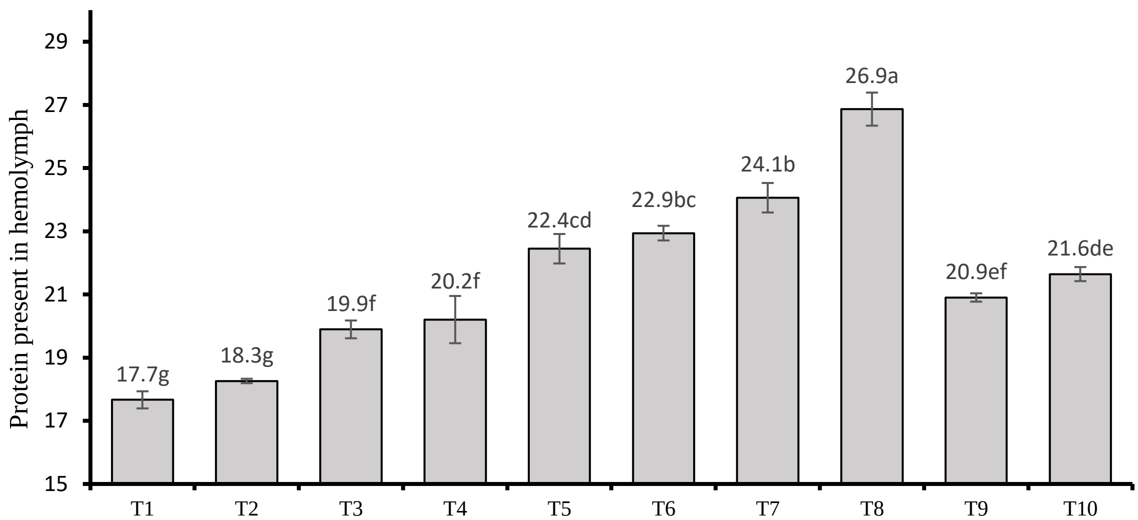

Figure-2. Protein present in the honeybee hemolymph in *ug/ul*

Diet digestibility

Treatments 6, 7 and 8 were statistically similar, while maximum diet digestibility (%) was recorded in T_2 (73.9 ± 1.3 %) followed by T_1 and T_4, while minimum was observed in T_9 (59.0 ± 1.22 %).

Protein present in hemolymph

Statistically significant differences were observed among all the treatments in terms of protein availability in hemolymph (df= 9, F= 49.0, $p < 0.001$). However, maximum presence of protein in hemolymph was recorded in T_8 (26.9 ± 0.52 *ug/ul*) followed by T_7 and T_6, while minimum was observed in T_1 (17.7 ± 0.27 *ug/ul*).

Correlation matrix

Correlation between variables i.e. Diet digestibility, Protein present in hemolymph with respect to diet consumption is explained in Table (3).

Table-3. Correlation of diet digestibility, protein levels in hemolymph, and diet consumption

Variables	Diet Digestibility	Protein hemolymph µg/µl
Protein hemolymph µg/µl	-0.640 (0.046)	
Consumption	-0.525 (0.119)	0.974 (0.0001)

There is a moderate negative correlation between diet digestibility and protein levels in hemolymph, indicating that as diet digestibility increases, the protein concentration in hemolymph tends to decrease. This relationship is statistically significant, with a p-value of 0.046. Similarly, there is a moderate negative correlation between diet digestibility and consumption; however, this relationship is not statistically significant, as indicated by a p-value of 0.119. On the other hand, a very strong positive correlation exists between protein levels in hemolymph and consumption, suggesting that higher consumption is associated with increased protein levels in hemolymph. This correlation is highly significant, with a p-value of 0.0001.

Effect of artificial diets on weight of queen bee

After feeding on the artificial diets, there were observed statistically significant differences among all the diets in terms of weigh of queen ($p < 0.001$). The queen emerged after feeding on T_8 weight was highest (198.65 ± 0.36 mg) followed by T_6 and T_7; while minimum performance was observed in control group, T_0 (182.25 ± 0.55 mg).

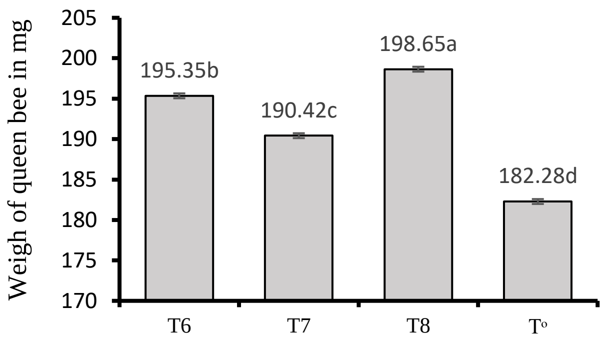

Figure-4. Comparison of bee's longevity in colonies offered selected diets with respect to control treatment

Effect of artificial diets on brood

Effect of artificial diets on number of eggs of honeybees

At the time of diet placement the diet on 1st day, there was no-statistically significant difference between all the treatments. After 14 days of diet feeding the position was different. All the treatments had statistically significant difference ($p<0.001$). T_8 had the maximum number of eggs (62 ± 2.03 eggs) followed by T_6 and T_7, while the minimum eggs were recorded in T_0 control group (43 ± 1.5 eggs). After 14 days, there was also a significant difference between all the treatments ($p<0.001$). Maximum number of eggs were recorded in T_8 (85 ± 2.03 eggs) followed by T_6 and $T_{7,}$ while the minimum was observed in control group T_0 (51 ± 1.5 eggs).

Effect of artificial diets on number of larvae of honeybees

At the time of diet placement (i.e. day-1), there were no significant differences between the treatments. After 14 days of diet feeding; the position was different. All the treatments were statistically significant difference ($p<0.001$). Diet-T_8 had the maximum number of larvae (47 ± 1.15 larvae) followed by diet-T_6 and T_7, while the minimum larvae were recorded in control group-T_0 (23 ± 1.5 larvae). After 14 days, there was also a significant difference among all the treatments ($p<0.001$). Maximum number of larvae were recorded in diet-T_8 (66 ± 1.15 larvae) followed by diet-T_6 and T_7, while the minimum were in control group-T_0 (26 ± 1.5 larvae).

Effect of artificial diets on number of pupae of honeybees

At the time of diet placement (i.e. day-1), there were no significant differences among all the treatments. While after 14 days of diet feeding the position was different. All the treatments were having statistically significant differences ($p<0.001$). Diet-T_8 had the maximum number of larvae (29 ± 1.15 pupae) followed by diet-T_6 and T_7, while the minimum larvae were recorded in control group-T_0 (19 ± 1.5 pupae). After 14 days, there was also a significant difference between all the treatments ($p<0.001$). The maximum number of larvae were recorded in diet-T_8 (78 ± 1.15 pupae) followed by diet-T_6 and T_7 while the minimum was in control group-T_0 (23 ± 1.5 pupae).

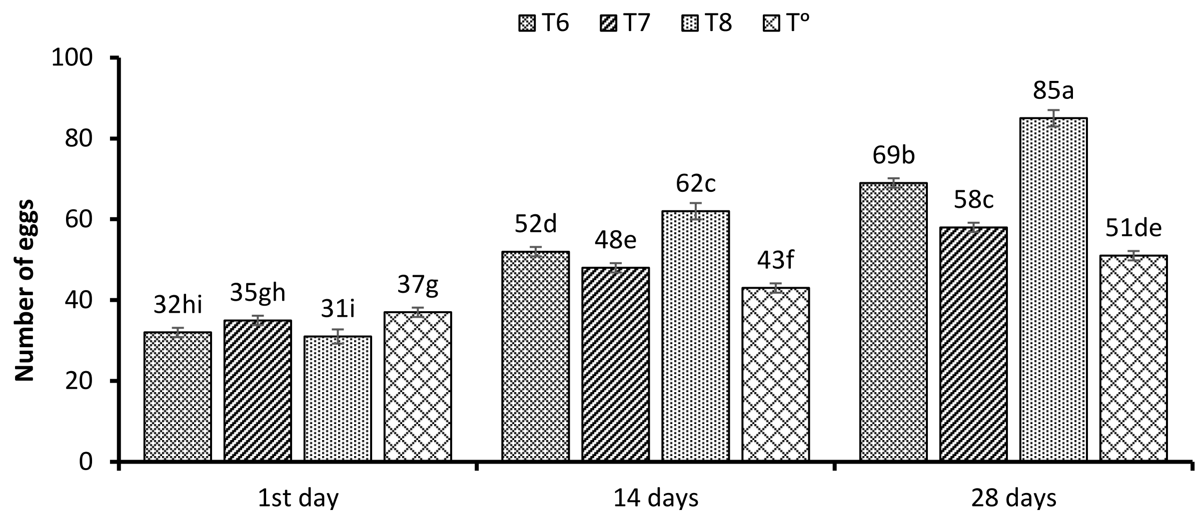

Figure-5. Comparison of effect of all the diets on number of eggs of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.

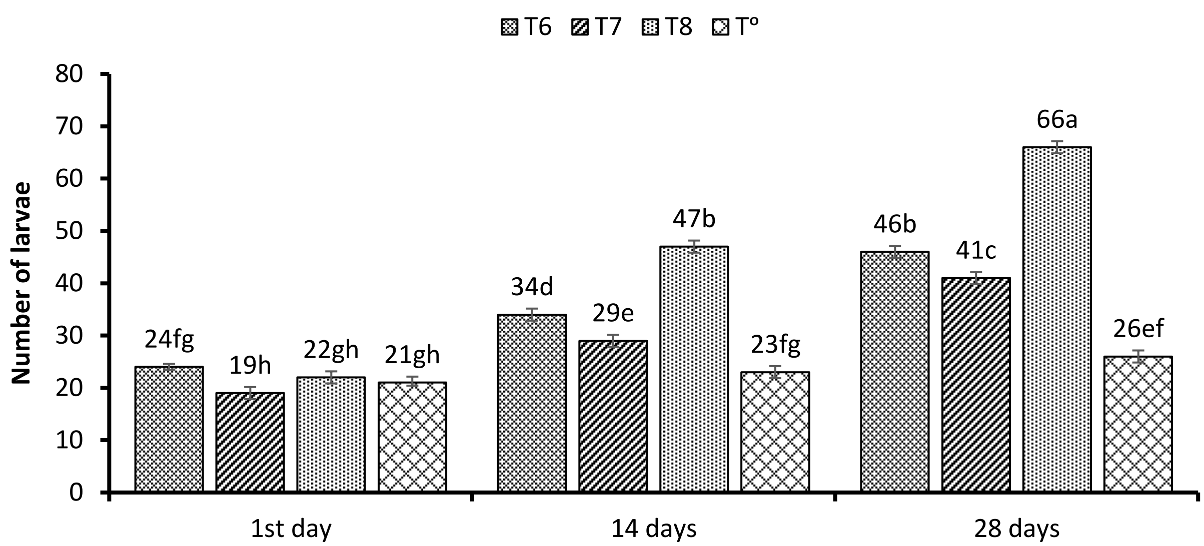

Figure-6. Comparison of effect of all the diets on number of larvae of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.

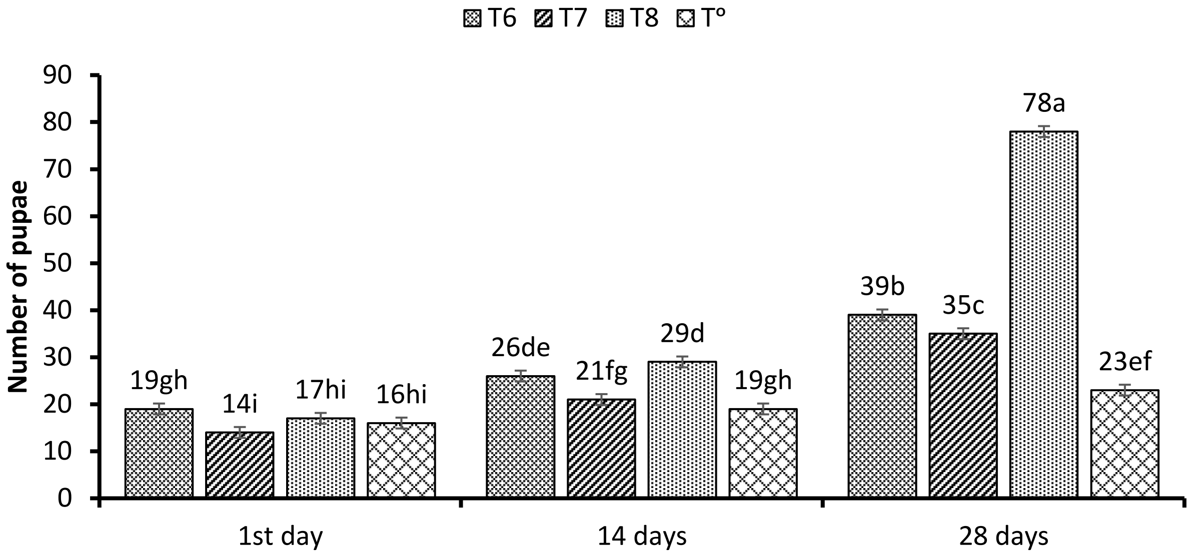

Figure-7. Comparison of effect of all the diets on number of pupae of honeybees in 1st, 14th, and 28th days, all the diets with respect to control group.

Discussion

Best artificial diet (among different diet combinations), the honeybee responses to artificial diet consumability and diet's digestibility in honeybee were evaluated during the current study. Results revealed that T_8 (i.e. 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil) had a significant impact. Honeybees consumed maximum diet-T_8 (41.29 ± 0.66 g) followed by diet-T_7 and diet-T_6; while minimum diet-T_1 (4.13 ± 0.50 g). The same trend was observed in the 2nd, 3rd, 4th, 5th, 7th and 8th week of diet consumption. The reason behind this honeybee preferred to eat candy diet in comparison to dry diet and also preferred to eat protein enriched diet; diet-T_8 was high in protein diet rather than other diets (Saffari et al., 2004; Mattila and Otis, 2006) so it was consumed maximum. The mean food consumption throughout the whole sample of honeybee colonies showed notable variability ($P < 0.05$). Similar findings from prior studies have shown that bees choose synthetic protein-rich foods over naturally occurring pollen (Saffari et al., 2004; Mattila and Otis, 2006).

Diet-T_7 also showed a good consumability after diet-T_8, in all eight weeks of feeding diet. The diet was in dry form not in the form of candy but showed good results after T8 rather than other treatments. The reason behind this consumption may be the ingredients were the same as diet-T_8, but the only difference was the addition of vegetable oil. Diet-T_6 was consumed mostly by bees after diets T_7 and T_8. It was a combination of the ingredients without dry apricot powder but in the form of candy. While the Diet-T_5 was the same as Diet-T_6 but not in the form of candy so its consumption was also less than diet-T_6. The same reason was with diet-T_3 and diet-T_4 as this diet combination was without date paste.

In the treatments where we had used skimmed milk as a source of protein and vitamins, the bees didn't prefer to feed on the diet having skimmed milk ever in the powder form or in candy. This contrasts with the findings of Paray et al. (2021). The lack of preference in our study may be due to the combination of skimmed milk with other ingredients, such as soy flour and powdered sugar, which the bees found less palatable, particularly when compared to other diets, especially diet T8, which was the most consumed.

In diet digestibility experiment, current outcomes resemble the findings of Omar et al. (2022), as they compared the digestibility of crushed and non-crushed pollens of the maize, we also used the crushed form of diet ingredients and found that our diet-T_8 (that was consumed maximum) showed the 62.6 percent diet digestibility that is lower as comparison to the other treatments. The maximum diet digestibility was recorded in diet-T_2 (73.9%) followed by diet-T_1 (71.3%) and T_4 (66.7%). Diet digestibility was good in diet-T_2 but it was not consumed even in a good quantity for the survival of the honeybee colony, so it was not recommended. The reason might be as the diet-T_8 had higher concentration of protein (13.2%) in comparison to the diet-T_6 and T_7 (i.e. 12.6 and 12.8 percent, respectively) as to Du Rand et al. (2020) high protein diets are difficult to digest.

The effectiveness of protein diets is often evaluated based on the hemolymph's protein content (Kunc et al., 2019; Noordyke and Ellis, 2021). High protein contents and nutrients increase bee lives and boost their defense against disease (Skowronek et al., 2021). Currently, hemolymph contents were found higher in diet-T_8 sample of hemolymph i.e. 26.9 μg/μl followed by diet-T_6 and T_7, as they had less protein contents in the diet so it proved the statement of Basualdo et al. (2014) the more protein available in the diet, the more protein contents should be available in the hemolymph.

The results showed that the artificial diets had a significant impact on the weight of queen bees. The queens that emerged after feeding on diet-T_8 had the highest weight (198.65 ± 0.36 mg), followed by diet-T_6 and T_7, while the control group (T_0) had the lowest weight (182.25 ± 0.55 mg). These findings were consistent with previous studies that had reported the influence of diet on queen bee weight and development (Keller et al., 2005; DeGrandi-Hoffman et al., 2010).

The longevity of worker bees was also significantly affected by artificial diets. Workers fed on diet-T_8 had the longest lifespan (53 ± 2.1 days), followed by diet-T_7 and T_6, while the control group (T_0) had the shortest lifespan (34 ± 1.7 days). This aligns with previous research highlighting the importance of diet composition on worker bee longevity and colony health. On the first day of diet placement, the control group (T_0) had the highest number of eggs (37 ± 1.5 eggs), followed by diet-T_7 and T_6, while diet-T_3 had the lowest (31 ± 1.5 eggs). However, after 14 and 28 days, the trend reversed, with diet-T_8 having the highest number of eggs (62 ± 2.03 and 85 ± 2.03 eggs, respectively), followed by diet-T_6 and T_7, while diet-T_0 had the lowest (43 ± 1.5 and 51 ± 1.5 eggs, respectively). These results suggest that the artificial diets, particularly diet-T_8, had a positive impact on egg production over time, which is consistent with previous studies on the effect of diet on brood production (Ullah et al., 2021; kumari and kumar, 2020).

Similar to egg production, artificial diets had a significant effect on the number of larvae produced. On the first day, diet-T_6 had the highest number of larvae (24 ± 0.58 larvae), followed by diet-T_8 and T_0, while diet-T_7 had the lowest (19 ± 1.5 larvae). However, after 14 and 28 days, diet-T_8 had the highest number of larvae (47 ± 1.15 and 66 ± 1.15 larvae, respectively), followed by diet-T_6 and T_7, while diet-T_0 had the lowest (23 ± 1.5 and 26 ± 1.5 larvae, respectively). These findings are consistent with the egg production results and further highlighted the positive impacts of the artificial diets, particularly diet-T_8, on brood production over time, which is consistent with previous studies on the effect of diet on brood production (Shurjeel et al., 2023; DeGrandi-Hoffman et al., 2010).

Overall, the results of this study demonstrated the potential of artificial diets, specifically T_8, in enhancing various aspects of honeybee colony performance, including queen bee weight, worker bee longevity, and brood production (eggs, larvae, and pupae). These findings contributed to the ongoing research efforts aimed at developing sustainable and efficient dietary solutions for improving honeybee health and colony productivity.

Conclusion

Diet-T_8 (i.e. 25 g soya flour + 25 g dry apricot powder + 25 g date paste + 25 g powder sugar + 8 ml vegetable oil) can be used as a suitable substitute in the dearth period for honeybee colony management and survival. It had higher consumption and good digestibility in comparison to other diets; study also demonstrated the potentials of diet T_8, in enhancing various aspects of honeybee colony performance, including queen bee weight, worker bee longevity, and brood production (eggs, larvae, and pupae). These findings contributed to the ongoing research efforts aimed at developing sustainable and efficient dietary solutions for improving honeybee health and colony productivity. The current study emphasized the value of providing honeybee colonies with additional foods when there is insufficient pollen supply. To learn how these artificial diets affect social behavior, the quality and quantity of honey under diverse climatic situations, more research work is required.

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Contribution of Authors

All the authors contributed equally for idea conception, designing research methodology, data collection, analysis and interpretation of results, manuscript write up and editing.
Moreover, all authors read and approved the final draft of the manuscript as well.

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