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Effect of Pollen Supplements and Substitutes on Honey Bee Queen Ovaries and Worker Hypopharyngeal Glands

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ABSTRACT

The study was carried out in a private outdoor apiary located at Meet Fares village, Bani Ebaid district, Dakahlia province. The study was conducted during winter and early spring, covering the dearth season prior to clover nectar flow season to investigate the effect of food supplements on the reared queens quality and the worker hypopharyngeal glands (HPGs). The results showed that the clover pollen diets had the largest effect on the mean queen weight and lengths also on the mean queen abdomen length and width in comparison with gluten and sugar syrup feeding. Also, the mean weight of queen ovaries and the mean number of ovarioles were the largest when honey bee fed pollen diets. Histologically, the nurse worker hypopharyngeal glands (HPGs) exhibited larger acini diameters and more secretions in case of clover pollen diets followed by corn gluten one in comparison with the sugar syrup. Generally, corn gluten exhibited moderate positive effects and could be a good protein nutritive, especially, when blended with other nutritive materials as yeasts and sugar. It is relatively cheaper and has high protein content, so it is recommended as a suitable pollen substitute in dearth periods.

Keywords: honey bees, feeding, substitutes, supplements

INTRODUCTION

Honey bees feeding is an essential process for brood rearing, colony development and maintenance. In dearth periods, pollen supplements and substitutes are the alternative solution, which may compensate the deficiency for colony survival until the nectar flow season. Honey bees need the main nutrients of carbohydrates, proteins, lipids, vitamins, minerals and water for development and maintenance. Nectar and honeydew are the chief sources of carbohydrates for bees. Pollen supplies them with the remaining dietary requirements that have indispensable constituents (Vivino and Palmer, 1994 and Schäfer *et al*., 2006). Honey bees need to ingest 10 amino acids described as essential to their diet and are highest for l-leucine, l-isoleucine and l-valine. Flowers are the mainstay of bees' life from where they collect pollen (protein rich food) and nectar (major source of energy). The only natural protein source for the colony is pollen, which its nutritive values vary widely according to species and region (2.5-61%). However, the availability of pollen depends on the plants' growing seasons during the year (Roulston *et al*., 2000 and Schäfer *et al*., 2006). In the colony, honey bees mix pollen with regurgitated nectar, honey and glandular secretions to produce bee bread, which has lower pH and less starch than freshly collected pollen (Herbert and Shimanuki, 1978 and Ellis and Hayes, 2009). The nutritive value of bee bread to honey bees is higher than that of fresh bee collected, laboratory stored or frozen pollen with few exceptions (Cremonez *et al*., 1998 and Pernal and Currie, 2000).

The success and quality of queen production depends on strong well-fed healthy nurse colonies, quality of parents, suitable equipment and colony management. The criteria related to the queen quality are the traits such as weight at emergence, ovarian weight, number of ovarioles and the diameter and the volume of spermatheca (Harbo, 1986 and Carreck *et al*., 2013). Pollen ingestion is also necessary to develop hypopharyngeal glands (Alqarni, 2006) and ovaries (Hoover *et al*., 2006). The development of both is positively correlated with protein consumption (Pernal and Currie, 2000). Nurse bees have developed hypopharyngeal glands and the enzymatic equipment to process protein derived from pollen into a high-quality larval food (Moritz and Crailsheim, 1987). It was recorded that different artificial diets affected the acceptance percentage of the adopted larvae and the fitness parameters of the resulted queens (Eremia *et al*., 2014). Larval nutrition may also affect behavior and physiology of workers (Mattila and Otis, 2006). The nutrition is not only important for colony development and bee longevity but also plays a vital role against pathogens and in maintaining gut fitness (Ritz and Gardner, 2006).

Colony strength before the nectar flow is a critical factor behind honey yield, allowing an effective use of early flow seasons. Pollen supplements or substitutes may accelerate colony development by stimulating egg-laying and maintain brood rearing under less-than-optimum conditions. It is to feed the colonies with protein-rich diets as bee bread, candy or with other substances (Mattila and Otis, 2006). In Egypt, there are two dearth periods; from November to January and from July to October (Shehata, 2016). Cage studies established that adult honey bees can survive for a very long time on carbohydrates, which they need for energy metabolism. Otherwise, bees allowed feed also on pollen show greater longevity (Schmidt *et al*., 1995; Alqarni, 2006 and Manning *et al*., 2007).

The most effective pollen substitutes and supplements are those that are most similar in chemical composition and physical consistency to stored pollen (Wilson *et al*., 2005 and Saffari *et al*., 2010). The corn gluten is underexploited protein due to its peculiar composition. For instance, zein is water insoluble because it contains many hydrophobic amino acids which are buried inside the molecules, its deficiency in lysine and tryptophan, and thus has limited uses in human nutrition (Jin *et al*., 2015). To determine the value to honey bees, it is necessary to conduct bioassays of different pollen or protein diets and their effects on lifespan (Schmidt *et al*., 1987), other physiological parameters with a peak at the age of nurses (Crailsheim *et al*., 1992) or brood production (Shehata, 2016). This research aimed to estimate the effect of pollen substitutes on the reared honey bee queens quality and the hypopharyngeal glands of the nurse workers.

MATERIALS AND METHODS

(A) Field Experiments:

1. Apiary and bees

The field portion of this study was carried out in a private outdoor apiary located at Meet Fares village, Bani Ebaid district, Dakahlia province (31°04'50.4"N & 31°35'51.8"E). The study was conducted during winter and early spring, covering the dearth season prior to clover nectar flow season to investigate the effect of food supplements on the honey bee biology and products. The apiary was surrounded with tall walls and covered with a ceiling from reed grass for protection from winds during winter and early spring. The flora found in the apiary area comprised *Eucalyptus* sp., *Poplus* sp., citrus, palms, beans, flax and clover. The tested honeybee race was the local Carniolan of *Apis mellifera carnica*. The hives were one-chambered typical Langstroth type. Every five days, all hives received sugar syrup (1 : 1, w/v) or sugar candy every 5 days for general enhancement of the honey bee colonies development.

2. Honey bee diet components

The pollen supplement was represented by clover pollen as a natural source of honey bees protein feeding. Pollen was collected by the beekeeper using the pollen traps from the apiary during the previous main nectar and pollen flow season, which is represented by the clover season in May. Pollen was freshly and directly put in the deep freezer at -14°C and kept for about 8 months. Such low temperature degree is the most suitable to preserve the main precious constituents of pollen without a great decline of its nutrition value. The constituents were 19.51 g/100 g total proteins, 13.5g/100g total amino acids, 19.67 g/100 g total carbohydrates and 9.4 g/100 g total lipids.

The pollen substitute was represented with corn gluten as an alternative natural botanical source rich with protein. Commercial American yellow corn gluten was purchased from Elbaraka-Gamasa Co. for fodder, Gamasa, Industrial area, Dakahlia, Egypt. Corn was imported from USA and reindustrialized in Egypt as poultry fodder. The constituents were 50.33 g/100 g total proteins, 14.19 g/100 g total amino acids, 13.34 g/100 g total carbohydrates and 24.16 g/100 g total lipids. Sugar used in these experiments was light brown cane sugar produced by the Egyptian Sugar and Integrated Industries Company. The main constituents of sugar were 0.3 g/100 g total proteins, 98.9 g/100 g total carbohydrates and lipids free.

3. Preparation of diets

The ratios of the diet contents were calculated to make the total proteins content nearly the same in case of the corn gluten- and clover pollen-fed colonies. The total proteins were about 20% because it is almost the percentage in the used clover pollen, which was lower than that of the used corn gluten. So, the diets were offered to honey bees in the form of patties containing cane sugar powder. Moreover, the sugar powder increases the flavor of the diet patties for honey bees. The corn gluten patty was made by grinding gluten with an electric grinder and the resulted powder was sifted, blended with grinded cane sugar (1 : 2, w/w) and provided with an adequate amount of water. The clover pollen patty was made by grinding pollen, mixing with cane sugar powder (1 : 1, w/w) and addition of a suitable amount of water. The diet components were blended with water until complete homogeneity of the resulted dough. The patties or cakes were made to be neither over-hardened nor over-softened. This made the patties more suitable for the honey bee workers to be easily manipulated and more edible. The cakes were directly placed on the wooden frames under the inner cover of the hives. All previous diets were freshly prepared in the same day of feeding.

4. Experimental design

a) A control colony was fed only a half-liter of sugar syrup (1 : 1, w/v) twice with 5 days interval.
b) A colony was fed a half kg of the corn gluten patty (1 gluten : 2 sugar powder, w/w) and a half-liter of sugar syrup (1 : 1, w/v) twice with 5 days interval.
c) A colony was fed a half kg of the clover pollen patty (1 pollen : 1 sugar powder, w/w) and a half-liter of sugar syrup (1 : 1, w/v) twice with 5 days interval.

5. Experimental procedure

In February, the experiments were started after two days of the apiary feeding with the cane sugar syrup (1 : 1, w/v). On the first day, three healthy colonies were randomly chosen, which were 8-frames contained and headed with newly and naturally mated egg-laying sister queens. They were dequeened and categorized as cane sugar syrup- (control), corn gluten- and clover pollen-fed colonies. The brood frames were removed and only 2 honey frames were retained in each hive (nucleus). More honey bee workers, covering the brood frames of different healthy colonies, were added for reinforcement the colonies. They were blended and distributed to the three hives to minimize the physiological differences. Also, the source hives were headed with sibling mother queens to decrease the genetic variation as possible. One grafting frame with empty plastic cups were added inside each experimental hive between the two honey frames and sprayed with sugar syrup (1 : 1, w/v). This step is necessary for familiarization of the plastic cups to be varnished and prepared by honey bee workers to increase the acceptance of the grafted larvae of the future reared queens. The colonies were fed with their diets according to the aforementioned experimental design to activate the hypopharyngeal glands of the young workers for secretion of royal jelly fed to the growing larvae. The patties were continuously added when consumed or in other words the feeding was *ad libitum*.

On the second day, 9-mm diameter yellow plastic cups were used for grafting honey bee larvae to be reared as queens. One strong colony was used as a donor of these larvae to all experimental groups during the time course of experiment to minimize the genetic variation. One opened brood frame, containing a plentiful amount of about one-day-old larvae, was chosen and shaken to remove bees. By means of a grafting metal needle, one larva was carefully transferred without injury to each plastic cup containing a small drop of royal jelly diluted by blending with warm water (1 : 1). Under a simple magnifying glass, the larvae must be quickly transferred to the cups and carefully placed on the bottom with the same original position in their source honeycomb.

The lower spiracles are nonfunctioning, so any change in the original position may cause suffocation of

the larvae and death. The grafting process should be carried out without exposing larvae to cooling. Hence, the grafting process was carried out inside a closed room, avoiding direct sunlight, wind and cold. A cold light source must be used for illumination, avoiding the increase of temperature that affects the larvae. The plastic cups were attached to the wooden bars of the standard grafting frame by means of molten beeswax. Each grafting frame had 3 bars with 15 cups for each with a total of 45 cups. The control, gluten- and pollen-fed colonies was provided with one grafting frame and fed the aforementioned diets according to the experimental design.

The grafting frames were quickly reintroduced into their source experimental hives without exposure to direct sunlight or wind. Each experimental colony was fed a half-liter of corn sugar syrup (1 : 1, w/v) twice at 5 days interval for enhancement of honey bees feeding. On the third day, 2 opened brood frames were added in each experimental hive, each one just at each side of the grafting frame and externally covered by the 2 honey frames. The brood frames act as brood pheromone source, activating royal jelly secretion and prohibiting workers egg-laying. On the fourth day, the accepted queen cups were counted and recorded for calculation of the acceptance percentages. From each colony, the growing queen larvae of 5 cups were removed by the metal needle. Their provisioned food of royal jelly was gathered by a spatula and put in 5-ml plastic containers and stored in a deep freezer at -14°C until analysis. About 20 nurse worker bees were picked up from each hive, which were moving over the accepted queen cups for nursing the growing queen larvae. These nurse workers were caged in wooden Benton cages with small pieces of candy. Then, they were quickly transferred to the laboratory and dissected for further morphometric and histological studies.

On the ninth day, all sealed queen cups were counted, removed from their grafting frames and reintroduced into their experimental colony by direct insertion into the honey comb. Individually, each queen cell was caged by a plastic half ball cage until queen emergence. On the twelfth and thirteenth days, all emerged queens were counted and the emergence percentages were calculated. Then, each newly emerged queen was individually caged in a wooden Benton cage with some attendants and a small piece of candy and quickly transferred to the laboratory to be dissected for the morphometric and histological studies. This experiment was repeated in March and April. The three experiments were carried out to compare between the effects of feeding with corn gluten and clover pollen patty diets on honey bees in dearth season months. The study comprised the effect of the diets on some morphometric and histological parameters of the newly emerged queens. Also, the morphometric and histological effects of feeding on the hypopharyngeal glands of the honey bee workers were investigated. Royal jelly harvested amounts during queens rearing in each experimental colony were analyzed.

(B) Laboratory investigations:

1. Queen parameters

The ovaries of the dissected queens and the hypopharyngeal glands of the nurse workers were removed and immediately fixed in 10% neutral formalin for 24 h. the specimens were routinely prepared for hematoxylin and eosin staining sectioning, investigation and photographing. From each hive, about 5 newly emerged queens (within 24 h) were obtained for morphological and histological studies. The measured morphological parameters were the queen weight and length as well as her abdominal length and width. Each queen was carefully dissected in a saline solution (NaCl, 0.09%). The right and left ovaries were removed and weighed using sensitive electrical balance. Isolated ovaries were carefully mounted in a drop of xylene on a glass slide according to Jackson *et al*. (2011). For clarification, a drop of Puri's medium (100 ml distilled water + 50 ml glycerin + 30 ml glacial acetic acid + 70 g chloral hydrate) was placed onto the ovaries for two minutes to separate the ovarioles, which were carefully washed with water to remove any residues. The loosened ovarioles were counted by a binocular microscope per ovary. The spermathecae were removed, freshly mounted, examined under a light microscope and the diameters were measured by a standardized ocular micrometer (Szabo, 1982).

2. Nurse worker hypopharyngeal glands

From each hive, about 20 nurse workers were obtained from the area around the accepted queen cups and transported to the laboratory. The worker heads were dissected in a saline solution (NaCl, 0.09%) under a binocular microscope and their hypopharyngeal glands were removed. The gland acini diameters were measured by the standardized ocular micrometer of a light microscope. About 10 acini were measured per each gland. For histological investigations, the glands were removed and immediately fixed in 10% neutral formalin for 24 h. They specimens were routinely prepared for sectioning, hematoxylin and eosin staining, investigation and photographing.

(C) Statistical analysis:

The obtained data were expressed as means ± standard deviation. The data were checked for normality and homogeneity of variance with Klomogrov-Smirnov and Levene tests, respectively. Parametric data were analyzed with One-Way ANOVA for analysis of variance followed by honest Tukey test for post-comparison of significant difference between groups. Non-parametric data were tested with Kruskal-Wallis test followed by Mann-Whitney (*U*) test. For correlation coefficient, Pearson and Kendall tau-b tests were used for parametric and non-parametric data, respectively. Statistical significance difference was accepted at $p < 0.05$ with double-sided type (two-tailed) distributions (Waller and Duncan, 1969 and Snedecor and Cochran (1971). All data were statistically manipulated using SPSS Statistics program 17.0© 1993-2007.

RESULTS

(A) Queen parameters:

1. Queen weight

The honey bee queen weights were measured after rearing by the experimental colonies fed with different proteinic diets. In February, the recorded means of queen weights were 148.14 ± 7.05, 140.86 ± 19.59 and 136.64 ± 8.45 mg in sugar syrup- (control), corn gluten- and pollen-fed colonies, respectively. Kolmogorov-Smirnov (normality)

test and Levene (homogeneity) test revealed that the data recorded in the experimental colonies in February were nonparametric. Thus, Kruskal-Wallis (K) test was carried out and showed insignificant differences in the honey bee queen weights between the experimental colonies (χ^2 = 3.472, P = 0.176). Though, Mann-Whitney (U) test showed a significant decrease in the mean weights in the clover pollen-fed colony when compared with the control one (P = 0.028). In March, the means of queen weights were 140.63 ± 13.10, 144.36 ± 27.82 and 136.24 ± 13.66 mg in sugar syrup-, corn gluten- and pollen-fed colonies, respectively. The data were parametric and One-Way ANOVA test was carried out, which revealed that there were insignificant differences between the experimental colonies (F = 0.351, P = 0.708). In April, the means were 152.80 ± 19.54, 141.29 ± 9.42 and 165.57 ± 23.85 mg in sugar syrup-, corn gluten- and pollen-fed colonies, respectively. The data were parametric and One-Way ANOVA test showed that there were insignificant differences between the experimental colonies (F = 3.00, P = 0.077). Though, post-hoc (LSD) test showed that there was only a significant increase in the queen weights in the clover pollen-fed when compared with the corn-gluten fed colonies (Fig. 1).

2. Queen length

The honey bee queen length was measured in all the experimental colonies fed with different diets. Kolmogorov-Smirnov and Levene tests showed that all the data were nonparametric. In February, the means of queen lengths were 14.67 ± 1.53, 17.83 ± 0.98 and 18.71 ± 2.36 mm in control, corn gluten- and clover pollen-fed colonies, respectively. Kruskal-Wallis (K) test showed that there were significant differences between the experimental colonies (χ^2 = 6.085, P = 0.048). Mann-Whitney (U) test exhibited significant increases in the corn gluten- and clover pollen-fed colonies when compared with the control (sugar syrup)-fed colony and P = 0.021 and 0.037, respectively. In March, the mean queen lengths were 18.50 ± 0.76, 15.86 ± 2.27 and 15.88 ± 2.85 mm in control, corn gluten- and clover pollen-fed colonies, respectively. Kruskal-Wallis test revealed that there were significant differences between the experimental colonies (χ^2 = 7.086, P = 0.029). The post-comparison were carried out by Mann-Whitney test, which revealed that there was a significant decrease in the corn gluten-fed colony when compared with the control one (P = 0.009). In April, the means of the queen lengths were 17.67 ± 1.21, 18.67 ± 1.21 and 19.14 ± 1.07 mm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were insignificant differences between the groups (χ^2 = 4.257, P = 0.119). Though, Mann-Whitney test revealed only a significant increase in clover pollen-fed colony in comparable with the control one (P = 0.034) (Fig. 1).

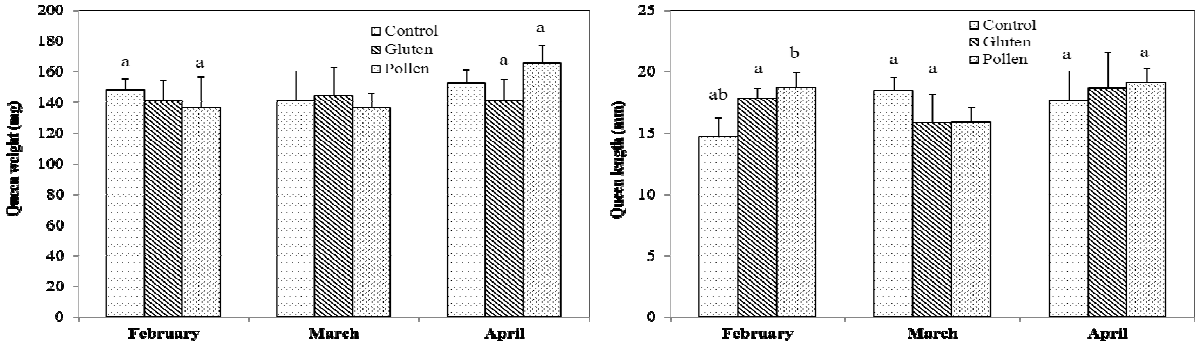

Fig. 1. Means of queen weights and lengths reared by the experimental colonies fed with different diets. The same letters mean significant differences in the same month.

3. Queen abdomen length

Kolmogorov-Smirnov and Levene tests showed that all the data were nonparametric. In February, the means of queen abdomen lengths were 8.33 ± 0.58, 10.50 ± 1.05 and 7.86 ± 0.69 mm in control, corn gluten- and clover pollen-fed colonies, respectively. Kruskal-Wallis (K) test showed that there were significant differences between the experimental colonies (χ^2 = 10.940, P = 0.004). Mann-Whitney (U) test exhibited significant increase in means of queen abdomen lengths of the corn gluten-fed colony when compared with the control and clover pollen-fed colonies, P = 0.026 and 0.003, respectively. In March, the mean queen abdomen lengths were 10.13 ± 0.64, 7.13 ± 3.14 and 8.88 ± 1.73 mm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test revealed that there were significant differences between the experimental colonies (χ^2 = 8.85, P = 0.012). The post-comparison were carried out by Mann-Whitney test, which revealed that there was a significant decrease in the corn gluten-fed colony when compared with the control one (P = 0.003). In April, the means of the queen abdomen lengths were 9.17 ± 1.33, 10.17 ± 0.98 and 10.14 ± 1.57 mm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were insignificant differences between the groups (χ^2 = 1.717, P = 0.424). Hence, Mann-Whitney test revealed insignificant differences between all the experimental colonies (Fig. 2).

4. Queen abdomen width

In February, the means of queen abdomen widths were 5.86 ± 0.32, 6.14 ± 0.46 and 5.86 ± 0.61 mm in control, corn gluten- and clover pollen-fed colonies, respectively. Kolmogrov-Smirnov and Levene tests showed that the data were nonparametric. Kruskal-Wallis (K) test showed that there were insignificant differences between the experimental colonies (χ^2 = 1.232, P = 0.540) and Mann-Whitney (U) test assured that. In March, the mean queen abdomen widths were 5.71 ± 0.21, 5.78 ± 0.29 and 6.09 ± 0.32 mm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test revealed that there

were insignificant differences between the experimental colonies (χ^2 = 5.563, P = 0.062). The post-comparison carried out by Mann-Whitney test assured that. In April, the means of the queen abdomen widths were 5.80 ± 0.45, 5.80 ± 0.08 and 6.93 ± 1.01 mm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were significant differences between the groups (χ^2 = 14.101, P = 0.001). Hence, the post-comparison Mann-Whitney test revealed that there was a significant increase in the clover pollen-fed colony when compared with the control and gluten-fed colonies, P = 0.017 and 0.002, respectively. Also, there was a significant increase in clover pollen-fed colony when compared with the corn gluten-fed one (P = 0.005) (Fig. 2).

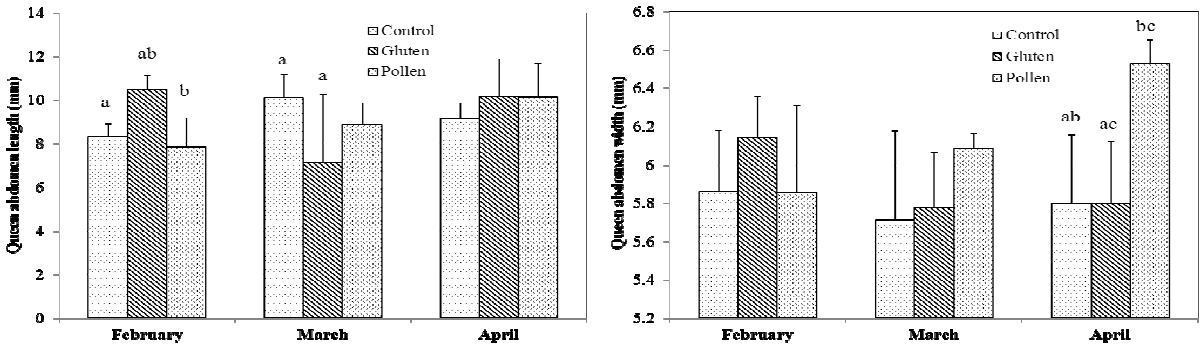

Fig. 2. Means of abdominal lengths and widths of queens reared by the experimental colonies fed with different diets. The same letters mean significant differences in the same month.

5. Queen ovary weight

In February, the recorded means of queen ovary weights were 6.80 ± 2.36, 5.40 ± 1.15 and 5.57 ± 2.66 mg in control, corn gluten- and clover pollen-fed colonies, respectively. Kolmogrov-Smirnov and Levene tests showed that the data were nonparametric. Kruskal-Wallis (K) test showed that there were insignificant differences between the experimental colonies (χ^2 = 1.361, P = 0.506) and Mann-Whitney (U) test assured that. In March, the means of queen ovary weights were 5.45 ± 1.41, 5.63 ± 1.41 and 5.59 ± 0.91 mg in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test revealed that there were insignificant differences between the experimental colonies (χ^2 = 0.310, P = 0.856). The post-comparison carried out by Mann-Whitney test assured that. In April, the means were 5.97 ± 1.48, 5.92 ± 2.26 and 6.86 ± 0.90 mg in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were insignificant differences between the groups (χ^2 = 2.824, P = 0.244). Hence, the post-comparison Mann-Whitney test assured that (Fig. 3).

6. Number of ovarioles of the queen ovary

In February, the recorded mean numbers of ovarioles were 56.67 ± 13.87, 82.00 ± 7.55 and 95.00 ± 1.41 ovarioles/ovary in control, corn gluten- and clover pollen-fed colonies, respectively. Kolmogrov-Smirnov and Levene tests showed that the data were nonparametric. Kruskal-Wallis (K) test showed that there were insignificant differences between the experimental colonies (χ^2 = 7.20, P = 0.027). The post-comparisons test of Mann-Whitney (U) revealed that there was a significant increase in number of ovarioles/ovary in clover pollen-fed colony in comparison with the control and corn gluten-fed ones (P = 0.05) for both. Also, it was deduced a significant increase in case of corn-gluten fed colony when compared with the control one (P = 0.05). In March, the mean numbers of ovarioles were 70.00 ± 3.00, 78.33 ± 3.51 and 80.00 ± 10.82 ovarioles/ovary in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test revealed that there were insignificant differences between the experimental colonies (χ^2 = 4.356, P = 0.113). The post-comparison carried out by Mann-Whitney test assured that. In April, the mean numbers of ovarioles were 63.33 ± 12.34, 83.67 ± 18.34 and 78.67 ± 9.50 ovarioles/ovary in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were insignificant differences between the groups (χ^2 = 3.467, P = 0.177). Hence, the post-comparison Mann-Whitney test assured that (Fig. 3).

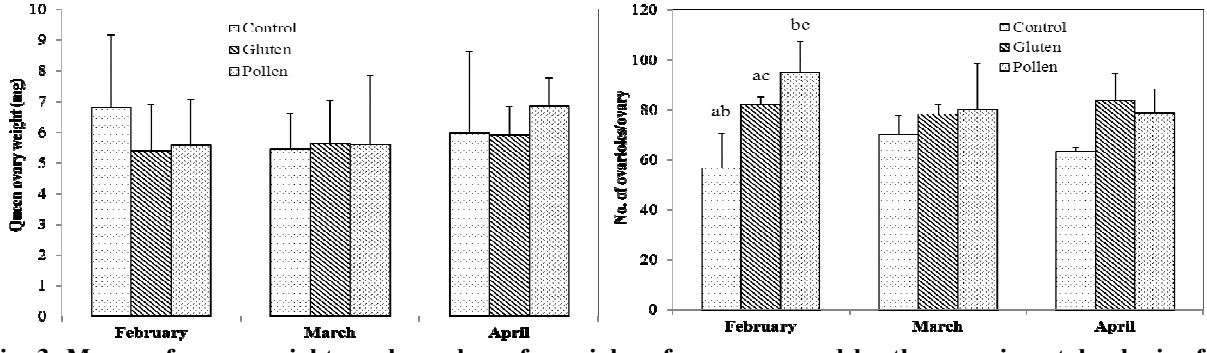

Fig. 3. Means of ovary weights and number of ovarioles of queens reared by the experimental colonies fed with different diets. The same letters mean significant differences in the same month.

(B) Nurse worker hypopharyngeal glands:

1. Hypopharyngeal gland diameters

The mean diameters of worker hypopharyngeal gland acini in February were 11.44 ± 2.37, 16.8 ± 5.30 and 19.13 ± 4.26 µm in control, corn gluten- and clover pollen-fed colonies, respectively. Kolmogrov-Smirnov and Levene tests revealed that the data were nonparametric. Kruskal-Wallis (K) test showed that there were very high significant differences between the experimental colonies (χ^2 = 25.470, P < 0.001). Mann-Whitney (U) test revealed that there were very high significant increases in the gland acini diameters in clover pollen- and corn gluten-fed colonies when compared with the control one (P < 0.001). In March, the gland acini diameter means were 68.68 ± 17.55, 88.64 ± 13.02 and 124.41 ± 26.92 µm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test revealed that there were very high significant differences between the experimental colonies (χ^2 = 33.689, P < 0.001). The post-comparison carried out by Mann-Whitney test showed that there was a very high significant increase in clover pollen-fed colony in comparison with the control and corn-gluten-fed colonies (P < 0.001). Also, there was a very high significant increase in corn gluten-fed colony when compared with the control one (P < 0.001). In April, the gland acini diameter means were 65.77 ± 13.05, 90.94 ± 22.82 and 102.22 ± 17.17 µm in control, corn gluten- and clover pollen-fed colonies, respectively. The data were nonparametric and Kruskal-Wallis test showed that there were very high significant differences between the groups (χ^2 = 19.080, P < 0.001). Thus, the post-comparison Mann-Whitney (U) test showed that there were very high significant increases in clover pollen-fed and corn gluten-fed colonies when compared with the control one (P < 0.001) (Fig. 4).

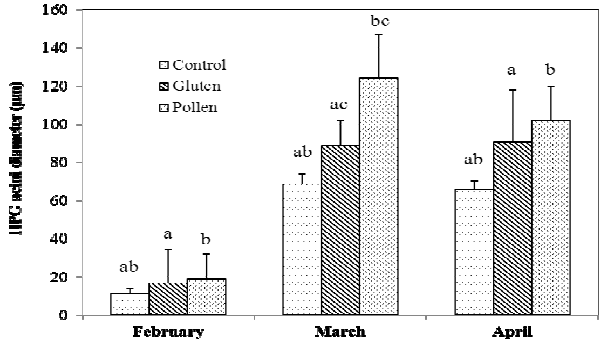

Fig.4. Mean diameters (µm) of worker hypopharyngeal gland acini of the experimental colonies fed with different diets. The same letters mean significant differences in the same month.

2. Hypopharyngeal gland histology

Hematoxylin and eosin stained sections of the hypopharyngeal glands of the honey bee workers, nursing the reared queen larvae, were investigated. The first treatment during February was chosen for picking up the workers for histological studies of their hypopharyngeal glands. In the control honey bee workers, the findings revealed the paired nature of the gland. It has two lateral lobes located in a fronto-lateral position on both sides of the worker brain close to the compound eyes. The typical structure of the gland was observed as each lateral gland lobe mainly consists of ovoid, spheroid or pyriform gland alveoli (acini) arranged around a main chain, in which they open by their ducts. A larger magnification explained more details about the gland acinus, which consists of the gland cells including the secretory vesicles of the royal jelly, the main secretion of this fascinating gland. The secretion aggregates inside structure called end apparatus acts as a reservoir for secretion until delivery into the main channel (Fig. 5A, B). The sections of corn gluten-fed honey bee worker hypopharyngeal glands showed enlarged gland acini in comparison with the control or sugar syrup-fed worker glands. The secretory vesicles are very large and the gland acini are turgid with the secretion. The end apparatus is filled with secretion, indicating a great secretory activity (Figs. 5C, D). In case of the clover pollen-fed honey bee workers, it was showed a good configuration of the gland acini sections. It was observed very huge secretory vesicles filling the acini cells cytoplasm so that they occupy nearly all the cytoplasm compressing the nuclei and end apparatus. Small minute duct cells were revealed connecting between the end apparatus and the main channel. Each of them collects secretion from the acinus for delivery into the main channel of each gland lobe (Figs. 5 E, F).

DISCUSSION

The queen weights and lengths were significantly increased when honey bees were fed clover pollen diets with significant increase in case of corn gluten diets in February when compared with the sugar syrup feeding (control). Also, it was observed that the weather conditions and food resources season in April had great effects in these increases. This is in agreement with Shimanova (1966) who stated that the prevailing weather conditions were among the factors controlling the weight of queens. She concluded that at the time of first flow, the queens were heavier and towards autumn, their weights decreased. Also, the obtained results are in agreement with those of Avetisyan *et al.* (1967) who recorded that the heaviest emerged queens were reared in early spring during the main nectar flow. Similarly, Khater (1998) found that the mean weight of newly emerged queens during spring was the highest when compared with other seasons. On the contrary, Morini and Bueno (1993) revealed that the queen weight was higher in September than in June and July. This variation could be attributed to the weather and botanical factors.

Also, El-Hanafy (1991) found that the mean weights of virgin queens produced from rearing colonies that fed with yeast gave the highest result, followed by nestogen, supramen and the sugar syrup 50% was the least. Also, Sharaf El-Din *et al.* (1999) studied the effect of different foods offered queen rearing colonies on the weight of newly emerged queens, where soya bean gave the best result, followed by yeast, mandarin cortex jam, semi dry date and sugar syrup again was the last one. The present findings agreed with those of Elaidy *et al.* (2010) who showed that the pollen, vitamin and wheat gluten diets increased the queen weights than the sugar syrup. Though, the weights of the queens were slightly larger than those reported in this study and this might be owed to the clover pollen was stored for more than nine months, decreasing

the proteins and vitamins content, which is the essential factor in queen development. Also, in case of the queen abdomen widths, the maximum mean was recorded with the clover pollen diet in April; otherwise, the maximum mean of queen abdomen lengths was recorded with corn gluten diet in February. This could be comparable to the results of Sharaf El-Din *et al.* (1999) who found that the yeast caused the highest significant effect, followed by mandarin cortex jam, sugar syrup, soya bean, semi-dry date. Also, the findings agreed with those of Elaidy *et al*. (2010) in which, it could be concluded that the winter-reared queens are the most preferable to gain the highest abdomen length and width of newly emerged queens. Diets containing wheat gluten gave the highest result of abdomen length and width.

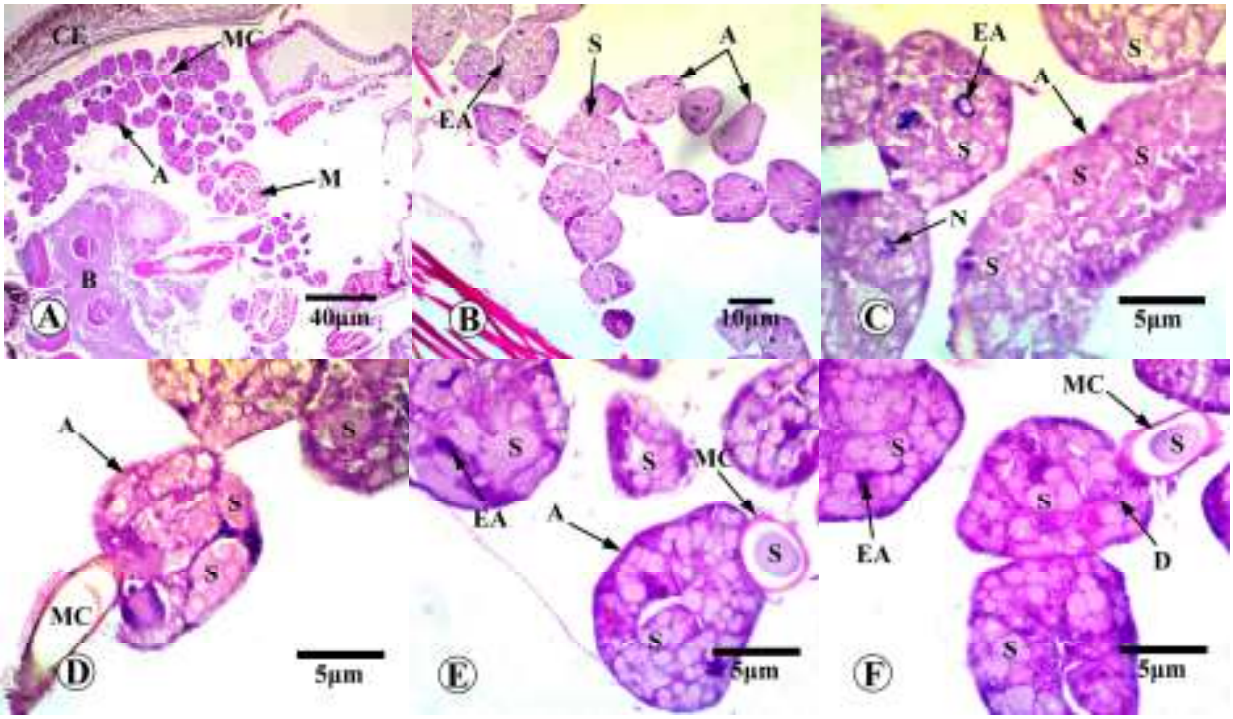

Fig. 5. Light micrographs of hypophryngeal gland sections of nurse workers. A & B: control (sugr syrup-fed); the gland acini (A) around the main channel (MC) of the gland lobe between the brain (B) and compound eye (CE) with muscle fiber bundles (M) around. The acini contain small and few secretory vesicles (S) besides the end apparatus (EA). C & D: corn gluten-fed; secretory vesicles increased in number and size and nuclei (N) are apparent. E & F: clover pollen-fed; good configuration and acini size increased and turgid with huge and numerous secretory vesicles, which were delivered by duct cells (DC) into the main channel (MC) (H & E).

The weight of the queen ovaries revealed insignificant differences between the experimental groups fed the different diets. The size of the queen ovary indicates to the ovary weight and both of them express the degree of its development. Our results were in agreement with Abd Al-Fattah (1996), who stated that twice amount of royal jelly was yielded from colonies provided with pollen supplement paste than those from unfed ones and that additional feeding with pollen substitute in bee colonies had a positive effect on the fresh weight of queens and newly emerged workers bees. Elaidy *et al*. (2010) reported that the mean lengths of ovaries of newly emerged queens were ranked as artificial diets, containing vitamins, wheat gluten, black seed oil and sugar syrup (control). During winter, the feeding with gluten gave the best result for the average number of ovarioles. While the lowest result of this concern appeared with the control. In addition, Khater (1998) stated a higher number of ovarioles of spring-reared queens when compared with those reared during summer. In addition, Krol *et al.* (1992) found that queens emerged from the colonies fed on sugar syrup supplemented with vitamin B_1 had 6.5% more ovarian tubules than those fed on sugar syrup alone (control).

The clover pollen and corn gluten diets significantly increased the hypopharyngeal gland acini diameters in the three months when compared with the sugar syrup. The maximum record was achieved with clover pollen diets in March. These data agreed with that of Ricciardelli *et al*. (1987) who reported that the development of the (HPG) was promoted by high protein concentration of diet. Also, Darhous (1990) found that feeding caged bees on defatted soya flour, wheat bran, chick pea flour and date paste in their sole sources induced more development of the hypopharyngeal glands. El-Dakhakhni and Metwally (1995) showed that Hypopharyngeal gland more developed in workers of 10-days-old fed wheat bran than those fed rice bran or mixture of wheat and rice brans. Mohanny (1999) found that the maximum development occurred was in the first group (the cake of wheat germ and honey) and the lowest one was in the control, while the second group, of wheat germ cake, pollen grains and honey, was in between. De Grandi-Hoffman *et al*. (2010) found that bees fed sugar syrup alone had lower protein concentrations and smaller hypopharyngeal glands compared with the other diets especially as the bees aged. The results in this work disagreed with El-Barbary (1980) who found that bees fed

wheat bran and rice germ failed in promoting growth of (HPG). Li *et al*. (2012) studied the effects of different levels of dietary crude protein on the development, antioxidant enzymatic activity, and total midgut protease activity of honey bees were investigated in the study. They indicated that the pollen substitutes used appeared to be a valuable proteinaceous food and provision of adequate dietary protein to a colony will improve brood rearing, weight of individual bees, body protein content, and antioxidant status of emerging workers. In this study, pollen substitutes with a protein level about 30-35% were recognized as an excellent diet for promoting bee development. These findings are particularly important for the successful beekeeping and management of colonies using pollen substitute when natural pollen is unavailable.

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تأثير مكملات وبدائل حبوب اللقاح على مبايض الملكات والغدد تحت البلعومية لشغالات نحل العسل

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تعتبر تغذية نحل العسل من الأمور الحيوية فى مجال النحالة حيث تعتمد عليها الخلية كليا فى تربية الحضنة ونمو الطائفة والتكاثر والنجاة خلال مواسم القحط بالإضافة للوقاية من الأمراض. موسم الشتاء فى مصر يتميز بانخفاض درجة الحرارة وزيادة الرطوبة وقلة مصادر الرحيق واللقاح مما يؤثر سلبا على طوائف النحل خصوصا مع زيادة التلوث الملحوظ فى البيئة وفقد النحل المتزايد على مستوى العالم، لذا من الأهمية بمكان أن نولى التغذية الصناعية بمكملات وبدائل حبوب اللقاح عناية خاصة فى مجال أبحاث النحل. تناولت الدراسة تأثير التغذية بحبوب لقاح البرسيم المجمدة وجلوتين الذرة فى موسم الشتاء وبداية الربيع على مبايض الملكات حديثة البزوغ والغدد تحت البلعومية للشغالات الحاضنة والتى تختص بإفراز الهلام الملكى (الغذاء الملكى) المسئول عن تغذية اليرقات حتى عمر ثلاثة أيام (للشغالات و الذكور) بالإضافة للملكات طوال حياتها. ولقد أسفرت النتائج عن أن حبوب اللقاح كان لها الأثر الأكبر على الخواص المورفوموترية للملكات العذارى حديثة البزوغ ومبايضها حيث تميزت بكبر وزنها وأطوالها وطول وعرض البطن، كما تميزت بزيادة وزن مبايضها وعدد وحداتها (أنابيبها) المبيضية مقارنة بالخلية الضابطة (المرجعية) والتى غذيت بالمحلول السكرى فقط. كما تميزت الغدد تحت البلعومية بكبر قطر حويصلاتها وزيادة الحويصلات الإفرازية داخلها وأيد ذلك الدراسات النسيجية. كان لجلوتين الذرة تأثيرا كبيرا مقاربا لتأثير حبوب اللقاح على كل المعايير السابقة فى نحل العسل مقارنة بالمحلول السكرى لاحتوائه على نسبة عالية من بروتين جنين الذرة لذا فهو جيد لتغذية النحل عند ندرة الغذاء ويمكن استخدامه مضافا لمواد غذائية أخرى مثل الخمائر والفيتامينات والسكر لرفع كفاءة تأثيره خصوصا أنه يتميز بسعره المناسب مقارنة بحبوب اللقاح وكثير من تلك الإضافات الغذائية.