Research Article

Comparative Study of the Influence of Artificial Diets on Protein Levels, Antioxidants and Midgut of Honey Bee Workers, *Apis mellifera* L.

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Abstract | Pollen replacement diets are growing more vital for the survival of healthy and powerful honey bee colonies. The current study revealed the impact of different protein-rich diets on protein and antioxidant contents and morphological changes in the midgut of *Apis mellifera* workers. Results showed substantial variations in terms of total protein (TP) and levels of antioxidants [total antioxidant content (TAX), glutathione (GSH), and lipid peroxidation (LPO) rates] between different diets for 6 and 12 days. In comparison to the other diets, honey bee colonies fed on diet 3 generally showed a significant decrease in their levels of TP, TAX, and GSH, whereas those fed on diet 5 and the control diet 1 showed a significant increase in these same levels. However, the highest LPO levels were in diet 2 and diet 3, as compared to the other diets. The histological structures of the midgut of honey bee workers fed on different diets showed significant differences. In contrast to control (Diet 1), the study observed alterations in the midgut of honey bee workers fed on all different diets for 6 and 12 days, as well as a variety of histological changes, particularly in the inner cell layer. The health of the morphological structure was also enhanced by comparable alterations in the peritrophic membrane, inner cellular layer, and epithelial cells in the midgut of honey bee workers fed on the control (Diet 1) and diet 5, respectively. In conclusion, these results indicate that diet 5 improved digestion and midgut development more than the other diets compared to the control.

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Introduction

The honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are famous for their important contributions to ecology, science, and agriculture. Honey bees represent the sole livestock species that collect nectar and pollen to produce honey (Paray *et al.*, 2020). Products made by honey bees, such as pollen, honey, royal jelly, propolis, and beeswax, are highly valuable both nutritionally and commercially.

Also, honey bees play a vital role in agricultural ecosystems as pollinators (Ye *et al.*, 2014). In response to colony collapse disorder, honey bee populations around the world have drastically decreased in recent decades due to the excessive application of antibiotics, poor breeding practices, nutritional deficiencies, and the careless use of pesticides, these are some of the possible causes of this phenomenon (Decourty *et al.*, 2010; Silva *et al.*, 2015; Borges *et al.*, 2021). In addition, disruption of the oxidative balance of honey bee workers that occurs due to pesticides, low temperatures, and pathogens in the hive which in turn affects the health and productivity of honey bee colonies (Li *et al.*, 2020; Mucci *et al.*, 2021).

To support both healthy honey bee workers development and rapid larval growth, protein is an essential part of honey bee nutrition (Zerbo *et al.*, 2001; Hoover *et al.*, 2006). A lack of protein results in starvation of honey bees and is considered the main cause of colony collapse disorder (Seitz *et al.*, 2016). Also, there are established connections between protein-rich nutrition and immune function (Alaux *et al.*, 2010), and honey bees' resistance to diseases is compromised by inadequate protein intake (Matilla and Otis, 2006). The only natural protein supply that honey bees can eat is pollen; maintaining the health of honey bee colonies is contingent upon having enough of it in quantities as well as quality (Brodschneider and Crailsheim, 2010; Nicolson, 2011). Many investigations have demonstrated that pollen possesses a variety of compounds exhibiting antioxidant qualities (Kieliszek *et al.*, 2018; Martinello and Mutinelli, 2021). It is an important supplier of hydrophilic antioxidants, which guard against oxidative stress in the extracellular fluid, cytoplasm, and cell organelles. Pollen-based bee bread offers immature honey bees the protein, fat, vitamins, and minerals they need for growth and development (Anđelković *et al.*, 2012; Borycka *et al.*, 2015). Additionally, a few pollen resources offer insufficient nutrition for the best colony development, according to Pernal and Currie (2001). It is imperative to identify solutions that mitigate pollen shortage in colonies of honey bees. To substitute the absence of the pure protein resource (pollen), alternative diets or supplements should be developed (Gamal Eldin *et al.*, 2018; Gregorc *et al.*, 2019). In situations where pollen is inadequate or of low nutritional value, synthetic diets can be used as a replacement for natural pollen, offering vital proteins, fats, vitamins, and minerals (Mortensen *et al.*, 2019). Pollen substitutes that are in the absence of natural pollen should be inexpensive, have a high nutritional value, and be easily embraced by honey bees all year long (Saffari *et al.*, 2010). For commercial beekeeping, numerous dietary formulations with different ingredient combinations have been developed and studied worldwide (Puškadija *et al.*, 2017; Stevanovic *et al.*, 2018; Wijayati *et al.*, 2019).

Dietary protein levels influence hemolymph protein amounts in honey bees, and the amount of protein in honey bee hemolymph can be used to gauge diet effectiveness (Basualdo *et al.*, 2013; Barragan *et al.*, 2016). Nutritionally optimized protein diets enhance both total protein levels and the concentration of vitellogenin, a primary honey bee preservation protein (De Jong *et al.*, 2009). Insects rely on a robust antioxidant system due to their elevated metabolic rate, which results in substantial free radical generation under normal physiological conditions (Candy *et al.*, 1997). The increased activity of antioxidant enzymes in total antioxidant (TAX), glutathione (GSH) levels detected in honey bee workers that fed a mixed diet of forage and pollen indicates an improved antioxidant defense, which enhanced the capacity of reactive oxygen species (ROS) reduction. The lower malondialdehyde (MDA) levels measured in honey bee workers fed a mixed diet (Diet and pollen) support this finding, as MDA is a recognized marker of oxidative stress (Del Rio *et al.*, 2005). Compared to other insects, honey bees appear to have a limited capacity to combat reactive oxygen species (ROS). The relatively tiny number of genes encoding antioxidant proteins found in their genome suggests this (Oakeshott *et al.*, 2010). According to Alaux *et al.* (2010), malnutrition in honey bees is a sign of a compromised immune system. Stress induces behavioral and physiological reactions that have developed to improve survival in such situations; these reactions frequently require the allocation of resources for their production and maintenance (Kourtis and Tavernarakis, 2011).

Protein digestion and absorption occur primarily in the honey bee midgut (Crailsheim, 1990). The honey bee midgut lacks a chitin lining and is therefore vulnerable to pathogens (Gregorc and Bowen, 2000) and toxic substances (Bielenin and Ibek, 1980). The abundance of probiotic bacteria (Szymaś and Przybył, 2007), the length of feeding periods (Crailsheim, 1988), or epithelial protection against noxious agents (Tellam *et al.*, 1999) have all been linked to high

periplasm content in insects.

Many researchers have speculated that nutritional stress caused by low-quality pollen is one of the causes of the current decline of honey bee colonies. The study aims to investigate how different protein diets affect honey bee workers' physiological functions. Also, to analyze the impact of these diets on the histomorphological features of the *A. mellifera* midgut, and to ascertain the relationship between the diet protein content and the antioxidant system of honey bee workers.

Materials and Methods

Diets preparation

Four artificial diets were employed as treatments for substitute pollen. These diets included both individual and combined forms of yeast, chickpeas, powdered sugar, date palm pollen, wheat germ, and skim milk. As shown in Table 1, the control group was fed on bee bread (pollen kept in comb cells).

Table 1: *Ingredients of various experimental diets given to laboratory-maintained honey bee workers (Apis mellifera L.)*

Ingredients (%)	Diet				
	D1 (control)	D2	D3	D4	D5
Bee bread	100	-	-	-	-
Date palm pollen	-	30	-	-	10
Wheat germ	-	-	30	-	10
Skim milk	-	-	-	30	10
Powder sugar	-	55	55	55	55
Chickpeas	-	10	10	10	10
Yeast	-	5	5	5	5

Experimental design

Colonies of the first hybrid Carniolan honey bees (*Apis mellifera* carnica) from the apiary of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt, provided the source of closed brood combs. In accordance with the procedure outlined by Williams *et al.* (2013), these combs were moved to regulated laboratory settings during the summer of 2024 to harvest freshly emerged honey bee workers. A total of 100 newly emerged honey bee workers (0–24 hours old) were housed in wooden cages (15 × 15 cm^3), each featuring a glass panel on one side and black muslin on the opposite side. Each cage was equipped with a piece of wax comb affixed to the side to serve as a clustering substrate, along with two vials—one containing tap water and the other a 1:1 (w/v) sucrose solution. Each cage contains 5 g of each diet, and the diet was changed every 3 days. Honey bee workers were fed on different diets for 6 and 12 days. Three replicas were made for each diet. Samples of treated and control whole honey bee workers were stored in 5.0 mL Eppendorf tubes contain 70% ethyl alcohol after the experiment was over. Before analysis, the samples were preserved for 2 hours in Carnoy's solution, which consists of 60% ethyl alcohol, 30% chloroform, and 10% glacial acetic acid.

Physiological studies

Preparation of tissue and biochemical analysis

Whole honey bee workers (6 and 12 days old) were tested for protein content and antioxidant enzyme activity. Following the procedure outlined by Tawfik *et al.* (2023), three pools of nine honey bee workers were homogenized and subjected to replicate of three analysis for each diet. The levels of total protein (TP) by the methods of Mahre *et al.* (2018), total antioxidant (TAX) by Koracevic *et al.* (2001), glutathione (GSH) by Beutler *et al.* (1963), and lipid peroxidation (LPO) by Ohkawa *et al.* (1979) were assessed in homogenates prepared from the whole bodies of honey bee workers. The assay was performed according to the instruction manual of reagent kits purchased from Biodiagnostic, Dokki, Giza, Egypt. The total protein (TP) was assessed at 550 nm. The total antioxidant (TAX) assay of honey bee workers was verified via spectrophotometry at 500 nm. GSH levels were measured at 340 nm by applying the protocol with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) as a substrate. Also, the levels of LPO were measured.

Histological studies

After whole honey bee workers being fixed in Carnoy's fluid, it is then dehydrated in absolute ethyl alcohol three times (15 minutes each time) before being cleared three times in methyl benzoate (8 hours each time) and toluene three times (2 hours total) before being embedded in paraffin wax at 60°C in an oven (2 hours each time) according to Drury and Wallington (1980). The Leica RM manual microtome was used to section paraffin wax blocks at 7µ thicknesses. Sections were dewaxed with xylene and rehydrated with descending ethyl alcohol (100%, 90%, 70%, and 50%), washed with distilled water, and then mounted

on glass slides. Haematoxylin and Eosin (H&E) staining was used according to Bancroft *et al.* (2013); and dehydration with ethyl alcohol in increasing concentrations (50%, 70%, 90%, and 100%); and then xylene was used to clean the surface before mounting it with DPX mounting medium (Drury and Wallington, 1980). Stained sections were photographed using a Carl Zeiss Microscope attached an Axio Camera Zeiss German company (Axio cam 712 color 20 mp, 2017), at Zoology Department, Faculty of Science, Assiut University (HE× 400, 25 µm)

Statistical analysis

Prior to statistical procedures, data were assessed for normality using the Shapiro–Wilk test. Subsequently, a one-way analysis of variance (ANOVA) was performed to examine the effect of dietary treatments. When significant differences were detected, pairwise comparisons among feeding diets were conducted using the Tukey–Kramer HSD test (Kramer, 1956). All statistical procedures were carried out using the Statistical Analysis System (SAS Institute Inc., 2018).

Results

Influence of different artificial diets on the total protein content of honey bee workers

Our results at 6 and 12 days showed that the total protein content of honey bee workers administered different protein-rich diets was affected (Figure 1). The highest levels of total protein (3.16 mg/dl) were found in honey bee workers, which were fed on diet 5 for 12 days, followed by those that were fed on diet 5 for 6 days (2.14 mg/dl). The lowest total protein level (1.77 mg/dl) was found in honey bee workers that were fed on diet 3 for 6 days. Total protein levels of honey bee workers that were fed the remaining diets ranged between 2.01 and 1.88 mg/dl. Statistical analysis shows significant differences in the effect of various diets on total protein content levels of honey bee workers ($F = 538$; $df = 9$; $P \leq 0.001$; Figure 1).

Influence of different artificial diets on antioxidants of honey bee workers

Total antioxidant levels

The findings show that the type of proteinaceous diet given to honey bee workers over a period of 6 and 12 days had a significant impact on their total antioxidant (TAX) levels (Figure 2). Honey bee workers fed on diet 5 for 6 days had the highest total antioxidant (TAX) level (0.36 Mm/l), followed by those fed on diet 1 for 12 days (0.34 Mm/l). Honey bee workers fed on other diets had TAX levels ranging from 0.31 to 0.14 Mm/l. A significant effect of diet type and TAX levels was found by statistical analysis ($F = 55.80$; $df = 9$; $P \leq 0.001$; Figure 2).

Glutathione levels

The results reveal that the type of proteinaceous diet given to honey bee workers over 6 and 12 days significantly affected their glutathione (GSH) levels ($F = 131$; $df = 9$; $P \leq 0.001$; Figure 3). Honey bee workers fed on diet 5 and the control (Diet 1) for 12 days had the highest GSH concentrations, measuring 12.11 and 10.51 µg/mg protein, respectively. Conversely, honey bee workers fed on diet 3 for six days showed the lowest GSH level (4.76 µg/mg protein). Honey bee workers fed the other diets had GSH levels ranging from 6.52 to 9.49 µg/mg protein.

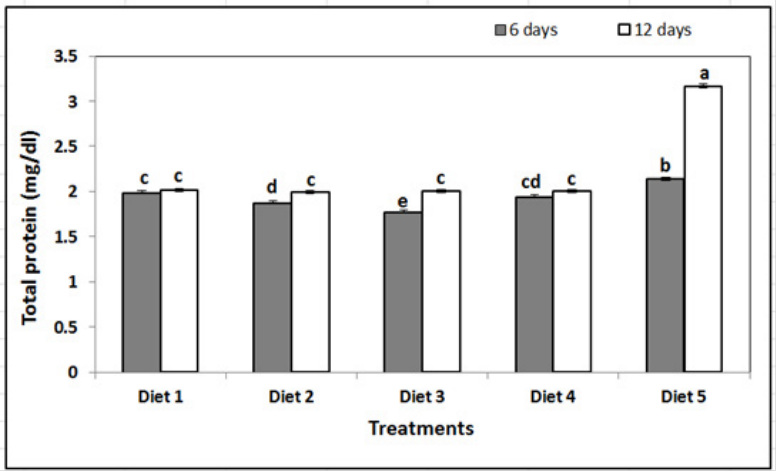

Figure 1: *The total protein content (mg/dl) of honey bee workers that were fed on various artificial diets for 6 and 12 days. Data as mean ± SE. The Tukey–Kramer HSD test at P ≤ 0.05 indicates that columns with the same letter are not significantly different.*

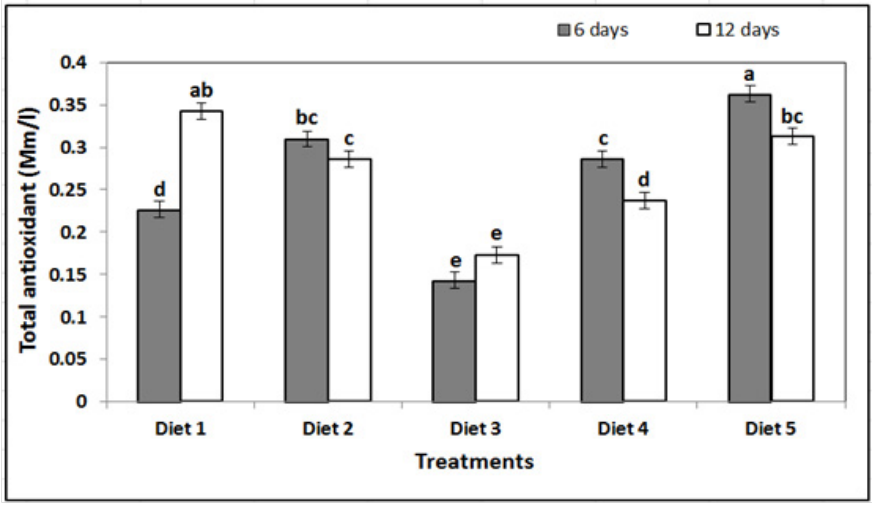

Figure 2: *The total antioxidant (Mm/l) of honey bee workers that were fed on various artificial diets for 6 and 12 days. Data as mean ± SE. The Tukey–Kramer HSD test at P ≤ 0.05 indicates that columns with the same letter are not significantly different.*

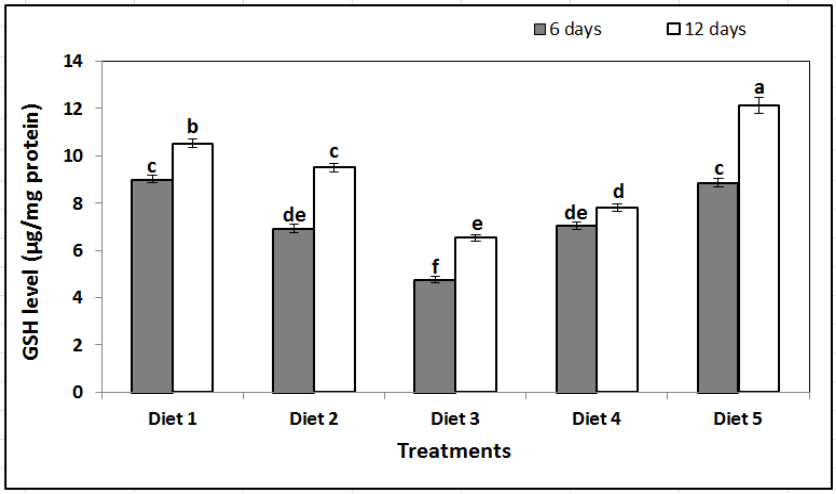

Figure 3: *Glutathione (GSH) levels (µg/mg protein) in honey bee workers fed on various artificial diets for 6 and 12 days. Data as mean ± SE. The Tukey–Kramer HSD test at P ≤ 0.05 indicates that columns with the same letter are not significantly different.*

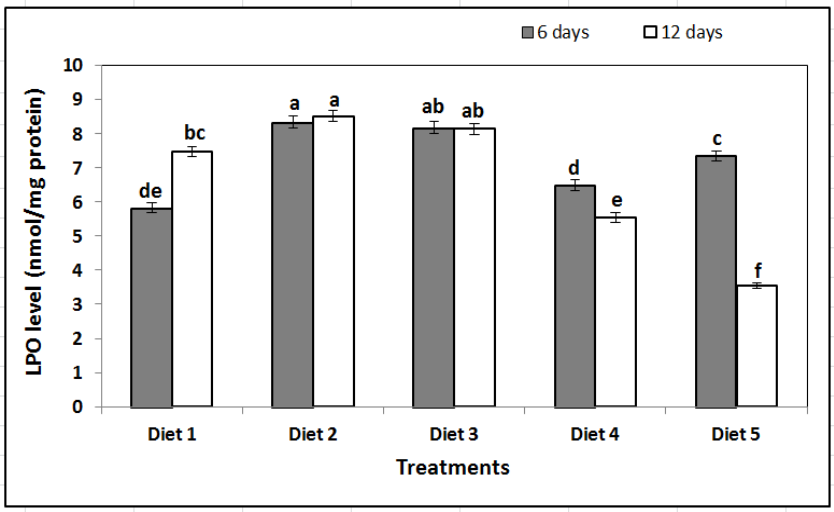

Figure 4: *Lipid peroxidation (LPO) levels (nmol/mg protein) in honey bee workers fed on various artificial diets for 6 and 12 days. Data as mean ± SE. The Tukey–Kramer HSD test at P ≤ 0.05 indicates that columns with the same letter are not significantly different.*

Lipid peroxidation levels

The results demonstrated that the type of proteinaceous diet given to honey bee workers over a period of 6 and 12 days had a significant impact on their levels of lipid peroxidation (LPO) ($F = 109$; $df = 9$; $P \leq 0.001$; Figure 4). Honey bee workers fed on diet 2 for 12 and 6 days had the highest LPO levels, measuring 8.50 and 8.33 nmol/mg protein, respectively. On the other hand, honey bee workers fed on diet 5 for 12 days had the lowest LPO level (3.54 nmol/mg protein). The range of LPO levels in honey bee workers fed on other diets was 5.54 to 8.17 nmol/mg protein.

Influence of different artificial diets on histological structures of honey bee workers

Histological analysis of honey bee workers midguts showed clear morphological changes in response to various proteinaceous diets. The midgut showed a typical architecture in honey bee workers fed on the control (Diet 1) for 6 and 12 days (Figure 5A and 6A). This included epithelial cells with intact, well-defined boundaries, homogeneous cytoplasmic inclusions, and distinctly visible apical striated borders, as well as a well-organized, multilayered peritrophic membrane and prominent epithelial folds along the entire mid

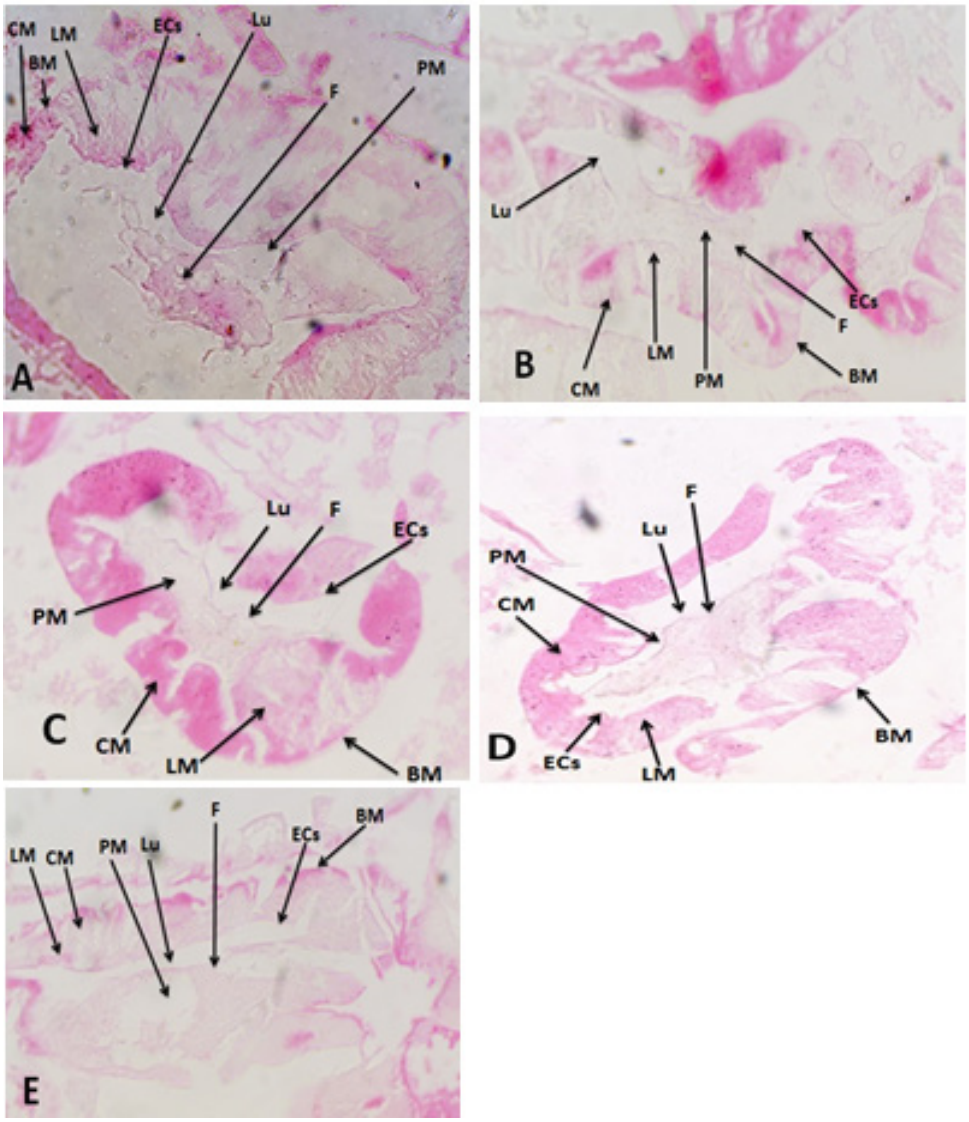

Figure 5: *Light microscopic image of midgut of honey bee workers fed on diet 1 (control) for 6 days (Fig. 5A), showed a well-structured of bees fed on diet 1 for 6 days, a well-structured, visible peritrophic membrane and epithelium folds are present normal nuclei, cells that are arranged on the basement membrane; in bees fed on diet 5 (Fig. 5B), epithelial cells was slight changes than diet 1 and marked presence of peritrophic membranes, cytoplasm slightly vacuolized; in bees fed on diet 2 (Fig. 5C), strong changes occur in the intestinal epithelium; in bees fed on diet 3 (Fig. 5D), number of peritrophic membranes decreased a few cells were destroyed; in bees fed on diet 4 (Fig. 5E), epithelial folds are less and absent the peritrophic membrane appears thinner and less. BM: Basement Membrane; CM: Circular Muscle; ECs: Epithelial cells; F: food; H&E: Hematoxylin and Eosin; LM: Longitudinal Muscle; Lu: Lumen; MG: Midgut; PM: Peritrophic Membrane.*

gut length. The majority of the cells were columnar epithelial cells, which were anchored to the basement membrane and organized in a single layer.

In honey bee workers fed on diet 1 for 6 days (Figure 5A), the epithelial midgut appeared elevated in certain areas, with granular cytoplasm and discernible peritrophic membranes. Both merocrine and holocrine secretions were clearly visible, and the intestinal lumen was slightly dilated. The peritrophic membrane was more noticeable, but the epithelial structure stayed mostly the same after 12 days of feeding on diet 1 (Figure 6A). Honey bee workers fed on diet 5, in contrast, showed only slight histological alterations. The epithelium looked elevated at 6 days (Figure 5B), with slightly vacuolated cytoplasm, especially at the basal region. It was easy to identify the peritrophic membrane. Within the intestinal lumen, homogeneous masses encircled by multiple peritrophic membranes were seen after 12 days (Figure 6B).

Honey bee workers fed on diet 2 showed more noticeable changes. In comparison to the control, the epithelial midgut displayed notable structural abnormalities at 6 and 12 days (Figure 5C and 6C), including decreased epithelial thickness and compromised peritrophic membrane integrity. Likewise, histological alterations in honey bee workers fed on diet 3 were similar to those seen in those fed

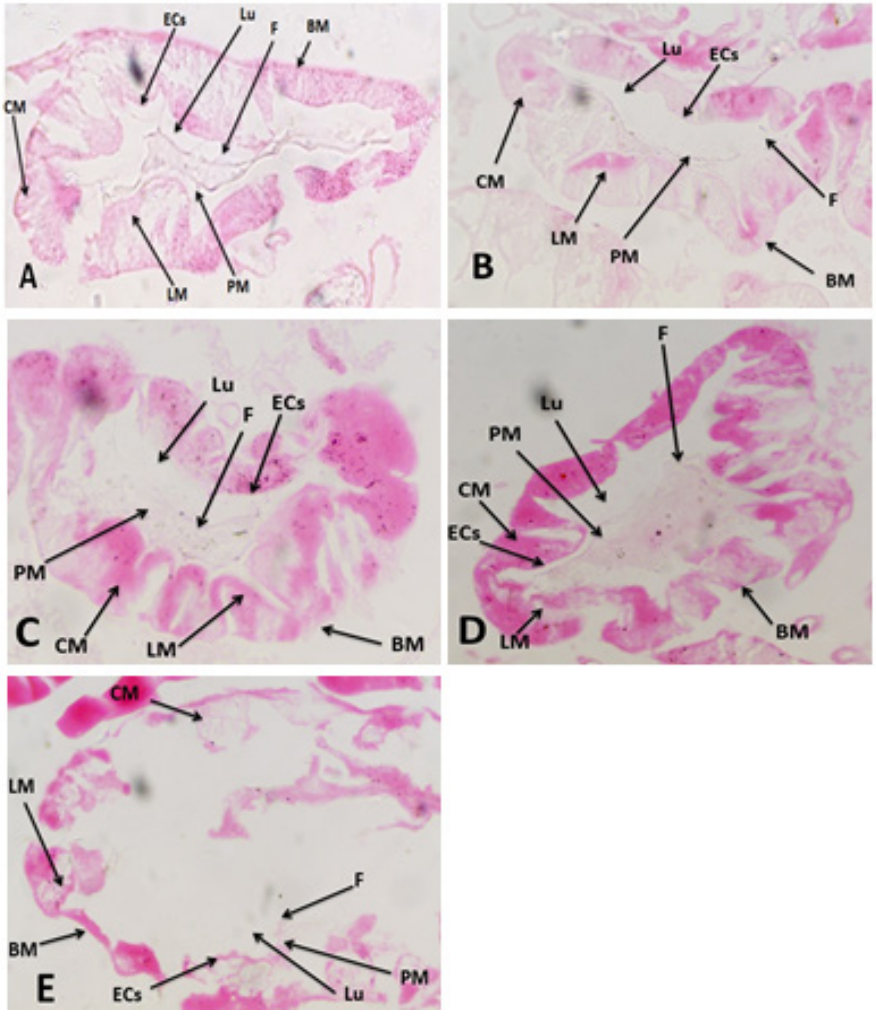

Figure 6: *Light microscopic image of midgut of honey bee workers fed on diet 1 (control) for 12 days (Fig. 6A), showed a similar and improve in cell epithelium and Peritrophic membranes was numerous; in bees fed on diet 5 (Fig. 6B) epithelial cells clearly visible, very numerous peritrophic membranes; in bees fed on diet 2 (Fig. 6C) was similar to those fed for 6 days, and peritrophic membrane decrease; in bees fed on diet 3 (Fig. 6D) was showed the number of epithelium in the inside diameter of the midgut decreased, some gut epithelial cells degenerated; in bees fed on diet 4 (Fig. 6E) there are differences as the epithelial folds are less and cells were severely necrosis and degenerated. . BM: Basement Membrane; CM: Circular Muscle; ECs: Epithelial cells; F: food; H&E: Hematoxylin and Eosin; LM: Longitudinal Muscle; Lu: Lumen; MG: Midgut; PM: Peritrophic Membrane.*

on diet 2. There were significantly fewer peritrophic membranes at 6 days (Figure 5D), and the epithelial lining displayed degenerative symptoms, with some cells separating but still loosely attached to the intestinal wall. Further degeneration was visible by 12 days (Figure 6D), with extensive cellular disintegration throughout the midgut and a discernible drop in the number and integrity of epithelial cells. Honey bee workers fed on diet 4 showed the most significant histological damage. Six days later, the peritrophic membrane looked noticeably thinner and the epithelial folds were either absent or sparse (Figure 5E). Out of all the diets, the epithelial cell density was the lowest. The quantity and size of ciliated columnar epithelial cells were significantly reduced at 12 days (Figure 6E). In addition to widespread necrosis and degeneration of gut epithelial cells, which included gut wall disintegration and epithelial cell separation, the peritrophic membrane was noticeably reduced.

In summary, the histological results show that the midgut integrity of honey bee workers was significantly impacted by the composition of their diet. Conversely, diets 2, 3, and particularly 4 caused varied degrees of structural damage, underscoring the significance of diet quality in preserving gut health. Diet 5 and the control (Diet 1) promoted healthier midgut morphology.

Discussion

The study showed that the individuals fed on diet 5 for 12 and 6 days had the highest total protein concentrations in honey bee workers, measuring 3.16 mg/dl and 2.14 mg/dl, respectively. The total protein level of honey bee workers fed on bee bread (Diet 1) for 12 days was 2.01 mg/dl. Individual development, longevity, foraging behavior, brood production, and pathogen resistance are all significantly impacted by the nutritional state of a honey bee colony (Brodschneider and Crailsheim, 2010; DeGrandi-Hoffman and Chen, 2015, Glavinic *et al*., 2017; Ahmed *et al*., 2019). These results, along with those of DeGrandi-Hoffman *et al*. (2010), point to a connection between diet and protein levels, suggesting that colony losses may be minimized by supplemental feeding to reduce protein stress. Basualdo *et al*. (2013) demonstrated that honey bee workers fed on a low-crude protein diet required a longer period to attain protein levels comparable to those of honey bees consuming a high-crude protein diet. Similarly, Ruth Archer *et al*. (2014) reported that protein-rich diets significantly enhanced honey bee survival rates. Supporting these findings, Tawfik *et al*. (2020) observed that newly emerged honey bee workers provided with a supplemental diet—comprising 200 g of feed per liter of sugar syrup and vitamin C administered every 10 days over 18 weeks—exhibited markedly elevated protein levels compared to other treatment groups. These studies collectively emphasize the critical role of dietary protein quality and supplementation in promoting optimal physiological development and longevity in honey bees. Tawfik *et al*. (2023) mentioned that honey bee workers fed on broad bean and clover bee bread had significantly higher concentrations of protein content than bees fed on cucumber, fennel, and maize bee bread.

It has been noted that honey bee workers fed on diet 5 had significantly higher levels of glutathione (GSH) and total antioxidant (TAX) than honey bee workers fed on other diets. On the opposing side, honey bee workers fed on diets 2 and 3 had significantly higher levels of lipid peroxidation (LPO), suggesting that these dietary conditions resulted in increased oxidative stress. These findings are in line with earlier research showing that vitamin C supplementation increases the activity of antioxidant enzymes like glutathione S-transferase (GST), catalase (CAT), and peroxidase (POX), as well as protein and GSH levels (Farjan *et al*., 2012). Furthermore, Khider *et al*. (2013) found that maize pollen had better antioxidant qualities than pollen from date palm, and alfalfa. The impact of diet on honey bee antioxidant defense mechanisms is further supported by the observation that the enzymatic activities of alkaline phosphatase (ALP), GST, and phenoloxidase (PO) differ based on the pollen source (Di Pasquale *et al*., 2013; Bobis *et al*., 2017). Bee bread has been found to contain lipophilic antioxidants like coenzyme Q10 and alpha-tocopherol, which support vital cellular metabolic and regulatory processes (Hryniewicka *et al*., 2016). Particularly in situations of biotic and abiotic stress, the essential antioxidant enzymes GST, CAT, and SOD are essential for neutralizing reactive oxygen species (ROS) (Li *et al*., 2020). Colonies given an 18-week supplemental diet showed improved antioxidant responses, according to Tawfik *et al*. (2020). Also, when compared to honey bees fed on bee bread from different floral sources like fennel, maize, cucumber, clover, and broad beans; honey bees fed only on sugar syrup showed noticeably lower levels of protein and

GSH and higher levels of LPO (Tawfik *et al*., 2023). These results support the idea that supplementing diets high in protein can boost antioxidant capacity and increase honey bee workers' resistance to oxidative stress.

Significant structural variations linked to dietary treatments were found by histologically analyzing the midgut of honey bee workers. The midgut epithelium of honey bee workers fed on the control (Diet 1, bee bread) retained a well-organized architecture, exhibiting prominent apical striations, a distinct peritrophic membrane, intact epithelial cell borders, and homogeneous cytoplasmic inclusions. After six and twelve days of feeding, columnar epithelial cells were the most common type. The nutritional value of bee bread, which offers vital proteins, fats, vitamins, and minerals, is in line with the findings of Herbert (1992). The development of young honey bees and the creation of essential tissues like glands and muscles depend on the proteins found in bee bread (Crailsheim *et al*., 1992).

Honey bees fed on different diets, especially pollen substitutes, on the other hand, showed varied degrees of epithelial disruption, most likely as a result of variations in nutrient composition and digestibility (Szymaś, 1976). Increased dry matter content and improved fat body development demonstrated improved midgut morphology and feed utilization following supplementation with a combination of date palm pollen, wheat germ, and skim milk (Szymaś, 1994). However, some substitutes caused minor epithelial changes and increased perigastric membrane formation, particularly when combined with sugar syrup (Szymaś and Przybył, 2007). Despite these changes, honey bees fed on pollen substitutes for up to 14 days showed no discernible degeneration of epithelial structures, with regeneration centers and cellular integrity largely intact (Szymaś *et al*., 2012). These findings support the importance of dietary protein quality for maintaining midgut health and cellular structure in honey bee workers (Zheng *et al*., 2014) and suggest that effective alternatives can mimic some benefits of natural pollen when honey bees face stress or pollen shortages (DeGrandi-Hoffman *et al*., 2010; Human and Nicolson, 2003).

Diets 2, 3, and 4 induced the most pronounced histological changes in the midgut epithelium of honey bee workers after 12 days, including extensive vacuolization, epithelial deformation, and reduced peritrophic membrane formation. These changes could be brought on by inadequate nutrient absorption and extended food retention in the gut, which could harm midgut cells. According to Crailsheim and Pabst (1988), even trace amounts of minerals in honey can cause the peritrophic membrane to rupture and the midgut to dissipate within six days. The observed variations demonstrate the dynamic interplay between honey bee physiology and nutritional inputs as well as the differing effects of diet composition on midgut integrity.

Conclusions and Recommendations

This study reveals that the physiological and histological health of honey bee workers is greatly influenced by their dietary composition. The most advantageous results were obtained with diet 5, which improved midgut structure, protein content, and antioxidant levels. Conversely, oxidative stress and epithelial damage were linked to diets 2, 3, and 4. These results emphasize the significance of balanced, nutrient-rich diets for honey bee health and colony productivity, particularly during times of pollen scarcity. Future studies should examine the field applications and long-term effects of optimal supplemental diets.

Novelty Statement

The study provides new insights into how diet composition affects honey bee nutrition, enhance antioxidant defenses and midgut health, contributing to the development of improved artificial diets for honey bee colonies sustainability.

Author's Contribution

All authors are in agreement with the content of the manuscript and were involved in all steps of its preparation.

Generative AI or AI-assisted technology statement
The authors declare that no generative AI and AI-assisted technologies were used for this manuscript.

Conflict of Interest
The authors declare no conflict of interest.

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