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Effect of feeding with different proteinaceous diets on some physiological parameter of honeybee workers , *Apis mellifera* (Hymenoptera :Apidae)

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Abstract

The effect of nutrition value of different diets on mortality, head weight and absorbed protein in gut of caged honeybee workers, *Apis mellifera* L. **(**Hymenoptera :Apidae) were studied. The experiment was carried out by used five bee bread (Broad bean, clover, cucumber, fennel, and maize), artificial diet, artificial diet plus syrup with ascorbic acid to fed newly emerged honeybee workers against control (Fed sugar solution only) under laboratory conditions. Head weight was increased gradually to reach maximum value at nine days then decreased gradually and again increased slightly at 18 and 21 days for all tested groups. The head weight was recorded in bees fed with broad bean and Egyptian clover bee bread 9.625 and 9.489 mg/bee, respectively significantly difference in comparison to control bees (8.100 mg/bee) at nine days. In this study we determined 75 % of cumulative mortality rates for all tested groups. At 39 days of experiment beginning for all treatments data were statically analysis. The lowest mortality rate recorded in clover, bean, cucumber, diet and diet plus ascorbic acid were 51.5, 51.75, 53.5, 51.25 and 52.25 % respectively in comparison to control bee mortality rate 75 % was recorded at 39 days of experiment beginning. Maize bee bread and artificial diet were the lowest percentage of absorbed protein in gut.

Introduction

The honeybee, *Apis mellifera* L. (Hymenoptera :Apidae) is among the most important economic insects not only for their ability to produce honey but also due to their role in pollination of many economically important crops such as berries, nuts, fruits and vegetables. It has been well studied compared to other bee species because of its ability to increase the yield in about 96% of animal-pollinated crops (Klein, 2007). Adequate nutrition in honeybee colonies is essential for development and growth. The survival and development of honeybee colonies are thus intimately related to the availability of those nutrients in the environment (Brodschneider and Crailsheim, 2010; Keller *et al*., 2005 and Haydak, 1970) which suggests that the change of bee foraging area down to the present in densification of landscape and agriculture alterations may provide a poor nutrition and so affect populates of honeybee (Decourty *et al*., 2010 and Naug, 2009). This is further stayed by Upper Egypt beekeepers, which are categorized starvation or poor nutrition as one of the main causes of losses of the colony (Abdel-Rahman and Moustafa, 2012). Colonies with limited protein intake decline from the combination of shorter lifespan and reduced brood rearing for the adult worker.

The lack of pollen or a lowering of its nutritional value over a long-term feeding negatively affects the physiological development of the honeybees. In the absence of protein food and a small amount of brood, bees formed from 13% to 28% smaller balls of pollen than the bees that are in optimal conditions of development (Haydak, 1961). A protein source is essential for biosynthesis of glandular protein causing a larger amount of hypopharyngeal glands (HGs) protein extractable (Huang and Otis, 1989). Workers of honeybee gather pollen from the flowers and keep it in comb cells inside the hive. During gathering and storing, pollen composition alterations during the addition of nectar and glandular secretions also. Then and there it undergoes a process of fermentation to transform into bee bread, thereby acquiring a specific smell and color according to the plant species (Winston, 1987 and Roulston, 2005).

Bee bread is the main source of several important nutrients and provides the bees with most of the nutrients needed e.g., vitamins and minerals, proteins (amino acids), lipids (Fatty acids and sterols). Such components, particularly protein in collected pollen from various plants are different and variety from 8 to 40% (Herbert, 1992). The derived protein from bee bread is mostly used to feed young bees up to the ages of 15-18 days and developing larvae, to provide bees with the structural elements of glands, muscles, and other tissues, and is necessary for the developing of hypopharyngal glands which making royal jelly (Haydak, 1970 and Crailsheim *et al*., 1992). Colonies that consume more bee bread can rear more bees (Campana and Moeller, 1977). Growth of a colony can be limited by either a lack of bee bread or, by the available bee bread lack of the essential nutrients (Loper and Berdel, 1980).

Some factors are used to value the physiology of honeybee workers under varied feeding conditions for example hypopharyngeal gland development (Hrassnigg and Crailsheim, 1998 and Khodairy and Moustafa, 2008). Head weight is used as an indicator for hypopharyngeal gland development. A significant correlation between head fresh weight and the acini volume of the hypopharyngeal gland and food consumption rate (Elaidy and Zedan, 2011).

Most studies of this nature have used diets based on bee-collected pellets of pollen. Bee bread as stored pollen packed into the comb has been seldom tested for its effects on honeybee performance. Poor quality pollen leads to reduced longevity and an incomplete brood food glands development, which in turn, reduces royal jelly production, and a failure to support the growth of the queen and larvae (Zaytoon *et al*., 1998).

The purpose of the present study was to determine the effects of some bee bread types besides one of pollen substitute diet alone and substitute plus sugar solution with vitamin C on the changes in three parameters, head weight (Hypopharyngeal gland), absorbed protein in the gut and mortality in caged honeybee workers (*A. mellifera*).

Materials and methods

Study experiments were conducted in Assiut, Insect Research Laboratory, Plant Protection Research Institute, Egypt over the years of 2023. The first hybrid of Carniolan bee colonies (*A. mellifeara carnica*) was used to gather bee bread (Pollen stored in comb cells) and as well as a source of newly young workers. The following experiments were performed:

1. Bee tested diets sources:

1.1. Bee bread separation:

Throughout flowering periods, bee bread was removed from collectively cultivated plants of honeybee colonies, according to the following protocol: A number of bee colonies were placed in five farms in Assiut governorate having monofloral species: broad bean (*Vicia faba*), fennel (*Faeniculum vulgare*), Egyptian alfalfa (*Trifolium alexandrinum*), corn (Zea *mays*), and cucumber *(Cucumis sativus*). At the flowering period end, beebread was extracted from the honeybee colonies of each monofloral culture. Each source of bee bread was preserved under freezing conditions until use. The determination of bee-stored pollen species was certainly microscopically prepared by comparison with standard pollen collected from the anthers of flowers (Hussein, 1983). Making bee bread in the form of a dough, meals of bee bread mixed with a tablespoon of bee honey for each until it becomes homogeneous.

1.2. Artificial diet preparation.

Artificial diet (Diet) was done according to the method (Moustafa, 2000). It contains 4.5 amounts of powdered sugar, 3 amounts of skimmed soybean flour, 1 dried brewer's yeast and 0.5 amounts of skimmed milk powder. All components have been mixed. To form the dough, hot water is added gradually, then saved in the freezer until used. The same artificial diet + sugar solution supplied with vitamin C (1.8 mg / 1 kg syrup) was prepared as described by Farjan *et al*. (2012). Each cage was provided with diets in small plastic feeders. Every 3 days the diet was changed. All cages were placed in the dark in an incubator at 32 ± 1 °C and 65 RH.

Groups of experiment.

- Group (1) was provided with sugar syrup only (Control).
- Group (2) was provided with bean bread and sugar syrup.
- Group (3) was provided with Egyptian alfalfa bread and sugar syrup.
- Group (4) was provided with cucumber bee bread and sugar syrup.
- Group (5) was provided with fennel bread and sugar syrup.
- Group (6) was provided with corn bee bread and sugar syrup.
- Group (7) was provided with an artificial diet (Diet) in addition to the sugar syrup.
- Group (8) was provided with artificial food in addition to a sugar syrup with ascorbic acid (Diet + Asc.).

2. The honeybee used:

Newly emerged (0–24 hrs.) honeybee workers were acquired through the brood combs of covered incubated worker bees at 32 ± 1 °C in standard environments (Williams, 2013). Newly emergent bees were mixed from modified brood combs before being introduced into test cages and bee samples were randomly selected.

3. Experimental cages design:

The experiment was done in wooden cages measuring 15 x 15 x 5 cm, surrounded by glass on one side and the other covered with black gauze. Each cage was supplied with one vial of tap water and another vial of a 3:1 (W/V) sugar solution and a piece of wax comb was attached to one side of the cage as a cluster plate for bees. Eight cages were used for each diet tested, control, four cages for mortality and four cages for head weight and total protein of gut contents.

Group (A) was used to determine mortality of bee worker:

One hundred bees per cag were used for mortality experimental. Each cage of dead bees was calculated and removed every day until 75 % of the original number of honeybees have died. The mortality was calculated after the death of 75% of control bees at 39 days of the experimental.

Group (B) was used to determine the head weight and protein digestion of honeybee worker:

-Head weight:

Two hundred bees per cage were used for head weight experiments. The head capsule of honeybee workers was dissected under binoculars in physiological saline. The head weight of honeybee workers was weighted 3, 6, 9, 12, 15, 18 and 21 days of bees. Ten honeybees were used to assimilate each age from each treatment. The head weight of honeybee workers was calculated as mg/bee.

- Protein digestion estimation of bee bread:

The concentration of protein was measured in the midgut and hindgut contents of seven days old bees fed different proteinaceous diets. Midgut and hindguts were removed from the abdomens of 5 bees / cage using methods described by DeGrandi-Hoffman *et al.* (2016) standard curve using bovine serum albumin.

4. Statistical analysis:

ANOVA was done and means±sd were compared using **(Duncan, 1955).** Duncan's new multiple range tests at 5% level of probability (SPSS version 16). Four independent replicates for each treatment. The mortality calculated after death was 75% of the experimental.

Results and discussion

1. The head weight:

Data illustrated in (Figure 1) showed that the mean head weight (Mg) varied according to diet type and bee age. All tested diets were significantly different from the control (Fed syrup only). Head weight was increased gradually to reach maximum value at nine days then decreased gradually and again increased slightly at 18 and 21 days for all tested groups. The head weight was the highest value in bees fed with broad bean and Egyptian clover bee bread 9.625 and 9.489 mg/bee, respectively significantly different compared to control bees (8.100 mg/bee) and all tested groups at nine days. Control bees were the lowest value at all days in comparison to all tested diets.

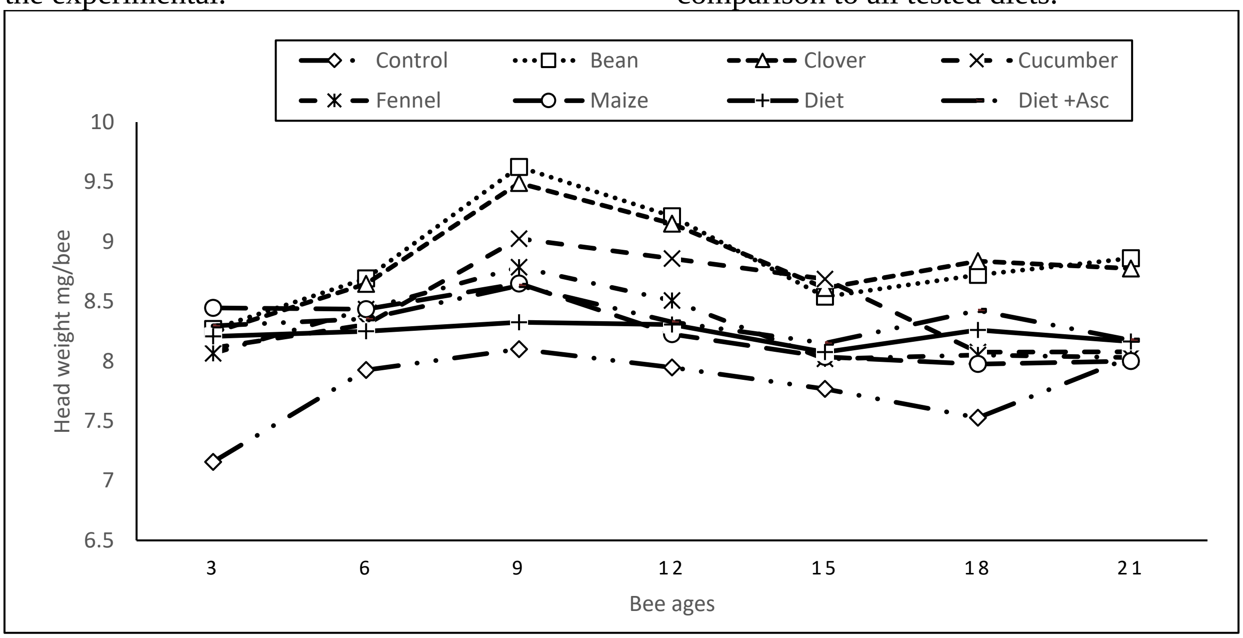

Figure (1): Mean of head weight (mg/3 days) comparison between caged nurse bees (*Apis mellifera*) fed with different proteinaceous diets and control.

2. Mortality of adult workers:

In this study, we determined cumulative mortality when reaching 75 % percentage of mortality for all tested groups. While mortality reached 75 %, the bee workers at 39 days of experimental beginning for all treatments data were statically analysed. The lowest mortality rates recorded in clover, bean, cucumber, diet, and diet plus ascorbic acid were 51.5, 51.75, 53.5, 51.25 and 52.25 %, respectively. There are significant differences in comparison to the control bee mortality rate of 75 % which was recorded at 39 days of experimental beginning (Table 1).

Table (1): Cumulative mortality of honeybee workers fed of bee bread types, artificial diet, artificial diet plus sugar solution with vitamin C and control fed sugar solution only at 39 days of all groups.

Bee age by Days	Cumulative mortality after 75 % of diets fed bee (Means ± SD)							
	Control	**Bean**	**Clover**	**Cucumber**	**Fennel**	**Maize**	**Diet**	**Diet +Asc.**
At 39 Days	75.1± 1.02c	51.75± 2.87a	51.5± 2.65a	53.5± 2.081a	67.75± 7.88b	69.25± 4.78b	51.25± 1.26a	52.25± 2.63a

Each column means have the same letter(s) do not significantly different at 0.05 level of probability.

3. The soluble protein of honeybee worker gut:

The concentration of soluble protein contents of the midgut, hindgut and different between mid and hindgut contents (Absorbed protein) at seven days. Midgut (mg) varied according to diets type (Figure 2). All tested diets had significant differences in soluble protein concentration contents of midgut, hindgut and absorbed protein. The highest soluble protein content of midgut maize and broad bean bee bread were 0.841 and 0.838 respectively. Broad bean bee bread had the highest soluble protein content of hindgut. While in artificial diet the lowest soluble protein content was 0.191 of hindgut. The difference between the midgut and hindgut of maize bee bread soluble protein content (Absorbed protein) was the highest value. Control bees had the lowest percentage of the difference between mid and hindgut contents (Absorbed protein).

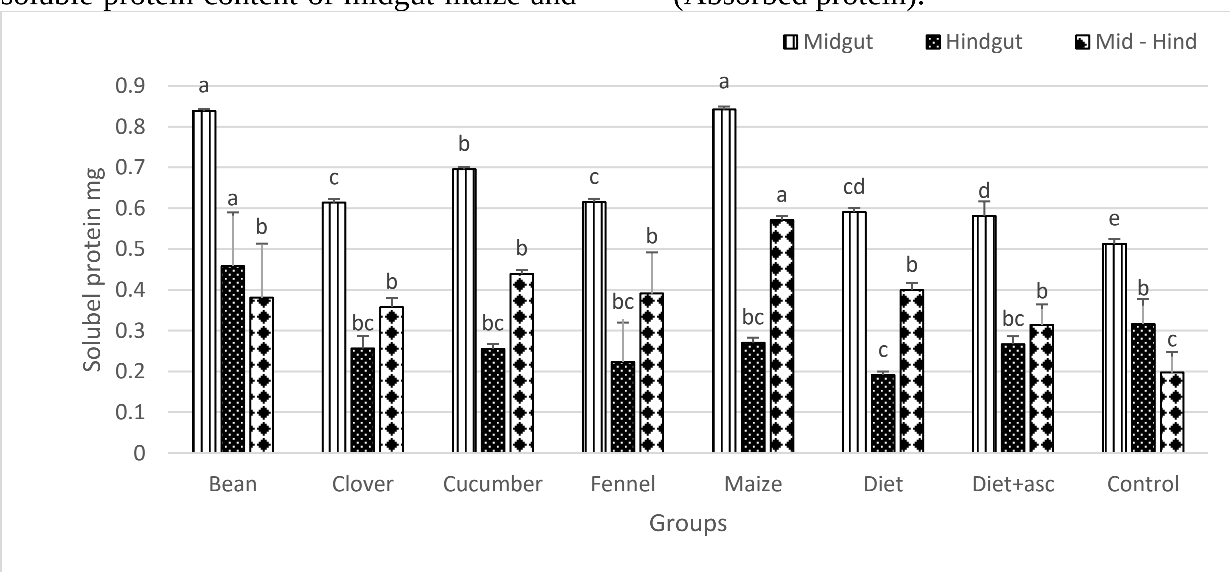

Figure (2): Concentration of soluble protein contents of midgut, hindgut and difference between mid and hindgut contents (Absorbed protein) at seven days and control. Bars with different letters indicate significant differences ($p < 0.05$) of honeybee workers fed bee bread types, artificial diet, artificial diet plus sugar solution with vitamin C and control fed sugar solution only.

Overall, newly emerged honeybee workers fed with broad bean and Egyptian clover bee bread had significantly higher head weight than bees fed pollen cucumber, fennel, maize bee bread diet and pollen substitute diet alone and with sugar solution 3:1 with (Vitamin C).

This means that broad bean and Egyptian clover bee bread stimulated hypopharyngeal gland development, while other diets stimulated less (Lower head weight). The size, hence, the head weight of the hypopharyngeal gland does positively correlate to the royal jelly secretion activity when the hypopharyngeal gland is larger than that of emergent bee (Deseyn and Billen, 2005). This is not to say other diets cannot stimulate the development of functional hypopharyngeal glands.

Poor pollen digestibility could have contributed to lower absorption of protein from the pollen diet, despite similar crude protein intake. Differences in pollen digestion and absorption are dependent on the age of the bee (Szolderits and Crailsheim, 1993), the type of pollen and the seasonality of pollen. In general, only 77% of pollen is digested (Crailsheim, 1990) but it can range from 30-90% (Black, 2006). Another factor that reduces digestibility is the absence of microorganisms in the pollen. Microorganisms are found in bee bread (Gilliam,1979 and Fernandes *et al.,* 2000) and it is believed that they are responsible for pre-digestion (Fermentation) and the secreting digestive enzymes which enhance pollen digestibility in bees. Because artificial diet was highly probable that a number of beneficial microorganisms were low.

Fresh weight and width of the head capsule are positively correlated with hypopharyngeal gland acinic lengths (Hrassnigg and Crailsheim, 1998 and Elaidy, 2008). The hypopharyngeal gland develops in size upon emergence to allow nurses to produce brood food. Smaller hypopharyngeal glands have reduced amounts of protein synthesis (Huang and Otis, 1989). While overaged nurses (>15 days old) rear smaller adults with developed ovaries (Wegener *et al.*, 2009), they have been related to rearing queen larvae (Jung-Hoffman, 1966). Data recorded by (Khodairy and Moustafa, 2008) found that the maximum hypopharyngeal gland development was on 9th day of worker age.

The same conclusion was found by (Al-Ghamdi *et al.*, 2011). Di Pasquale *et al.* (2013) found that certain aspects of nurse bee physiology, (Such as hyphopharygeal gland development) were affected by pollen quality. The peak of hypopharyngeal glands development was at 9 days according to Khodairy and Moustafa (2008) these results agree with our data 9 days for the highest head weight. The bee bread types have been classified into three groups depending on their nutritional values to hypopharyngeal gland development as follows: the first group (Most effective), the gland degree was more than 3.5, included canola and broad bean bee bread. The second category (Considerably effective) ranged from 3 to 3.5, included Egyptian clover. Meanwhile, the third one (Slightly effective) included maize, caper, coriander, and fennel bee bread (Khodairy and Moustafa, 2008).

Pollen types (*Zea mays, Trifolium alexnadrinum*, *Elaeis guineensis* and *Vicia faba*) were collected by honeybee colonies to investigate its feeding effects on amount of total protein on some of their characters by Fawzey *et al.* (2008). They concluded that the highest total protein percentage (30.50%) was found in date palm pollen, while the lowest (15.62%) was found in maize pollen. The protein concentrations of the head and thorax capsules increased significantly ($P<0.05$) with increasing dietary protein levels (Zheng *et al.*, 2014). It was clear that bee bread types have different effects on hypopharyngeal gland development or head weight and related to protein content of diet types.

When the food of bees is poor in protein, their lifespan decreases markedly and vice versa, when the pollen that bees consume contains a high amount of protein, it positively affects their lifespan (Maurizio, 1950). The increase in survival was related to the amount and types of protein content of the pollen consumed (Schmidt *et al*., 1987). Results showed that caged bees fed on sugar syrup only were the shorted longevity in comparison with these feds on bee bread types.

These results showed that protein sources played a vital role in the survival of workers honeybees. This conclusion is in agreement with those of (Abdel-Rahman *et al*., 2013 and Erickson and Herbert, 1980). The Changes in dietary protein are the main reasons affecting on honeybee worker longevity (Zheng *et al*., 2014). DeGrandi-Hoffman *et al*. (2010) found a relationship between bee immune response and protein - level, protein nutrition artificially by providing nutrition supplements or pollen substitutes for bee colonies in periods of scarcity or lack of pollen as well as in periods of disease spreads. In this process, the chance of incidence of disease can be reduced and improve teammate system of bees.

This is in addition to reducing the loss incident in bee colonies because of starvation (Abdel-Rahman, 2014). Our data supported also, by the results obtained by Irandoust and Ebadi (2013) showed that the longevity of caged honeybee *A. mellifera* in an incubator was significantly affected by dietary treatments. Also, they reported that, wheat gluten and lentil substitute supplements caused the highest (61 days) and the lowest longevity of bees (9.2 days) with 50% mortality, respectively.

While 100% morality pollen and lentil substitutes caused the highest (143.5 days) and the lowest longevity of bees (20.7 days), respectively. Also, Omar (1989) in the Assiut area, Egypt, indicated that the total protein content of bee bread collected from honeybee colonies during the active season ranged between 16.88 to 20.97%, and the water content varied from 18.50 to 22.46%. According to the protein fractions isolated from the bee bread, the bread collected in early summer was of a better quality than the bread collected in late summer. The total protein percentage decreased to 14.86% and 13.54% during August and September, respectively.

We found that soluble protein of midgut and absorbed protein were the highest in broad bean and clover. Mize bee bread had the lowest absorbed protein in the gut. Ahmed *et al.* (2019) reported that broad bean and clover had the highest protein value. While maize bee bread had the lowest protein value. Human and Nicolson (2003) said that osmotic pseudogermination or shock as mechanisms do not appear to be used by bees for pollen maize digestion. DeGrandi-Hoffman *et al.* (2016) said hindgut higher levels of protein indicated that protein lower levels of digestion.

The present study illustrates that the bee bread type has different effects on honeybee worker head weight (Hypopharyngeal glands indicator), comparing mortality at 75% of bees and absorbed of protein in the gut. Broad bean and Egyptian clover were the best effects on mortality and head weight of fed honeybee workers. The mortality and head weight in caged honeybees (*A. mellifera*) were affected by different diets of protein.

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