Bee Studies 17(1), 9-17
http://doi.org/10.51458/BSTD.2025.45

RESEARCH PAPER

Effects of Homemade Diets on *Apis mellifera caucasica*: Body Weight and Colony Productivity

Mustafa Güneşdoğdu [1,*], Ahmet Şekeroğlu [2], Samet Hasan Abacı [3],

[1] Department of Animal Production and Technologies, Faculty of Applied Sciences, Muş Alparslan University, 49250, Muş, Türkiye
[2] Department of Animal Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, 51240, Niğde, Türkiye
[3] Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University, 55270, Samsun, Türkiye

Article History
Received 24 May 2025
Accepted 29 July 2025
First Online 30 July 2025

***Corresponding Author**
Tel.: 004362494949
E-mail: m.gunesdogdu@alparslan.edu.tr

Keywords
Brood
Consumption
Proteinaceous diet
Population
Worker bee weight

Abstract

Honey bees (*Apis mellifera* L.) are insects that make a vital contribution to human life. In recent years, the honey bee population has declined due to various factors, including pathogens, chemicals, climate change, air pollution, and habitat loss. Among these, malnutrition, often resulting from habitat loss, plays a critical role in both population decline and colony development. Although the availability of floral resources varies by region, high-quality nutrition during the late summer and fall is known to enhance the overwintering success of bee colonies. In this study, we compared the characteristics of colonies fed solid diets prepared from six different protein sources (Diet I to Diet VI) with those of an unfed control group. A total of 49 experimental colonies (7 groups × 7 colonies per group) were monitored over 120 days. Colony- and individual-level parameters were assessed throughout the study. The results showed that Diet I and Diet II yielded the highest levels of diet consumption, honey production, population growth, sealed brood area, and foraging activity. Colonies fed fresh baker's yeast (Diet V) and inactive brewer's yeast (Diet VI, a commercial product) exhibited the highest rectum weights. The highest wet body weights in adult bees were observed in Diet I, Diet II, and Diet VI. These findings demonstrate that different protein sources significantly influence both individual bee health and overall colony performance. This study highlights the importance of selecting appropriate supplemental diets for sustainable beekeeping practices.

Introduction

Honey bees (*Apis mellifera* L.) are among the most essential pollinators, playing a critical role in maintaining ecological balance. However, their populations are increasingly threatened by a range of biological and environmental challenges, including climate change, habitat loss, poor nutrition, environmental pollution, pathogens, and parasites (Güneşdoğdu et al., 2023). Sustaining colony health and avoiding collapse requires an understanding of each colony's specific needs (Weinstock et al., 2006). Proper nutrition is fundamental for the vitality and productivity of both queen and worker bees (Jang et al., 2022). Like all organisms, honey bees rely on adequate food intake for survival (Haydak, 1970). The availability and quality of floral resources directly influence bee diets, which vary depending on geographic location and seasonal changes in flora (Donkersley et al., 2014). Bees require a balanced intake of carbohydrates (from nectar and honey proteins (from pollen), lipids, minerals, vitamins, and water to maintain their physiological functions (Nicholson, 2011). Worker bees gather nectar, pollen, propolis, and water from their environment, and the proportions of these resources shift throughout the year based on the colony's demands (Page, 2013). Carbohydrates serve as bees' main energy source, with glucose being metabolized for immediate energy and excess stored as body fat (Hrassnigg & Crailsheim, 2005).

Pollen, derived from flowering plant anthers, is the main protein source for bees and also supplies essential fats, vitamins, and minerals. Its protein content can range widely, from 2.5% to 61% depending on plant species (Roulston et al., 2000). To raise one larva, bees

require about 25–37.5 mg of protein, which equates to 125–187.5 mg of pollen (Hrassnigg & Crailsheim, 2005). Adult worker bees consume an estimated 3.4–4.3 mg of pollen daily (Crailsheim et al., 1992). Inadequate access to these nutrients negatively impacts colony development and resistance to disease. When natural pollen supplies are insufficient, artificial diets enriched with protein substitutes are essential for larval development (Saffari et al., 2010) and brood productivity (Mattila & Otis, 2006). Undernourished colonies are more susceptible to infections and pests (Aziz et al., 2015). To mitigate these risks, various researchers have developed synthetic diets aimed at supporting colony health (Mattila & Otis, 2006; Saffari et al., 2010; Kumar & Agrawal, 2014; Pande et al., 2015; Frias et al., 2016; Adgaba et al., 2020).

Without access to diverse and nutrient-rich food, colony strength may decline, potentially leading to population losses or colony collapse. Consequently, effective colony management practices that address dietary needs are of paramount importance. In temperate regions such as Türkiye, brood rearing typically intensifies from early spring through July, coinciding with increased nectar and pollen availability. However, brood production wanes toward late summer and halts almost entirely by September unless supplementary feeding is provided (Knoll et al., 2020).

In Türkiye, limited research has explored how artificial protein diets affect the performance of *Apis mellifera* colonies. This study was designed to evaluate the impact of different pollen substitute diets on *A. mellifera caucasica* colonies located in Muş during the summer and autumn months. As natural pollen sources diminish, the development of effective artificial diets becomes increasingly important. The study assessed the effects of various protein-rich diets on consumption levels, honey yield, rectum wet weight, and the wet and dry body weights of adult worker bees.

Materials and Methods

Experimental Design – Apiary Setup, Colonies, and Hive Conditions

This study was carried out at the apiary of Muş Alparslan University, located at coordinates 38°46'15" N and 41°25'42" E. The colonies were housed in standard 10-frame Langstroth hives equipped with pollen traps on the bottom boards; however, the traps were left open to allow unrestricted access to natural pollen sources throughout the trial.

A total of 49 colonies of Apis mellifera caucasica were used in the experiment. These colonies were randomly divided into seven groups: six treatment groups receiving different diets and one control group with no supplemental feeding. Each group consisted of seven colonies. To ensure genetic uniformity and reduce variability arising from queen age or source, all colonies were requeened in June using queens reared from a single breeder colony, in accordance with the protocol of Pirk et al. (2010). Climatic data for the study location are presented in Supplementary Table S1 (Anonymous, 2022).

Diet Preparation and Nutrient Composition Analysis

The ingredients, ratios, and proximate composition of each experimental diet are detailed in Table 1. Diet I and Diet II were identical in formulation except for the addition of spirulina to Diet II. Other formulations contained equivalent amounts of pollen and substitute components. Canola oil was included in all diets except for Diet III, which contained fresh egg yolk, to maintain suitable texture and limit fat content. ApiProtein, a commercially available inactive brewer's yeast product, was also used as a protein source. Frozen polyfloral spring pollen was included in the formulations.

All diets were mixed, portioned, and stored at 4 °C until administration. Colonies received 500 g of their respective diets weekly. Any uneaten portions were removed and replaced with fresh feed at each weekly interval.

The diets' nutritional profiles—including dry matter, crude protein, fat, ash, and crude fiber—were analyzed following the methods outlined by Dumlu and Bölükbaşı (2023), as summarized below:

Dry Matter (%): Approximately 5 g of each sample was dried in a redLINE moisture analyzer at 105 °C for 48 hours until constant weight was achieved. The residual weight was recorded as dry matter.

Crude Protein (%): The Kjeldahl method was used. Samples (0.20 g) were digested with sulfuric acid and catalyst tablets. After digestion, distillation was performed using the Gerhardt system, and titration was carried out to quantify nitrogen content, which was then converted to protein using a conversion factor of 5.6.

Crude Fat (%): Diet samples were pre-dried at 50 °C. A 2 g portion was wrapped in filter paper and placed into an oil extractor for 6 hours. After solvent evaporation, the fat content was calculated based on the remaining mass.

Crude Ash (%): 5 g of each diet was incinerated in a muffle furnace at 550 °C for 5 hours. The weight of the residual inorganic matter was used to calculate ash percentage.

Crude Cellulose (%): Following drying and sieving, 0.5 g of each diet sample was sealed in F57 filter bags and subjected to sequential boiling in acid and alkaline solutions using an ANKOM system. Bags were washed with acetone, dried, and then ignited in a muffle furnace at 600 ± 15 °C for 2 hours. The cellulose content was calculated based on pre- and post-combustion weight changes.

$$\%\ Crude\ Cellulose = \frac{100 \times [(A1 - A2) - (A3 - A2)]}{[(A1 - A2)] - (A3)}$$

A1=bag + fiber + crucible
A2 = crucible + ash
A3=crucible + empty bag

Table 1: Raw materials and chemical composition of diets

Components	Diets					
	Diet I	**Diet II**	**Diet III**	**Diet IV**	**Diet V**	**Diet VI**
P. (g)	250	250	125	125	125	125
P.S. (g)	250	250	250	250	250	250
Sp.(g)	-	2	-	-	-	-
F.E.Y. (g)	-	-	125	-	-	-
L.S.L.M.P. (g)	-	-	-	125	-	-
F.Y. (g)	-	-	-	-	125	-
Ap.® (g)	-	-	-	-	-	125
E.I.S.S. (ml)	150	150	50	150	150	150
A.V.M.C. (g)	1	1	1	1	1	1
C.O. (ml)	10	10	-	10	10	10
Price ($)	1.12	1.19	1.40	1.71	1.54	1.26
Crude Ash (%)	0.81	0.88	0.59	1.17	0.73	1.18
Crude Fat (%	9.33	10.12	9.62	9.63	11.52	7.93
Crude Protein (%)	6.97	8.41	6.36	6.65	6.85	11.04
Crude Cellulose (%)	1.24	1.49	0.74	1.05	1.74	2.23
pH	3.40	3.54	2.31	4.67	3.88	4.20
Dry Matter (%)	84.84	85.86	85.56	86.04	84.31	84.97

*P; Pollen, P.S; Powdered Sugar, Sp; Spirulina, F.E.Y; Fresh Egg Yolk, L.S.L.M.P; Lyophilized Skim Lactose-free Milk Powder, F.Y; Active Fresh Yeast, Ap.®; ApiProtein®, E.I.S.S; Enzymatic Invert Sugar Syrup, A.V.M.C; Amino acid-Vitamin-Mineral Complex for Bees, C.O; Canola Oil.

Table 2. The effect of different protein substitute diets on colony levels

Diets	Consumption (g/colony)	Honey Yield (kg/colony)	Frame Covered by Bees (number/colony)	Sealed Brood (cm^2/colony)	Foraging Effort (bee/min./colonies)
Diet I	418.59±15.27b	21.73±1.77a	12.62±0.77a	515.01±28.48b	49.37±4.10
Diet II	473.27±3.27a	21.54±1.96a	12.62±0.77a	559.75±30.40a	49.67±3.98
Diet III	63.07±10.86d	16.94±1.54b	9.38±1.21b	227.87±35.33e	43.92±4.43
Diet IV	289.45±30.51c	16.45±1.51b	10.40±1.08ab	368.75±44.00d	48.08±4.56
Diet V	269.37±15.27c	16.37±0.95b	11.76±0.89ab	448.47±33.52c	45.55±4.45
Diet VI	281.26±16.40c	20.51±0.74a	11.83±0.88ab	495.12±33.83b	46.96±3.77
Control	Non-diet	17.14±0.65b	11.67±0.90ab	420.48±35.15c	44.77±3.95
Overall Mean	299.17±20.47	18.67±0.38	11.47±0.25	440.78±13.60	46.18±0.81
P Value	<0.001	<0.001	<0.001	<0.001	0.590

* The difference between the means given with the different letters in the same column is statistically significant (*P < 0.05*), and the results are presented as the mean and standard error of the mean (mean ± SEMs, n=7).

Table 3. Weights of body parts of worker bees dependent on different diets (mg/bee)

	All body and parts characteristics (mg/bee; Mean±SEM)								
Diets	**Rectum Weight**	**Body Wet Weight**	**Body Dry Weight**	**Head Wet Weight**	**Head Dry Weight**	**Thorax Wet Weight**	**Thorax Dry Weight**	**Abdomen Wet Weight**	**Abdomen Dry Weight**
Diet I	30.44±1.53cd	124.60±2.49a	40.03±0.93ab	11.40±0.75bc	5.64±0.28ab	26.54±0.89c	11.61±0.34b	86.65±2.56a	22.77±1.00
Diet II	26.28±0.66d	129.78±2.91a	40.67±0.76ab	18.56±0.85a	6.07±0.22a	30.56±0.85ab	12.69±0.14a	80.65±3.90a	21.91±0.78
Diet III	32.59±2.13c	104.26±3.32b	37.97±1.10c	8.62±0.48d	5.35±0.27ab	34.19±1.32a	9.66±0.30d	61.43±2.45c	23.93±0.86
Diet IV	27.49±0.73cd	124.50±1.80a	39.23±0.68ab	11.19±0.92c	6.13±0.21a	32.06±0.50a	11.68±0.25b	81.24±1.61a	21.41±0.69
Diet V	61.40±1.39a	111.08±3.35b	38.09±1.33bc	12.83±0.94bc	5.61±0.26ab	31.71±0.55a	10.60±0.28c	66.72±2.80b	21.87±1.25
Diet VI	48.99±1.41b	131.62±2.57a	41.69±0.35a	14.27±1.31b	6.27±0.12a	30.19±0.45ab	11.54±0.22b	87.15±1.58a	23.87±0.36
Control	22.38±0.53e	105.21±1.86b	35.81±1.23c	11.36±1.10bc	4.92±0.25b	28.30±1.64bc	10.78±0.26c	65.54±2.53b	20.10±1.28
Overall Mean	35.23±1.97	117.61±2.03	38.73±0.46	12.49±0.55	5.66±0.11	30.19±0.45	11.08±0.19	74.96±1.83	21.99±0.27
P Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.189

* The difference between the means given with the different letters in the same column is statistically significant (*P <0.05*), and the results are presented as the mean and standard error of the mean (mean ± SEMs, n=7).

pH Measurement: For pH evaluation, 2 g of each diet was mixed with 15 mL of distilled water and allowed to sit for 24 hours before measurement, as described by Kim et al. (2024).

Diet Consumption

Each colony was supplied weekly with 500 grams of its assigned diet, which was placed on the hive frames inside a nylon freezer bag. A small hole was pierced at the bottom of each bag to enable bees to access the food while minimizing moisture loss. To prevent dehydration of the diets and ensure consistent presentation, the leftover feed was removed and replaced with fresh portions each week.

Diet consumption was monitored over a 16-week period. For each feeding interval, the amount of food consumed was calculated using the following formula:

Diet Consumed (g) = Initial Weight (500 g) – Residual Weight

This method followed procedures previously described by Kim et al. (2024) and Hoover et al. (2022). Importantly, throughout the experiment, bees also had unlimited access to naturally available resources including nectar, pollen, water, and propolis in their environment.

Assessment of Colony Population and Brood Area

The population of each colony was estimated by counting the number of frames fully covered by adult bees. The sealed brood area was measured every 21 days using the Puchta ellipse formula:

$$\text{Area (S)} = 3.14 \times (A/2) \times (a/2)$$

where A is the long axis and a is the short axis of the brood ellipse (Akyol et al., 2014).

Honey Yield Determination

Honey production was measured once during the study. The weight of the frames was recorded prior to and after honey harvest, and the difference was used to calculate yield (Akyol et al., 2014).

Foraging Activity

Foraging behavior was assessed by counting the number of bees exiting the hive over a one-minute period. Video recordings were made using mobile phone cameras between 10:00 and 14:00 on clear days. Videos were analyzed to count the number of foragers per colony. Hive inspections were not performed on the same days to avoid disturbance (Delaplane et al., 2013).

Rectum Wet Weight

In each colony, 30 adult worker bees were collected during the feeding period. Their rectums were dissected by gently pulling the sting with forceps and weighed individually using an analytical scale with a precision of 0.001 mg (Al-Qarni, 2006). This process was repeated in each replicate.

Worker Bee Body Weight

Twenty adult worker bees per colony were collected in glass vials and transported to the laboratory in a cooled container. Measurements were conducted in triplicate. The wet weights of the whole body and specific body parts (head, thorax, abdomen) were recorded using a precision scale. Dry weights were determined by placing the samples in a drying oven at 60 °C for 24 hours (Kim et al., 2024).

Statistical Analysis

All data were tested for normal distribution using the Kolmogorov–Smirnov test, confirming compliance with normality assumptions ($P > 0.05$). One-way analysis of variance (ANOVA) was used to evaluate treatment effects. Significant differences between group means were determined using Duncan's multiple range test at a significance level of $P < 0.05$. Results are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA), and visualizations were created using Microsoft Excel.

Results

Performance Results

Significant differences were found between the diet consumption, honey yield, number of bee-covered frames, and sealed brood areas of bee colonies fed with different protein substitutes over 112 days (P <0.05; Table 2). However, no statistical difference was found between foraging efforts (P >0.05; P=0.590; Table 2). Honey bees consumed significantly greater amounts of Diet II (473.27 ± 3.27 g), followed by Diet I (418.59±15.27 g), Diet IV (289.45 ± 30.51 g), Diet VI (281.26 ± 16.40 g), Diet V (269.37 ± 15.27 g), and Diet III (63.07 ± 10.86 g). Among our development diets, Diet I, II, and IV groups consumed more than the commercial diet (Diet VI) group. Diet I (21.73 ± 1.77 kg), Diet II (21.54 ± 1.96 kg) and Diet VI (20.51 ± 0.74 kg) had the highest total honey yield. Among our development diets, Diet III, Diet IV, and Diet V had less honey production than the control group (Figure 1).

The number of frames occupied by bees was highest in Diets I (12.62 ± 0.77) and II (12.62 ± 0.77), and lowest in Diet III (9.38 ± 1.21). The highest sealed brood area was determined for Diet II (515.01 ± 28.48 cm^2) to which spirulina powder was added. Surprisingly, it was higher than for the diet I (515.01 ± 28.48 cm^2) prepared with poly-floral fresh pollen. Although there was no statistical difference between the average foraging effort of the bee colonies fed with different diets, the maximum was determined in Diet I (49.37 ± 4.10) and Diet II (49.67 ± 3.98) (Table 2). Regarding the characteristics listed in Table 2, Diet VI prepared with the commercial product is performed worse than Diet I and Diet II. However, it was more successful than the other experimental groups.

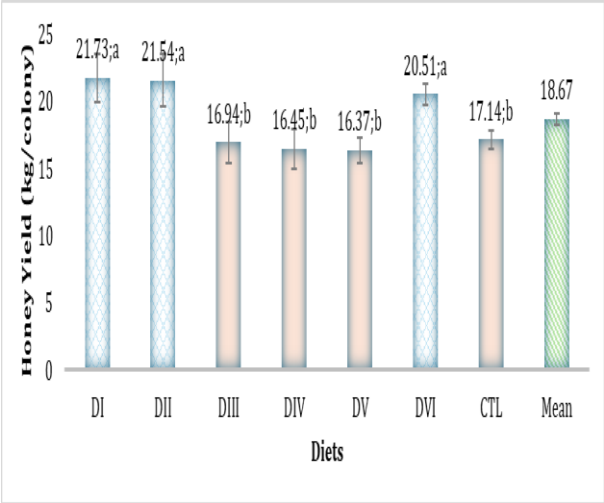

Figure 1. Average honey yield (kg/per colony) (*P <0.05*, mean, n=7)

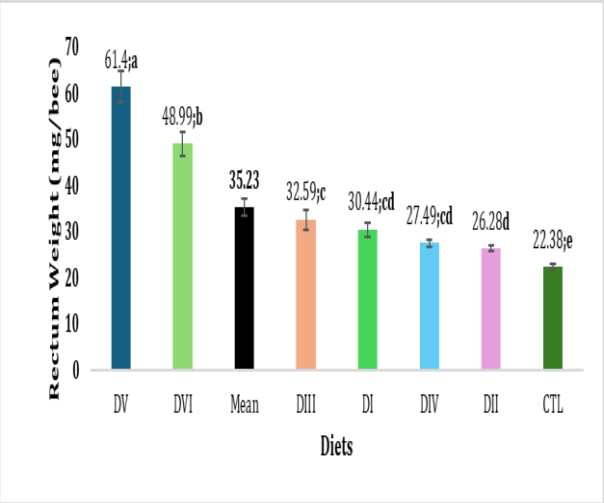

Figure 2. Average worker bee rectum weight (mg/per bee). The means followed by different letters are significantly different according to Duncan's multiple range comparisons (*P <0.05*, n=7).

Rectum and Body Weight Results

There were significant differences between wet and dried all bodies and parts (excluding abdomen dry weight) weight according to the diet (*P* <0.05; Table 3). The wet-dry weights of whole bodies and parts of worker honey bees under different protein substitutions varied greatly. The group with the highest all body wet weight of worker bees was Diet VI (131.62 ± 2.57 mg). Adult bee weight was also lowest in the group with Diet III group (104.26 ± 3.32 mg), in which consumption was lowest. The heaviest group in all 24-hour dried bodies of worker bees was Diet VI (41.69 ± 0.35 mg). The wet head weight of the worker bees in the group fed with Diet II group (18.56 ± 0.85) was the highest. However, in the dried state, the highest weights were recorded in Diets II, IV, and VI. Surprisingly, in Diet III, where consumption and wet all body weight were the lowest, wet thorax weight was the highest. Although there was a statistical difference between the wet abdomen weight of bees fed different diets, no difference was found after drying (Table 3).

Significant differences were found between the rectum weight of worker honey bee fed with different protein substitutes over 120 days (*P* <0.05; Table 3). The average rectum weight was found to be 35.23 ± 1.97 mg. The highest rectum weight was found in Diet V (61.40 ± 1.39 mg) and diet IV (48.99 ± 1.41 mg) compared to the other groups (Figure 2). Diet V and Diet VI contained fresh baker's yeast and inactive brewer's yeast (commercial product), respectively. The lowest rectal weight was observed in the control group that was not exposed to a diet (Table 3; Figure 3).

Drying the wet worker bee body and body parts resulted in a considerable weight loss. The loss of full body weight is approximately the same (Figure 3(A)). The amount of weight loss during drying of the wet worker bee head was determined in the highest diet II (67%) (Figure 3 (B)). The highest losses in the thorax (Figure 3 (C)) and abdomen (Figure 3 (D)) were found in Diet III (72%) and Diet I (74%), respectively. The average weight loss of the full body, head, thorax, and abdomen was determined as 67%, 53%, 63%, and %70, respectively.

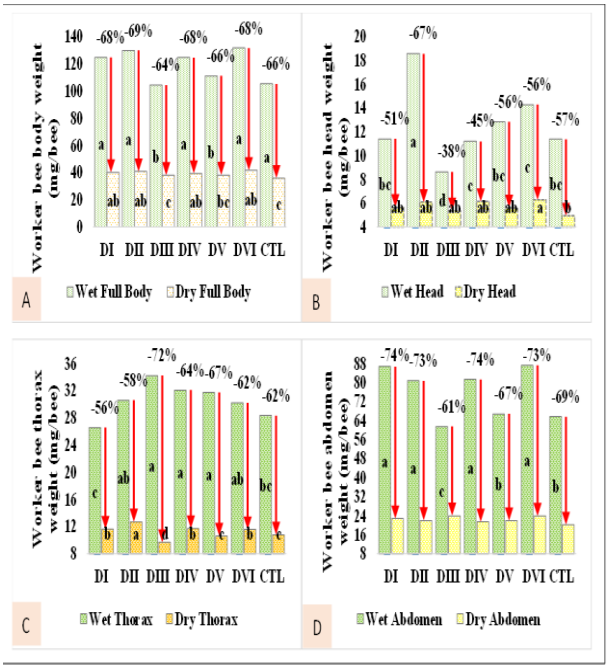

Figure 3. Weight loss (%) of body and parts of adult bees after drying. (A) Full body; (B) Head; (C) Thorax; (D) Abdomen. The means followed by different letters are significantly different according to Duncan's multiple range comparisons (*P* <0.05, n=7).

Discussion

In periods when natural pollen availability is insufficient, beekeepers commonly provide supplementary or commercially prepared diets to sustain colony development. Such nutritional support plays a significant role in stimulating brood rearing, which directly contributes to colony population growth and, ultimately, productivity (De Grandi-Hoffman et al., 2008; Akyol et al., 2014). For bees, the palatability, nutritional content, and digestibility of a given diet are critical factors influencing its consumption (Radev et al., 2014). Based on the findings of the present study, Diet III, which included fresh egg yolk, was the least favored among the tested alternatives.

This study offers comprehensive insights into how colonies respond to various protein substitute diets through parameters such as feed intake, honey production, brood area expansion, population increase, and foraging activity. On the individual level, physiological changes—reflected in rectum mass and the weights of body parts such as head, thorax, and abdomen—also revealed significant variations depending on the dietary formulation.

Feed intake serves as a practical indicator of both diet acceptance and its physiological impact. It is also closely related to the existing number of forager and nurse bees within a colony. To standardize the initial conditions, all colonies in this study were adjusted to ten-frame strength before the onset of the experiment. Results indicated that diets containing natural pollen (Diet I) and spirulina (Diet II) were consumed more readily than others. This finding aligns with earlier research by Haydak (1970), who found that bees showed high consumption of egg yolk-based diets containing approximately 30% protein. In contrast, Amro et al. (2019) observed the highest intake with yeast-based diets, while Ricigliano et al. (2021) noted that spirulina enrichment, when excessive, actually reduced diet palatability and consumption.

A clear association exists between feed consumption and both brood development and adult bee population growth (De Grandi-Hoffmann et al., 2008). Numerous studies have reported that colonies receiving pollen-enriched diets tend to display enhanced brood areas and increased worker populations (El Ghbawy et al., 2022; Kumar & Agrawal, 2014; Hoover et al., 2022).

In terms of honey production, Taha (2015) demonstrated that a blend of honey and multifloral pollen resulted in the highest yields. Similarly, Günbey and Cengiz (2021) and Cengiz and Erdoğan (2017) reported average honey yields for A. mellifera caucasica colonies as 7.58 kg and 23.36 kg, respectively. As colony populations expand due to improved nutrition, honey production can also increase in parallel (Wright et al., 2018).

Thorax weight is particularly important, as it reflects the development of flight musculature, which is essential for efficient foraging and colony resilience (Ricigliano et al., 2022). In terms of foraging activity, Gösterit et al. (2012) reported an average of 60.17 bees per minute leaving Caucasian colonies, while previous findings by Dodoloğlu and Genç (2002) and Sıralı et al. (2007) showed varying one-minute forager counts of 88.71 and 21.32 bees, respectively, under different conditions. Many researchers have reported that high-quality diets provide more energy for forager bees and lead to an increase in foraging effort. Avni et al. (2009), Delaplane et al. (2013), and Wright et al. (2018) results are consistent with our findings.

When examining the weight of the wet and dried bodies and parts of the honey bees, differences were found across the diets. The highest average body weight (wet; dry) of adult worker bees was determined in diet VI. The closest value was measured in feeding group II. In this case, the positive effect of these diets on individual body development is evident. This is consistent with the idea that the bees receive a high-quality diet during the larval stage (Eishchen et al., 1982). Amro et al. (2016) reported the wet and dry body weight of worker bees as 96.5 mg and 15 mg/bee, respectively. According to our findings, this is quite low. There are positive correlations between dry weight and lifespan (Eishchen et al., 1982). It is assumed that as the thorax weight of worker bees increases, their flight muscles also increase and they become stronger and more agile in flight (Brodschneider et al., 2009). As in our study, Ullah et al. (2021) reported maximum thorax weight as 33.12 and 32.54 mg/bee. Greater dry head weight was found in workers fed diets II, IV, and VI. The greater dry bee head weight the head reflects the development of the glands in the head of the bees (Ricigliano et al., 2022). Many researchers have reported the dried head weight as more than 8 mg/bee (Es'kov and Es'kova, 2013; El Ghbawy et al., 2022). In our study, the maximum is 6.27 mg/bee. There is a positive correlation between the development of hypopharyngeal glands and the head weight of bees (Peng et al., 2012). The wet head weights of bees fed with pollen, yeast extract powder and lactose-free milk powder are approximately the same (10.6 mg) (Yang et al., 2021). When comparing the abdominal weight of worker bees fed with pollen and egg yolk diets, the bees fed with milk powder, soy flour and yeast had more abdominal weight. In our study, the average wet and dry abdominal weight of worker bees were 74.96 and 21.99 mg/bee, respectively. In a study, Yang et al. (2021), this weight was reported as a maximum of 48.8 mg/bee. Abdominal weight is related to the amount of abdominal fat. Adipose tissue is a dynamic tissue involved in lipid-carbohydrate metabolism, protein synthesis, amino acid and nitrogen metabolism, the detoxification system and detoxification of nitrogen metabolism. It is in the dorsal and ventral parts of the abdomen. The fat tissue is larger in nurse bees than in forager bees (Alaux et al., 2010).

In our study, the average weight of the rectum was 35.23 mg. Many researchers report that this weight is

significantly lower than our results (Al-Qarni, 2006; Amro et al., 2016; Amro et al., 2019). The rectum weight is different in forager bees and nurse bees. Forager bees weigh less. The reason for this is that they consume little food and empty their rectum by flying. The transit time of pollen through the intestine varies between 2-24 hours (Crailsheim et al., 1992). Al-Qarni (2006) found that gut weight directly reflects nutritional suitability.

Conclusion

This study demonstrates that the performance of honey bee colonies and individual bees is influenced by the type of protein substitute included in their diet. Results revealed that the effectiveness of the diets varied according to the protein source used. Notably, the fresh egg yolk-based diet performed poorly across most evaluated parameters (with the exception of thorax wet weight) and is therefore not recommended as a substitute. Further research is warranted to fully elucidate the impact of alternative protein sources on honey bee nutrition and overall colony health.

Ethical Statement

This research did not involve any procedures that required ethical approval, and there are no ethical concerns related to the publication of this work.

Funding

This work was financially supported by the Scientific Research Projects (BAP) Coordination Unit of Niğde Ömer Halisdemir University under the project number TGT 2022/3-LUTEP.

Conflict of Interest

The authors declare that there are no conflicts of interest related to the execution or reporting of this study.

Author Contributions

MG was responsible for data collection; MG and AŞ jointly contributed to writing, reviewing, and editing the manuscript; HSA carried out the statistical analyses. All authors have read and approved the final version of the manuscript.

Acknowledgments

This article is derived from the doctoral dissertation of the first author. The research was supported by funding from the Scientific Research Projects (BAP) Coordination Unit of Niğde Ömer Halisdemir University, under project number TGT 2022/3-LUTEP.

References

Adgaba, N., Al-Ghamdi, A., Tadesse, Y., Mohammed, S. E. A., & Al-Attal, Y. (2020). Brood-rearing enhancing potential of manually packed pollen feeding in comparison with pollen and pollen supplements in patty forms. *Journal of Apiculture Science*, 64(1), 189–198.

Akyol, E., Unalan, A., Yeninar, H., Ozkok, D., & Ozturk, C. (2014). Comparison of colony performances of Anatolian, Caucasian and Carniolan honeybee (*Apis mellifera* L.) genotypes in temperate climate conditions. *Italian Journal of Animal Science*, 13(1), 637–640.

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, 6(4), 562–565.

Al-Qarni, A. S. (2006). Influence of some protein diets on the longevity and some physiological conditions of honey bee *Apis mellifera* L. workers. *Journal of Biological Sciences*, 6(4), 734–737.

Amro, A., & Omar, M., & Al-Ghamdi, A. (2016). Influence of different proteinaceous diets on consumption, brood rearing and honey bee quality parameters under isolation conditions. *Turkish Journal of Veterinary & Animal Sciences*, 40(4), 468–475.

Amro, A. M., Younis, M. S., & Ghania, A. A. (2019). Physiological effects of some pollen substitutes diet on caged honey bee workers Apis mellifera (Hymenoptera: Apidae). *Egyptian Journal of Plant Protection Research Institute*, 2(4), 741–750.

Anonymous. Turkish State, Meteorological Service. (2022). Available online: https://www.mgm.gov.tr/eng/forecast-cities.aspx/ (accessed on 1 February 2024).

Avni, D., Dag, A., & Shafir, S. (2009). The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. *Journal of Apicultural Research*, 48(1), 23–28.

Aziz, M. A., Azeem, M., Ahmed, M. S., Siddique, F., & Jamal, M. (2015). Control of Varroa destructor Anderson and Trueman (Acari: Varroidae) on *Apis mellifera* ligustica by using thymol and formic acid in Pothwar region of Punjab Pakistan. *Asian Journal of Agriculture and Biology*, 4(2), 150–154.

Brodschneider, R., Riessberger-Galle, U., & Crailsheim, K. (2009). Flight performance of artificially reared honey bees (*Apis mellifera*). *Apidologie*, 40(4), 441–449.

Cengiz, M. M., & Erdoğan, Y. (2017). Comparison of wintering ability and colony performances of different honeybee (*Apis mellifera* L.) genotypes in eastern Anatolian/Türkiye conditions. *Journal of the Faculty of Veterinary Medicine*, Kafkas University, 23(6), 865–870.

Cutler, C. G., Scott-Dupree, C. D., Sultan, M., McFarlane, A. D., & Brewer, L. (2014). A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health, development, and overwintering success. *PeerJ*, 2, e652.

Crailsheim, K., Schneider, L. H. W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R., & Schöffmann, B. (1992). Pollen consumption and utilization in worker honeybees: dependence on individual age and function. *Journal of Insect Physiology*, 38(5), 409–419.

De Grandi-Hoffman, G., Wardell, G., Ahumada-Segura, F., Rinderer, T., Danka, R., & Pettis, J. (2008). Comparisons of pollen substitute diets for honey bees: Consumption rates by colonies and effects on brood and adult populations. *Journal of Apicultural Research*, 47(4), 265–270.

Delaplane, K. S., van der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research*, 52(1), 1–12.

Dodologlu, A., & Genç, F. (2002). Some physiological characteristics of the Caucasian and Anatolian honeybee *(Apis mellifera* L.) breeds and their cross hybrids. *Turkish Journal of Veterinary & Animal Sciences,* 26(3), 715–722.

Dumlu, B., & Bölükbaşı, Ş. C. (2023). Effect of solid state fermentation with whey on nutrient composition of pomegranate peel supplemented sunflower meal. *Journal of the Hellenic Veterinary Medical Society*, 74(3), 6163–6170.

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., & Wilson, K. (2014). Honeybee nutrition is linked to landscape composition. *Ecology and Evolution*, 4(21), 4195–4206.

Eischen, F. A., Rothenbuhler, W. C., & Kulincevic, J. M. (1982). Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios. *Journal of Apicultural Research*, 21(1), 19–25.

El Ghbawy, I. A., Omar, R. E., Khattab, M. M., & Nowar, E. E. (2022). The novelty of Azolla pinnata as a promising alternative feed for honeybee *Apis mellifera* (L). *Egyptian Academic Journal of Biological Sciences (A. Entomology*), 15(1), 35–49.

Es'kov, E. K., & Es'kova, M. D. (2013). Factors influencing wing size and body weight variation in the western honeybee. *Russian Journal of Ecology*, 44(5), 433–438.

Frias, B. E. D., Barbosa, C. D., & Lourenço, A. P. (2016). Pollen nutrition in honey bees (*Apis mellifera*): Impact on adult health. Apidologie, 47(1), 15–25.

Günbey, B., & Cengiz, F. (2021). Comparison of physiological adaptation and performance of local honey bee (*Apis mellifera* L.) breeds and ecotypes in the Black Sea region. *Agriculture Engineering Journal*, 371(1), 113–123.

Güneşdoğdu, M., Bayram, A., Işıkber, A. A., Şekeroğlu, A., & Bozkurt, H. (2023). Insecticidal effect of a natural Turkish diatomaceous earth formulation on greater wax moth. *Turkish Journal of Agriculture - Food Science and Technology*, 11(1), 2372–2376.

Gösterit, A., Çıkılı, Y., & Kekeçoglu, M. (2012). Comparison of Yığılca local honey bee with Caucasian and Anatolian honey bee crosses in terms of some performance characteristics. Süleyman Demirel University, *Journal of the Faculty of Agriculture*, 7(1), 107–114.

Haydak, M. H. (1970). Honey bee nutrition. Annual Review of Entomology, 15(1), 143–156.

Hoover, S. E., Ovinge, L. P., & Kearns, J. D. (2022). Consumption of supplemental spring protein feeds by western honey bee (Hymenoptera: Apidae) colonies: Effects on colony growth and pollination potential. *Journal of Economic Entomology*, 115(1), 417–429.

Hrassnigg, N., & Crailsheim, K. (2005). Differences in drone and worker physiology in honeybees (*Apis mellifera* L.). *Apidologie*, 36(2), 255–277.

Jang, H., Ghosh, S., Sun, S., Cheon, K. J., Mohamadzade Namin, S., & Jung, C. (2022). Chlorella supplemented diet improves the health of honeybee (*Apis mellifera*). *Frontiers in Ecology and Evolution*, 10(1), 922741. https://doi.org/10.3389/fevo.2022.922741

Kim, H., Frunze, O., Maigoro, A. Y., Lee, M. L., Lee, J. H., & Kwon, H. W. (2024). Comparative study of the effect of pollen substitute diets on honey bees during early spring. Insects, 15(1), 101. https://doi.org/10.3390/insects15010101

Knoll, S., Pinna, W., Varcasia, A., Scala, A., & Cappai, M. G. (2020). The honey bees (Apis mellifera L.; 1758) and the seasonal adaptation of productions: Highlights on summer to winter transition and back to summer metabolic activity. A review. Livestock Science, 235(1), 104011. https://doi.org/10.1016/j.livsci.2020.104011

Kumar, R., & Agrawal, O. P. (2014). Comparative performance of honeybee colonies fed with artificial diets in Gwalior & Panchkula region. Journal of Entomology and Zoology Studies, 2(1), 104–107.

Mattila, H. R., & Otis, D. W. (2006). Influence of pollen diet in spring on the development of the honey bee (Hymenoptera: Apidae) colonies. Journal of Economic Entomology, 99(3), 604–613. https://doi.org/10.1093/jee/99.3.604

Nicolson, S. W. (2011). Bee food: The chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology,* 46(2), 197–204. https://doi.org/10.3377/004.046.0201

Page, R. E. (2013). The spirit of the hive: The mechanisms of social evolution. Harvard University Press.

Pande, R., Karnatak, A. K., & Pande, N. (2015). Development of nectar supplements for death period management of honeybees (*Apis mellifera* L.) colonies in foothills of Shivalik range of Himalayan. *The Bioscan*, 10(1), 1599–1603.

Peng, Y., D'Antuono, M., & Manning, R. (2012). Effects of pollen and artificial diets on the hypopharyngeal glands of newly hatched bees (*Apis mellifera* L.). *Journal of Apicultural Research*, 51(1), 53–62. https://doi.org/10.3896/IBRA.1.51.1.07

Pirk, C. W. W., Boodhoo, C., Human, H., & Nicolson, S. W. (2010). The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). *Apidologie*, 41(1), 62–72. https://doi.org/10.1051/apido/2009049

Radev, Z., Liolios, V., Tananaki, C., & Thrasyvoulou, A. (2014). The impact of the nutritive value of pollen on the development, reproduction and productivity of honey bees (*Apis mellifera* L.). *Bulgarian Journal of Agricultural Science*, 20(1), 685–689.

Ricigliano, V. A., Dong, C., Richardson, L. T., Donnarumma, F., Williams, S. T., Solouki, T., & Murray, K. K. (2021). Honey bee proteome responses to plant and cyanobacteria (blue-green algae) diets. *ACS Food Science & Technology,* 1(1), 17–26. https://doi.org/10.1021/acsfoodscitech.0c00026

Ricigliano, V. A., Williams, S. T., & Oliver, R. (2022). Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. BMC *Veterinary Research*, 18(1), 1–15. https://doi.org/10.1186/s12917-022-03174-1

Roulston, T. H., Cane, J. H., & Buchmann, S. L. (2000). What governs protein content of pollen: Pollinator preferences, pollen–pistil interactions, or phylogeny? *Ecological Monographs*, 70(4), 617–643.

Saffari, A., Kevan, P. J., & Atkinson, J. (2010). Consumption of three dry pollen substitutes in commercial apiaries. Journal of Apicultural Science, 54(1), 13–20. https://doi.org/10.2478/v10045-010-0002-6

Sıralı, R., Şılbır, Y., & Sıralı, B. (2007). Comparison of some behavioral characters of Ordu, Posof and Ikizdere honeybees (*Apis mellifera* L.) under the conditions of Anzer high plateau. *Uludag Bee Journal*, (1), 20–25.

Taha, E. A. (2015). The impact of feeding certain pollen substitutes on maintaining the strength and productivity of honeybee colonies (*Apis mellifera* L.). *Bulletin of the Entomological Society of Egypt, Economic Series*, 41(1), 63–74.

Ullah, A., Shahzad, M. F., Iqbal, J., & Baloch, M. S. (2021). Nutritional effects of supplementary diets on brood development, biological activities and honey production of *Apis mellifera* L. *Saudi Journal of Biological Sciences*, 28(1), 6861–6868. https://doi.org/10.1016/j.sjbs.2021.07.026

Weinstock, G., Robinson, G., Gibbs, R., Worley, K., Evans, J., et al. (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature*, 443(1), 931–949. https://doi.org/10.1038/nature05260

Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honey bees. *Annual Review of Entomology,* 63, 327–344. https://doi.org/10.1146/annurev-ento-020117-043423

Yang, K. C., Peng, Z. W., Lin, C. H., & Wu, M. C. (2021). A new design of bee cage for laboratory experiments: Nutritional assessment of supplemental diets in honey bees (*Apis mellifera*). *Apidologie*, 53(1), 418–431. https://doi.org/10.1007/s13592-021-00874-8