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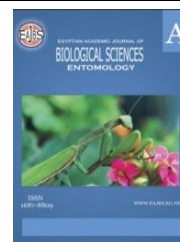
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**Physiological Impacts of Some Food Additives on Honeybee Workers
(*Apis mellifera* L.)**

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ABSTRACT

Honeybees *Apis mellifera* L. has an important role in pollination of agricultural crops. To enhance the performance and improve the physiological characteristics of honeybee workers, seven pollen substitutes were used and administrated at 7-day intervals for two years from February 2020 to January 2022. The Consumption pattern of diets, sealed brood area, and some physiological characteristics of honeybee individuals (proteolytic enzyme activity, HPG and wax glands development, abdominal lipid content, and protein content of the larvae and adults) were measured. After providing diets to honeybee colonies, we observed a positive effect on colony growth, where colonies fed with protein diets produced more brood than control colonies. Physiologically, the bees that were fed with protein diets had a higher percentage of abdominal lipid and soluble protein content than the control group and also recorded the longest development of the hypopharyngeal and wax glands was higher than the control group; and the proteolytic enzyme activity was higher in the midgut of bees in the colonies supplied with protein diets than control. Hence, pollen alternative diets can boost the physiology of honeybees leading to increased honey yield and profit.

INTRODUCTION

Honeybees are the most important insects that play a critical role in our natural ecosystem (Klein *et al.*, 2007). Proper and good nutrition is necessary for maintaining the health of honeybee colonies (Jang *et al.*, 2023) and workers' performance. Recently, honeybee colonies have experienced significant declines all over the world; This is due to Colony Collapse Disorder (CCD), a phenomenon that may occur for many reasons including inadequate diet (Borges *et al.*, 2021 and Elenany & Hassan 2023).

Honeybees consume floral nectar and pollen as natural food (Michener, 2000). Nectar is a source of carbohydrates, often provides energy to bee workers and contributes to the production of bees' wax (Wright *et al.*, 2018), and bees store it in the form of honey. On the other hand, bees don't consume fresh pollen; Instead, they prepare it as bee bread and store it in combs around the brood area (Barene *et al.*, 2015; Ghosh and Jung, 2022 and Jang *et al.*, 2023), which is the main source of protein, fat, minerals, sterols, and micronutrients for brood rearing and development (Nicholson, 2011). In the colony, nurse

bees consume honey and bee bread themselves, while the rest of the individuals depend on them for nourishment (Elenany and Hassan 2023), larval development also depends on the protein secretion of the cephalic glandular system (hypopharyngeal gland) of nurse bees (Schmitzova *et al.*, 1998 and Wright *et al.*, 2018).

Furthermore, the effects of pollen deficiency result in decreased metabolism, immunity, and stress tolerance of the honeybee colony (Di Pasquale *et al.*, 2013), and if pollen scarcity continues, no more brood can be produced (Brodschneider and Crailsheim 2010). Several diets have been developed by combining different ingredients and examining their suitability for honeybee colonies (Paray *et al.*, 2021); They usually use wheat flour, eggs, yeast, soybeans, and lentils as ingredients to prepare protein-rich bee forage (Mortensen *et al.*, 2019), but the standard protein diet for honeybee colonies has not been determined yet.

Recently, utilized germinated pulses as a substitute for pollen (Ghramh and Khan, 2023). Protein is one of three major classes of storage products in seeds (protein, lipids and oleosins). After extraction of oil, this is a valuable source of protein for animal nutrition (NOVOD, 2009), Legume seeds also contain stored protein of up to 40-70%. The protease in the seeds, which is activated when they germinate, breaks down the stored protein into amino acids by hydrolysis (Muntz *et al.*, 2001), which meets the needs of bees from the minimum essential amino acids as described by (De Groot, 1958).

Pollen consumption has been reported to be positively associated with gland development (Corby-Harris *et al.* 2016). Thus, Hypopharyngeal glands (HPG) growth and development can be considered important parameters that can be used to assess the suitability of natural pollen meals or protein supplements fed to young bees (Standifer *et al.*, 1960 and Omar *et al.*, 2017). HPG acini diameter has been effectively used to estimate the physiological status of honey worker bees (Moritz and Crailsheim 1987).

The main aim and scope of this study was to investigate the impact of different pollen substitutes on the activity, and some physiological aspects of honeybee workers.

MATERIALS AND METHODS

1. Apiary Site and Experimental Groups:

The experiment was conducted on twenty-four *A. mellifera* L. colonies in the apiary of the Faculty of Agriculture, Benha University at Moshtohor, Qalyoubia Governorate, Egypt, (Elev. 52 ft., 30°21'22.4"N and 31°13'21.5"E). Those colonies were led by queens of similar ages of medium strength and relatively similar (five combs, three sealed broods and two honey with bee bread covered by bees).

These colonies were divided into eight groups, each group containing three replicates seven of them that were fed on seven different protein diets, and one not supplied with protein as a control. Throughout the trial, all colonies were managed in accordance with the best beekeeping techniques and management practices.

2. Diet preparation and nutritional analysis:

Weekly 150 g of pollen replacement/experimental colony was added with sucrose syrup (50%), while the control colonies were fed on sucrose syrup (50%) only.

The prepared diets were analyzed for total lipid, carbohydrates, and protein contents according to the methods of AOAC (2005).

Table 1. Seven different protein substitutes were prepared in the honeybee lab at the Faculty of Agriculture, Benha University as following:

Diets	Components									
	Germinated faba bean (g)	Germinated chickpea (g)	Germinated fenugreek (g)	Lactic acid (ml)	Glycerol (ml)	Sodium chloride (g)	Brewer yeast (g)	Honey (ml)	Powdered sugar (g)	Soybean flour (g)
Diet1	25	--	--	--	--	--	20	15	25	15
Diet2	--	25	--	--	--	--	20	15	25	15
Diet3	--	--	25	--	--	--	20	15	25	15
Diet4	--	--	--	5	--	--	20	15	35	25
Diet5	--	--	--	--	5	--	20	15	35	25
Diet6	--	--	--	--	--	2	20	18	35	25
Diet7	--	--	--	--	--	--	20	20	35	25

3. Measurements of Colony Parameters:

3.1. Food Consumption Amounts:

An amount of each pollen substitute (150 g/colony) was administered in a perforated polyethylene bag and placed over the top of the midframes above the brood nest. The amount of food consumption (g/colony/7 days) was quantified as the difference between the weight of the surrogate patties before and after application (Ghazala and Nowar, 2013):

$$\text{Consumption amount (g)} = \frac{DWb - DWa}{DWb}$$

Where: DWb: diet weight before application.

DWa: Diet weight after application.

3.2. Workers Sealed the Brood Area:

In all experimental colonies, the workers' sealed brood areas were measured every 13 days using an empty Langstroth frame divided into square inches (Buchler *et al.*, 2013 and Nowar *et al.*, 2018).

4. Physiological Parameters:

4.1. Proteolytic Enzyme Activity in Midgut:

Ten nurse bees were taken from brood frames from each treated colony to dissect and extract the midguts. Then it was homogenized using 1.0 ml glycine buffer (0.1M, pH 7.9) in ice, and transferred into falcon tubes 15ml, then centrifuged at 8,000 rpm for 10 min. After that, one ml of supernatant was transferred to a test tube of 15 ml, and then added 1.0 ml of casein solution (1.0 g soluble casein dissolved in 100 ml distilled water) and taken the mixture in a water bath at 37°C for 10min. Then 0.2 ml of 24% trichloroacetic acid (TCA) was added to stop the reaction and incubated for 30 min. at 37°C. Casein fragments soluble in trichloroacetic acid were quantified by adding 3.0 ml of 0.5M NaOH and 0.5 ml of Folin reagent to 0.5 ml of the reaction mixture and left for 10 min. at 37°C, then transferred to spectrophotometer plastic cuvettes and read at 660 nm on a spectrophotometer (Keay and Wildi, 1970).

4.2. Hypopharyngeal glands (HPG):

Five workers were taken at 3, 6, 9, 12, 15 and 18 days after emergence to determine the development of the hypopharyngeal glands (HPG). The HPG was dissected and mounted using saline (0.9% NaCl) according to the method described by (Wang and Moeller, 1969). The following parameters were measured according to the method described by (Omar *et al.*, 2017):

$$\text{Acinal surface} = \frac{4}{3} \pi \times \left(\frac{a+b}{4}\right)^3$$

Where: **a** = maximum length of the acini

b = maximum width of the acini

$\pi = 3.14$.

4.3. Wax Glands:

Five workers from each group were taken at 12, 14, 16, 18 and 20 days of age to

estimate the length and width of a 2nd and 4th wax mirror. The 2nd and 4th wax mirrors were removed from the 5th and 7th abdominal sternites. The maximum mirror length and width were measured and recorded according to (Ruttner, 1988).

4.4. Soluble Protein in All Bodies of Larvae and Adults:

Ten adults (nurse bees) and ten larvae fourth instar were collected from each colony of the experiment, then placed in Eppendorf tubes and immediately cooled on ice and then stored in a frozen freezer until analyzed and when analyzed, the frozen individuals (larvae or adults) were crushed to a fine powder using liquid nitrogen and 0.2 g of bee powder was mixed with phosphate-buffered saline PBS pH7.4 and complete volume to 100 ml, (as described by Hartfelder *et al.*, 2013) buffer solution containing 1% (v/v) protease inhibitor in a ratio of 1:3 and then centrifugation was carried out the mixture at -4°C, 20000 rpm for 10 min and the resulting supernatant was used for protein determination according to the Bradford Protein Assay (Bradford, 1976), and measured by a spectrophotometer at 595 nm.

4.5. Abdominal Lipid Content:

The abdomens of Fifteen honeybee workers were collected from the treated colonies and thirty bees were collected from three replicates on the 5th, 7th, and 10th days from the cages. After that, cuts to remove the abdomens of these bees. Each sample contained 15 abdomens dried at 45°C for 3 days in a drying oven to reach a dry mass, weighed and transferred in a clean falcon tube. Then add 4.5ml of ethyl ether to each tube and shake for 24 h on a shaker to solve lipids. Then, the contents of all tubes were emptied into Petri dishes and dried for 3 days and weighed again. Abdominal lipid content was calculated according to the method described by (El-Ghabawy *et al.*, 2022):

$$\text{The abdominal lipid content (mg/g)} = \frac{W_{ABB}}{W_{ABA}} \times 1000$$

WABB: Weigh of the abdomens of bees after drying and before extraction.

WABA: weight of the abdomens of bees after extraction and drying for 3 days.

Statistical Analysis:

The effects of consumption of proteinaceous diets on worker-sealed brood area, development of HPG and wax glands, proteolytic enzyme activity, protein concentration, and abdominal lipid content, were evaluated using ANOVA tables one-way ANOVA, by MSTAT-C version 1.41 according to Snedecor & Cochran (1980).

RESULTS AND DISCUSSION

1. Chemical Composition of Prepared Diets:

Protein, carbohydrates, and lipids are macro-nutrients for honeybees, which honeybees need about 20-21% crude protein, 2-4% lipids and 4 mg of utilizable sugars per day in diets (Brodschneider and Crailsheim 2010). (Fig. 1) shows the ratio of macro-nutrients in seven diets were introduced for experimental colonies. Based on the protein, lipids and carbohydrate percentages of the diets, after mixing; diet1 contained about 22.4% protein, 2.68% lipid and 68.42% carbohydrate, diet 2, 22.23% protein, 4.13% lipid and 63.88% carbohydrate, diet 3, 19.78% protein, 4.04% lipid and 68.65% carbohydrate, diet 4, 18.63% protein, 4.16% lipid and 68.65% carbohydrate, diet 5, 19.73% protein, 4.06% lipid and 68.73% carbohydrate, diet 6, 19.08% protein, 4.43% lipid and 67.93% carbohydrate, diet 7, 24.5% protein, 4.06% lipid and 63.88% carbohydrate.

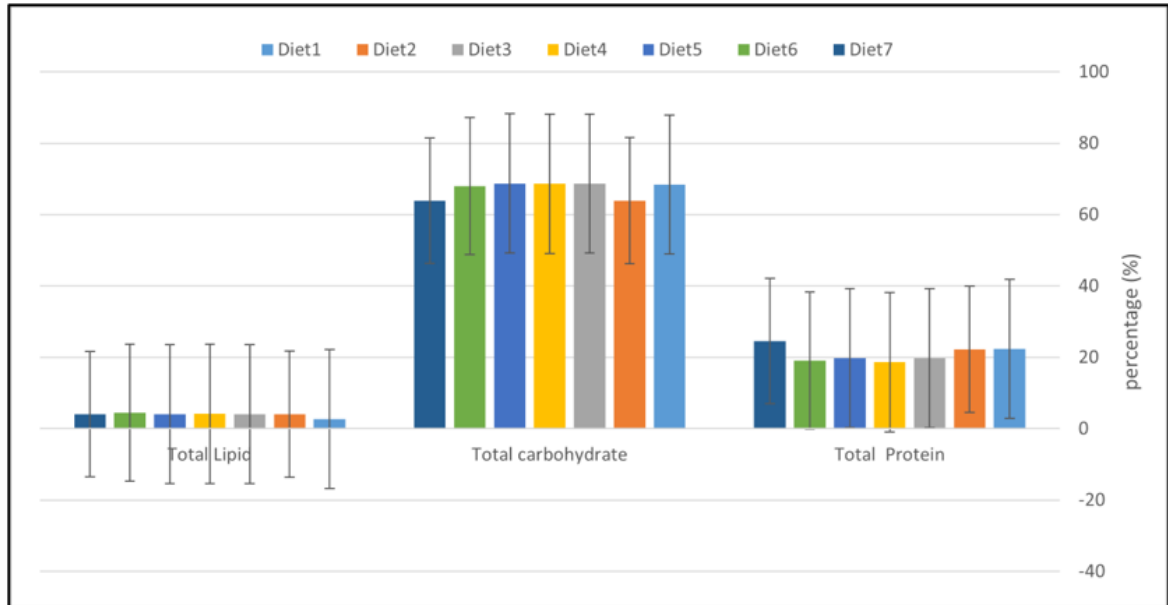


Fig. 1: Concentration (%) of total protein, lipids, and carbohydrates in prepared patties.

2. Consumption Pattern of Diets:

To determine the bees' palatability and acceptance of different protein alternatives, the consumption of these alternatives was estimated during the two years of the study 2020/2021 and 2021/2022. The food consumption amount (g/colony) for each alternative fluctuated during the experimental period (Fig. 2). Seven curves for food consumption amounts were recorded during the feeding period (Fig. 2a, and 2b).

The highest one was observed in the winter season 295.17 and 253.74 g/colony followed by summer 247.13 and 247.80 g/colony, and spring 238.25 and 239.07 g/colony, then autumn 231.85 and 234.42 g/colony in first and second years, respectively (Fig. 2c, 2d). The highest total mean consumption amount was recorded for diet 6 followed by diet 7, then rest diets in the first and second years, recording 179.21 and 171.10 g/colony in diet 1, 186.55 and 180.39 g/colony in diet 2, 203.71 and 197.79 g/colony in diet 3, 276.51 and 254.35 g/colony in diet 4, 253.37 and 244.12 g/colony in diet 5, 359.66 and 342.77 g/colony in diet 6 and 312.69 and 315.77 g/colony in diet 7, respectively (Fig. 2 e and f). The consumption amount is affected by the protein content of the diet and its palatability by bees. There is no doubt that the consumption rate is a good indicator of the bees' acceptance of the diet. In the current study, the proportion of protein was close in diets, so the difference in consumption is due to the extent to which bees accept the diet.

Several previous studies indicated that the rate of honeybee diet consumption does not depend primarily on protein content (Saffari *et al.*, 2010 and Amro *et al.*, 2020). Also, Saffari *et al.* (2010) stated that the palatability of pollen alternatives has a major role in the consumption rate. However, Schmidt *et al.* (1995) indicated that the carbohydrate content of diets is an attractive factor for honeybees.

Sodium is one of the mineral salts required by bees to regulate the activity of individuals (de Sousa *et al.*, 2022). Additionally (Khan *et al.*, 2021) indicated that the bees drank more sodium (Na Cl) than the other salts during the summer and winter study period, this supports our results in consuming diet6 more than other diets.

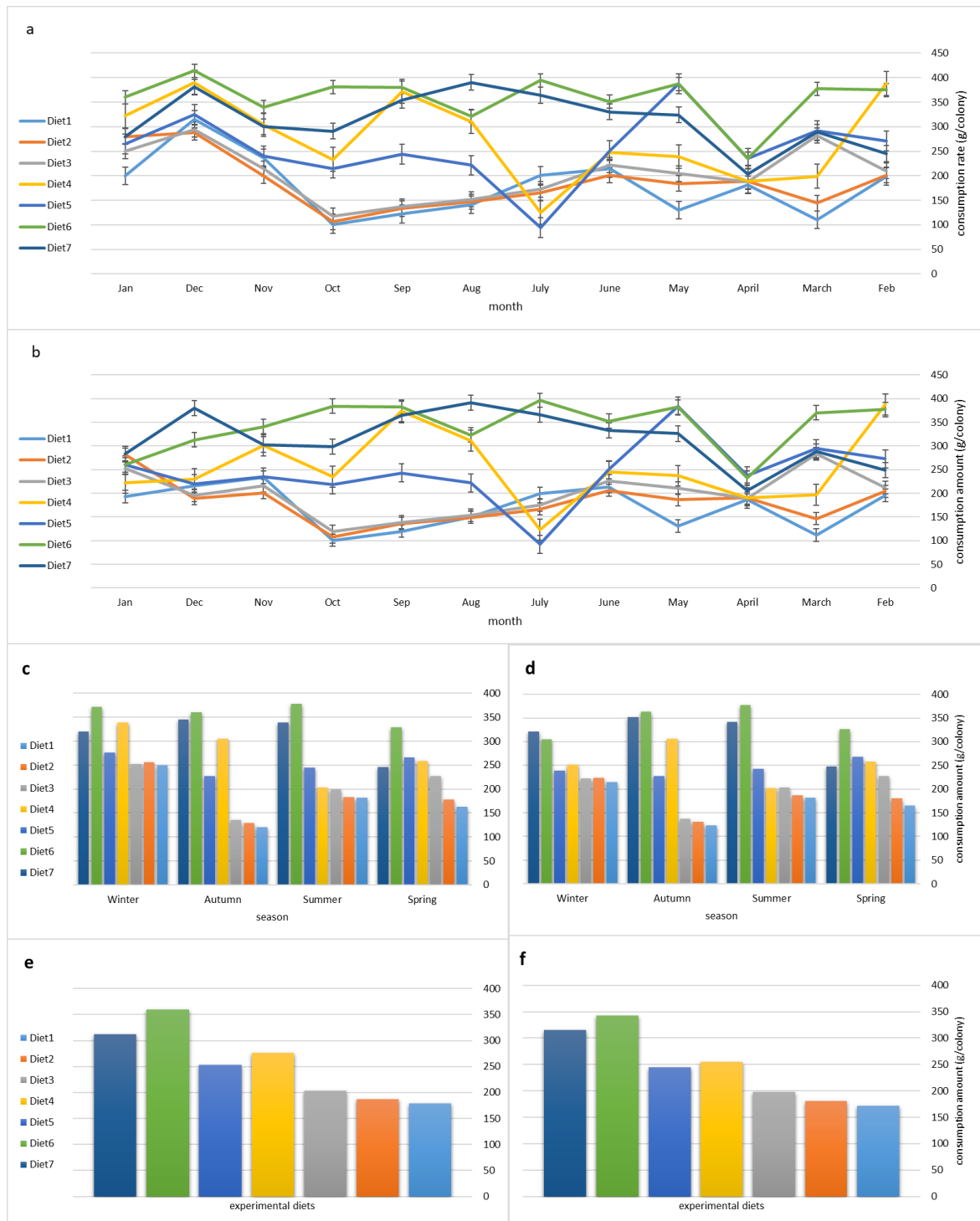


Fig. 2: Variation of pollen substitute consumption amount by honeybee colonies during two years 2020/2021 and 2021/2022. Monthly consumption amount for different diets **a)** in the first year and **b)** in the second year; Seasonal consumption for different diets **c)** in the first year and **d)** in the second year; and total mean consumption amount for diets during the experimental period **e)** in the first year and **f)** in the second year.

3. Worker's Sealed Brood Area:

To determine the extent to which protein alternatives enhance colony growth in the tested honeybee colonies, sealed brood areas were estimated at the beginning to end of the experiment during the two years 2020/2021 and 2021/2022 (Fig. 3). In the winter, sealed brood areas were identified in all experimental colonies, which were the least brood

area in the seasons of the year (Fig. 3a and b). The maximum sealed brood area was in the summer, followed by autumn then spring. The first- and second-year results were 3155.92 and 3152.12 inch²/colony in spring, 5413.25 and 5414.67 inch²/colony in summer, 3435.50 and 3435.38 inch²/colony in autumn, and 1090.12 and 1088.79 inch²/colony in winter, respectively (Fig. 3c, d, g, and h).

However, the increase in the mean of sealed brood areas was uneven in all groups tested in the summer period. Higher averages were recorded in the colonies fed with the protein diet compared to the control colonies. The results showed the ability of all tested diets to increase the area of the sealed brood significantly at the end of the experiment. The largest improvement was detected in unable with diet 1. This diet achieved a highly significant increase in the areas of sealed brood (4110.83 and 4111.50 inch²/colony) in the first and second years, respectively) compared to the results of the rest of the diets, which were 3663.42 and 3665.42 inch²/colony in diet 2, 3606.33 and 3608.08 inch²/colony in diet 3, 3313.92 and 3310.92 inch²/colony in diet 4, 3283.17 and 3282.67 inch²/colony in diet 5, 3253.25 and 3245.25 inch²/colony in diet 6, 2512.33 and 2512.92 inch²/colony in diet 7 and 2446.42 and 2445.25 inch²/colony in control colonies, respectively (Fig. 3e, and f). These results indicate that, the rate of change of brood area in colonies supplied with protein diets was higher than that in the control group, which agrees with the findings of (Younis 2019; Tawfik *et al.*, 2020; Ullah *et al.*, 2021 and Israr *et al.*, 2022) who concluded that the brood area of workers in colonies supplemented with artificial protein diet was higher than that control group.

Germinated seeds can be a good protein source for honeybees (Sihag and Gupta 2011) that stimulate brood production. In our study, it was found that brood rearing in colonies supported with alternatives containing germinated seeds raised brood at a greater rate than the rest of the colonies. This is consistent with the results of (Pande and Karnatak 2014) who used four distinct diets of germinated legume (horse gram, chickpeas, green gram/mung bean and pea), and found all diets significantly superior to the control.

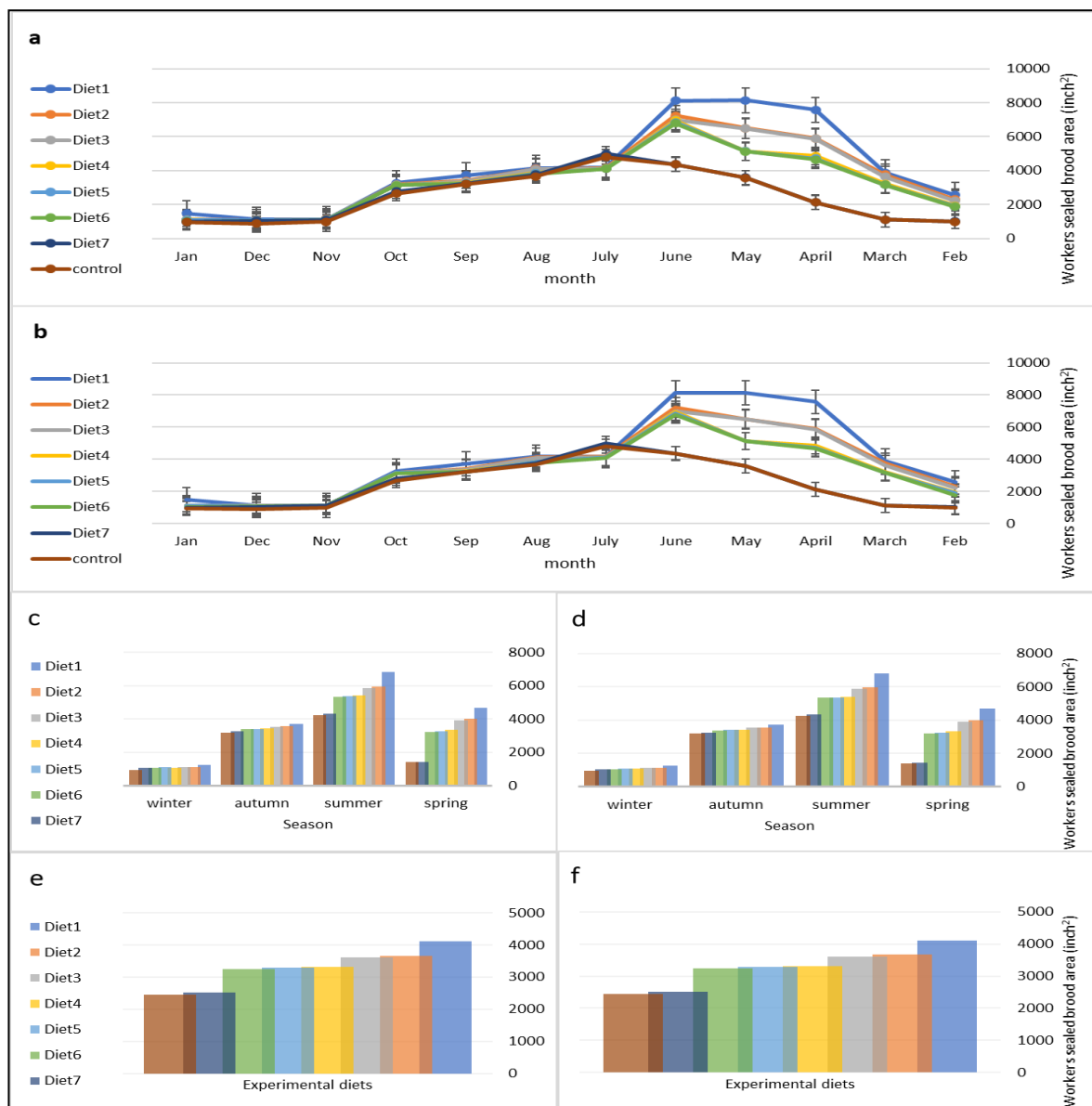


Fig. 3: Total mean of brood rearing areas after administration of proteinaceous diets to experimental honeybee colonies during two years 2020/2021 and 2021/2022. Monthly brood rearing **a)** in the first year and **b)** in the second year; Seasonally brood rearing **c)** in the first year and **d)** in the second year; and total mean brood rearing during the experimental period **e)** in the first year and **f)** in the second year.

4. Proteolytic Enzyme Activity:

Protease is an enzyme that digests protein in the midgut of honeybees. The results showed that the pollen substitutes affected midgut proteolytic enzyme activity (Fig. 4). The highest proteolytic enzyme activity was obtained in the midgut of workers who were fed protein diets. The concentration of tyrosine in the midgut of the workers in experimental groups were 0.0062 (mg/ml) in diet 1, 0.0073 (mg/ml) in diet 2, 0.0065 (mg/ml) in diet 3, 0.0058 (mg/ml) in diet 4, 0.0073 (mg/ml) in diet 5, 0.0060 (mg/ml) in diet 6, 0.0079 (mg/ml) in diet 7 and 0.0270 (mg/ml) in control group.

Protease enzymes convert natural and synthetic protein systems into usable amino acids (Zheng *et al.*, 2014), thus, the biosynthesis of HPG protein and body proteins in bees is affected by proteolytic midgut enzyme activity (Sagili *et al.*, 2005). Proteolytic midgut

enzyme activity increases when bees are fed high-protein meals, and this increase results from bees using excess dietary amino acids for growth (Zheng *et al.*, 2014), likewise, the results obtained in the current study. Additionally, Li *et al.*, (2012) indicated that higher levels of proteolytic enzyme activity in bee-fed diets are likely to contain more protein due to the greater amount of protein in these groups of bees.

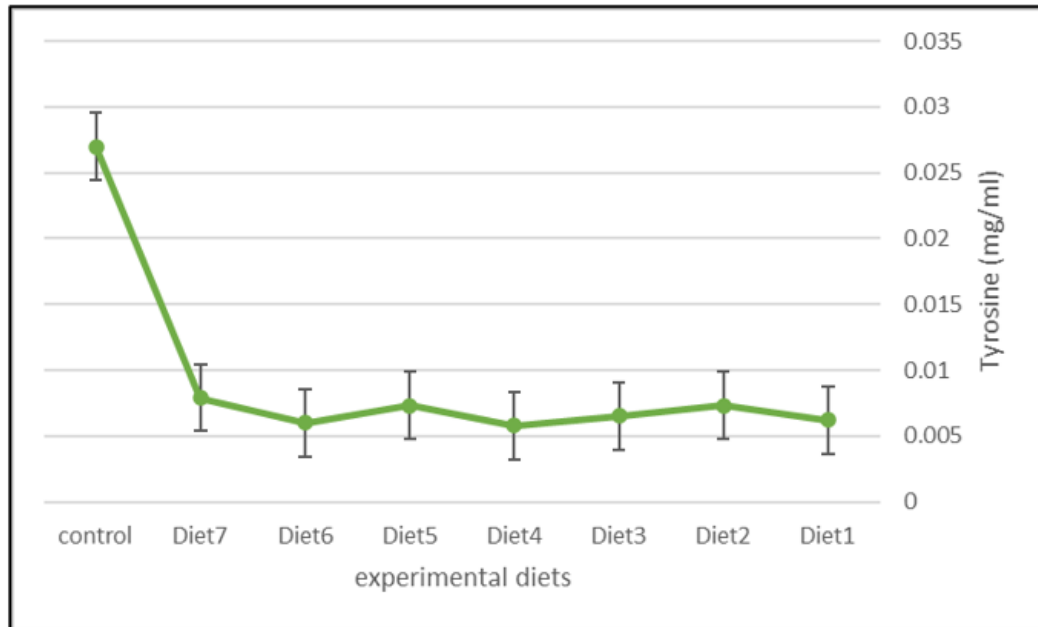


Fig. 4: Influence of pollen substitute patties on midgut proteolytic enzyme activity of honeybee workers.

5. Hypopharyngeal Gland (HPG) Development:

Low differences were found among the two trials of the first and second year. While HPG size differed highly whereas bees-fed pollen substitutes have larger acini areas than those without fed one's protein diets, being 0.153 and 0.157, 0.231 and 0.233, 0.244 and 0.245, 0.289 and 0.292, 0.158 and 0.158, 0.277 and 0.281, 0.308 and 0.308, 0.094 and 0.094 mm² in the first and second years for diet 1, diet 2, diet 3, diet 4, diet 5, diet 6, diet 7, and control group, respectively. The highest average of the acinal surface area of HPG was recorded at 12 days old (0.253 and 0.257) mm² in the first and second years, respectively, this is consistent with the results found by (Omar *et al.*, 2017), stated that the activity of the HPG began on the 5th day, and its maximum activity was on the 12th day. In addition, the area of the acini was higher in diets 7 and 4 because it contains sodium, which in turn activates the hypopharyngeal gland.

It has been reported that good nutrition is positively associated with HPG development (Corby-Harris *et al.*, 2016), this is consistent with our results as well as with (De Grandi-Hoffman *et al.*, 2010 and El-Ghabawy *et al.*, 2022) found that bees fed sucrose alone had significantly less protein and significantly weaker hypopharyngeal glands than other tested diets.

The percentage of protein in the head is evidence of the hypopharyngeal gland protein content and development. The protein content in the head of the colonies supported by protein diets was significantly higher than the control (Chakrabarti *et al.*, 2020), and this is consistent with found in our findings that HPG development was higher in colonies supplemented with protein, and this disagrees with (Zheng *et al.*, 2014) found that there were no significant differences between the control and the protein-supplemented colonies in the head protein content.

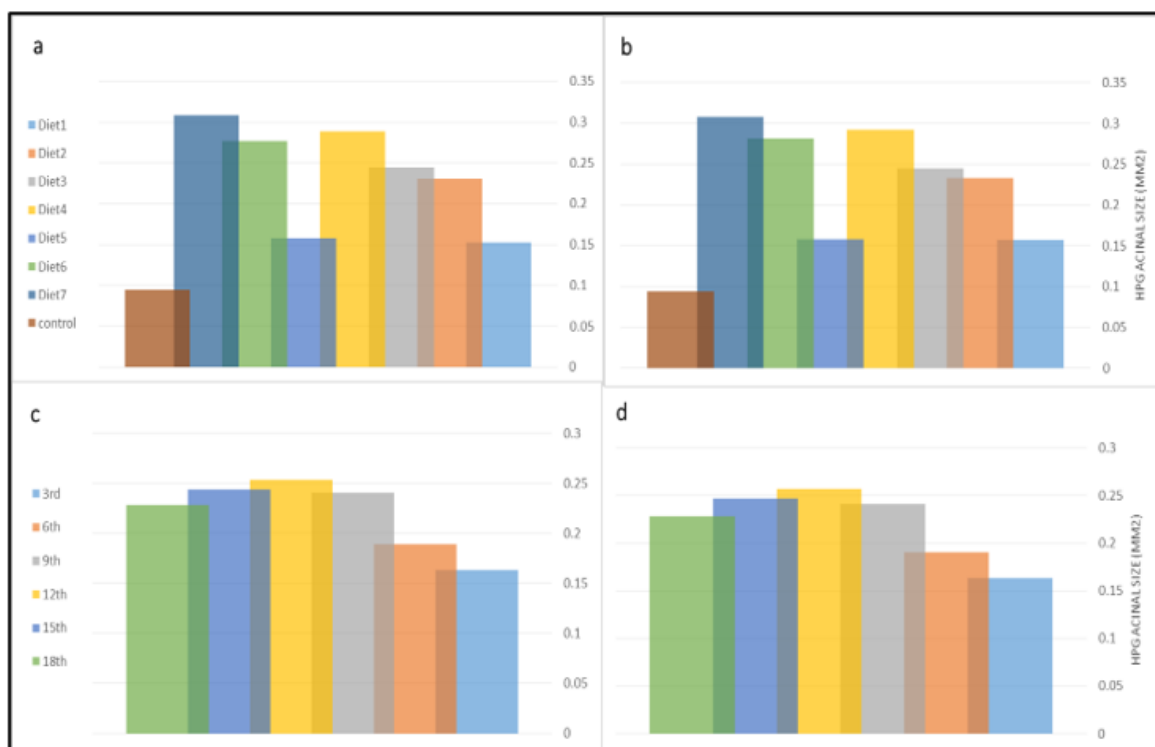


Fig. 5: Impact of protein diets on HPG development (acinal surface mm^2) for two years. Total mean acinal surface for each diet during experimental period **a**) in the first year and **b**) in the second year. Total mean of daily development **c**) in the first year and **d**) in the second year.

6. Wax Gland Development:

Wax is a product of the honeybee, which is secreted from the wax glands in the abdomen of worker bees. The total mean of wax mirror surface was lowly different between experimental groups (Fig. 6). In general, the bees fed on the artificial protein diets had a higher wax mirror area than the control bees, which was lower than the other treatment groups, that represented 11.35 and 6.43, 11.36 and 6.65, 11.37 and 6.61, 11.49 and 6.94, 10.94 and 6.46, 11.64 and 6.80, 11.66 and 6.76, and 10.88 and 6.37 mm^2 L and W in diet 1, diet 2, diet 3, diet 4, diet 5, diet 6, diet 7, and control group.

In a study by (Ahmed *et al.*, 2020) bees fed on protein diets (pollen substitutes and supplements) had significantly higher wax-built cells than control bees. In the same trend, (Barragán *et al.*, 2022) found that the large amount of protein fed to 27-day-old bees fed a free amino acid diet promoted the growth of wax glands, this is consistent with our results obtained

Additionally, (Esanu *et al.*, 2018) found that there was an increase in the amount of beeswax produced by introducing protein sources into the bee's diet, and the best results were obtained with pollen and the lower values of this parameter were with the control group. On the other hand, our results disagree with (Shawer and Mousa 2016), who indicated that the area of the four wax glands was not affected by the type of diet.

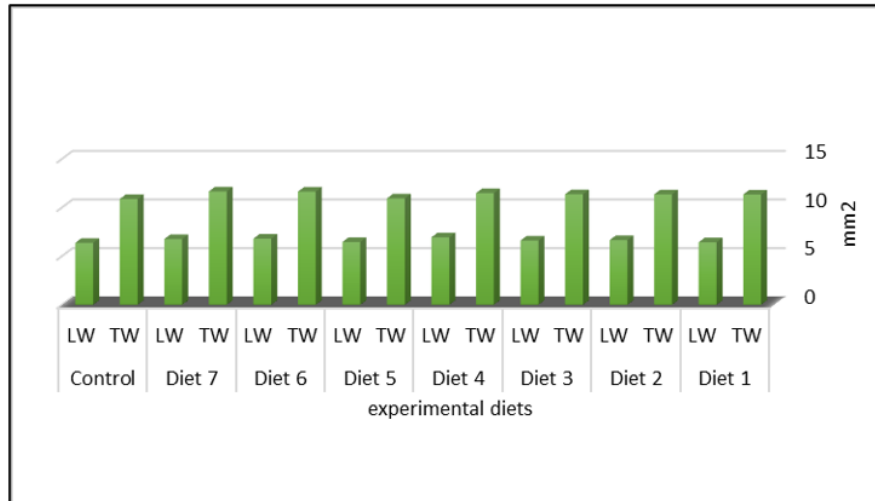


Fig. 6: Total mean of wax mirror surface for workers between experimental groups fed on different tested diets.

7.Soluble Protein in All Body:

The effects of different diets on the protein content of the larvae and adults of honeybee workers are illustrated in Figure 7. There was a difference in the protein content in larvae and adult workers in the control colony and supplemented colonies with alternative diets. Workers fed a pollen substitute had a higher body protein content than bees that were not fed protein. The values of protein content in larvae were 50.66 in diet 1, 51.06 in diet 2, 49.19 in diet 3, 49.11 in diet 4, 45.11 in diet 5, 47.55 in diet 7, and 42.14 in the control group. Likeness in adults the values were 37.69 in Diet 1, 38.27 in Diet 2, 34.22 in Diet 3, 33.22 in Diet 4, and 23.22 in the control group.

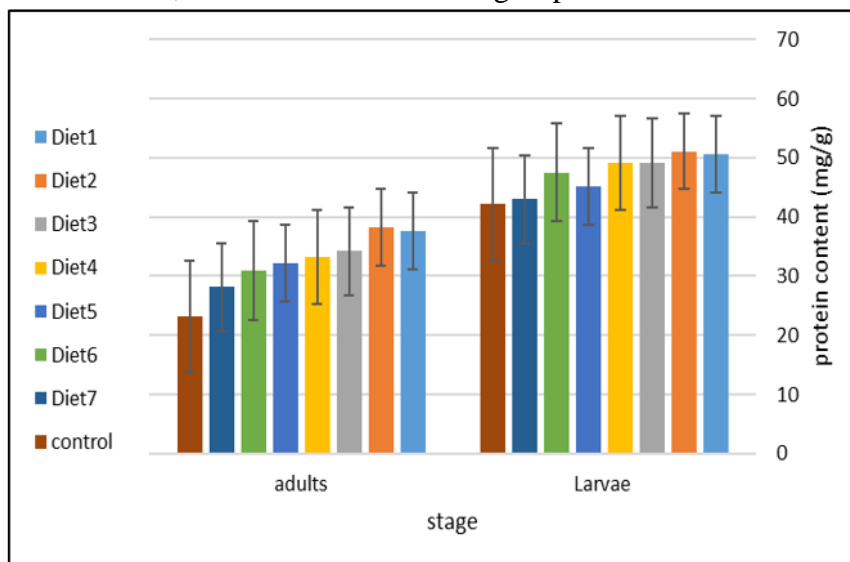


Fig. 7: Effect of different diets on the protein content of the larvae and adults of honeybee workers.

The present results agree with those found by (Ricigliano and Simone-Finstrom, 2020) that protein levels were higher in bees-fed pollen substitutes than in bees-fed sucrose. Also, the soluble protein of newly emerged adults and larvae was higher in colonies that had access to natural pollen than in colonies that were pollen-limited and fed substitutes (Amro *et al.*, 2017). In another study, newly emerged workers and larvae had higher soluble body protein with increasing dietary protein (Li *et al.*, 2012 and 2014). On

the other hand, our findings disagree with (Lamontagne-Drolet *et al.*, 2019) who found that nurse bees from colonies that had access to natural pollen had lower soluble body protein than nurse bees from colonies provided pollen substitutes.

8. Abdominal Lipid Content:

Abdominal lipid content is an indicator of the quality of tested proteinaceous diets. The experimental groups that fed on protein diets had higher abdominal lipid content than the control groups in colony and cage trials (Fig. 8). In colonies trial, after feeding on the experimental diets, mean lipid content (mg/g dry abdominal weight) of bees fed on experimental diets 1 and 2 had the highest mean abdominal lipid content of 237.21 and 225.42 mg/g, respectively, followed by diet 5 (191.83 mg/g), diet 3 (166.11 mg/g), diet 4 (154.92 mg/g), then diet 6 (150.41 mg/g) and diet 7 (119.28 mg/g), While it was the lowest content in the control groups (110.22 mg/g) (Fig. 8d).

Likewise, in the laboratory trial, dietary protein-supplied bees had higher mean abdominal lipid content than bees in the control group in 5th, 7th, and 10th days (245.20 mg/g in diet 1, 192.25 mg/g in diet 2, 175.34 mg/g in diet 3, 177.98 mg/g in diet 4, 189.80 mg/g in diet 5, 168.58 mg/g in diet 6, 164.98 mg/g in diet 7 and 161.19 mg/g in control group. Figure 8 (a and c). Also, the results show that the abdominal fat content started to increase up to its maximum average on the 10th day (217.80 mg/g) (Fig. 8b).

The abundance of proteins in the diets provided to bees has a role in fat metabolism (Chan *et al.*, 2011), which explains our findings that the bees fed on protein alternatives contained higher abdominal lipid content than the control group, this agrees with the results obtained by (El-Ghabawy *et al.*, 2022). Additionally, (Chakrabarti *et al.*, 2020) reported that honeybee activity and abdominal fat content were significantly higher in bees that had sterol supplement diets than in the control.

Moreover, lipid composition was associated with the lifespan of worker honeybees as reported (Toth and Robinson, 2005) that body fat content initially increases until it reaches a certain limit (before the bees start to forage) and then the fat body loses lipids. On the other hand, glycerides are neutral lipids in pollen that play an important role in fat body synthesis and supporting basic physiological functions in honeybees (Tsuruda *et al.*, 2021), and this is consistent with our findings that the glycerol-containing diet had high abdominal lipid content in the bees fed on it. Also, the percentage of abdominal fat was high in diets containing germinated seeds because of their amino acids, which have a role in the synthesis of fatty bodies (Archer *et al.*, 2021 and Ricigliano *et al.*, 2021).

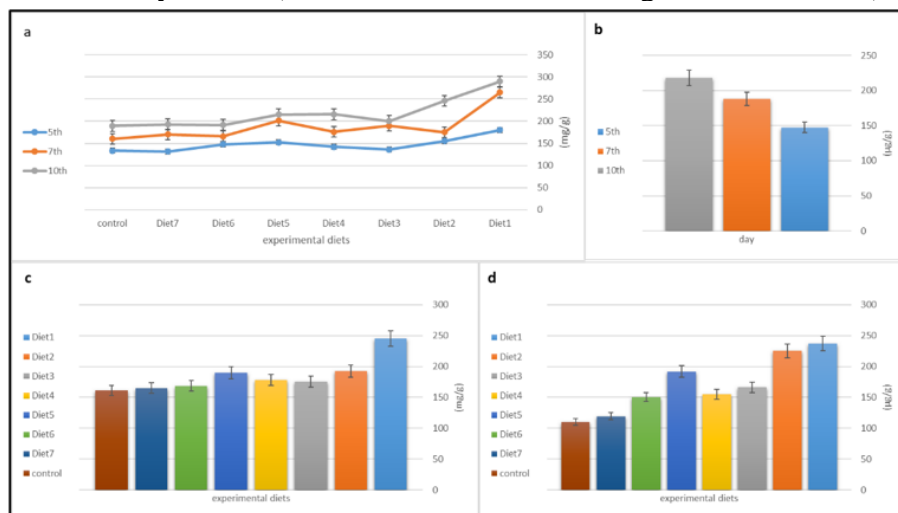


Fig. (8) Abdominal lipid content in the treated honeybee: **a)** 5th, 7th, and 10th days old. **b)** total mean on different days 5th, 7th, and 10th. **c)** total mean in different experimental diets of cage bees. **d)** total mean in different experimental diets of colony bees.

Conclusion

Providing honeybee colonies with protein diets affected the physiological status of honeybee workers positively. It might encourage bee immunity for its positive effects on abdominal lipid content. Also, helpful in developing honeybee colonies by increasing the activity of brood rearing and thus enhancing bee numbers, improving their physiological condition which encourages beekeepers to pay attention to pollen alternatives, support colonies and increase their productivity.

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ARABIC SUMMARY

التأثيرات الفسيولوجية لبعض الإضافات الغذائية على شغالات نحل العسل (*Apis mellifera* L)

نقى فتحي مشعل، ورضا السيد عمر، ومتولي مصطفى خطاب، والحسيني السيد نوار
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ان نحل العسل له دور مهم في تلقيح المحاصيل الزراعية. ولتعزيز الأداء وتحسين الخصائص الفسيولوجية لشغالات نحل العسل، تم استخدام سبعة بدائل لحبوب اللقاح وتقديمها على فترات زمنية مدتها 7 أيام لمدة عامين من فبراير 2020 إلى يناير 2022. تم قياس معدل استهلاك البدائل الغذائية، ومساحة الحضنة المغلقة، وبعض الخصائص الفسيولوجية لأفراد نحل العسل (نشاط الإنزيم المحلل للبروتين، وتطور غدة الغذاء الملكي وغدد الشمع، محتوى دهون البطن ومحتوى البروتين في البرقات والحشرات الكاملة). بعد توفير البدائل الغذائية لطوائف نحل العسل، لاحظنا تأثيرًا إيجابيًا على نمو الطوائف، حيث أنتجت الطوائف التي تتغذى بالبدائل البروتينية حضنه أكثر من طوائف الكنترول. من الناحية الفسيولوجية، أحتوي النحل الذي تم تغذيته بالبدائل البروتينية على نسبة أعلى من الدهون في البطن البروتين الذائب مقارنة بنحل الكنترول، كما سجلت شغالات النحل التي تم تغذيتها بالبدائل البروتينية أعلى نسبة تطور في غدد الغذاء الملكي وغدد الشمع من مجموعة الكنترول؛ وكان نشاط الإنزيم المحلل للبروتين أعلى في المعى المتوسط للنحل في الطوائف المزودة بالبدائل البروتينية من الكنترول. وبالتالي، يمكن القول ان البدائل البروتينية لحبوب اللقاح يمكن أن تعزز نحل العسل فسيولوجيًا مما يؤدي إلى زيادة إنتاج العسل والأرباح.