

## PHYSIOLOGICAL EFFECTS OF SOME POLLEN SUBSTITUTES DIETS ON CAGED

### HONEY BEE WORKERS (*Apis mellifera* L.)

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#### Abstract

Nutritional value of four proteinaceous diets and their physiological effects on honey bee workers were evaluated under laboratory conditions. The tested diets were as follows: diet 1 – date (*Phoenix dactylifera* L.) syrup, skimmed milk powder and dried brewer's yeast, diet 2 – Fenugreek (*Trigonella foenum-graecum* L.), Turmeric (*Curcuma longa* L.) powders and dried brewer's yeast, diet 3 – chickpea (*Cicer arietinum* L.) flour, wheat germ and dried brewer's yeast and diet 4 - soybean meal, skimmed milk powder and dried brewer's yeast, beside a control group (bee bread). Caged Carniolan honey bee workers were used in the experiments. The consumption rate, workers longevity, development degree of hypopharyngeal glands (HPG), and weight of rectal contents were determined. The greatest consumption rate was recorded for the control group while the lowest one was recorded for diet 4. Feeding bees on diet 3 gave the longest longevity ( $LT_{50} = 27.0$  days) among the tested diets and as a second rank after bees in the control group ( $LT_{50} = 29.0$  days) while diet 4 showed the lowest longevity ( $LT_{50} = 20.5$  days). The highest HPG development degree (3.78) was recorded for 9 days old bees in the control group, followed by diet 3 (3.24) while the lowest degree (2.14) was to diet 4. The weight of rectal contents of honey bee workers was 13.43, 16.03 and 16.12 mg/bee/3 days for diet 3, diet 1 and diet 2, respectively, suggesting the suitability of these diets to bees. In light of this study, diet 3 and 2 have the best physiological effects for bees with good nutritional values.

Key words: *Apis mellifera*; Hypopharyngeal glands; Nutritional value; Pollen substitutes; Workers longevity

List of abbreviations:  $LT_{50}$  (the time at which 50% mortality occurred); HPG (Hypopharyngeal glands); CCD (Colony collapse disorder); RH (Relative humidity)

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## Introduction

Pollens are indispensable food for honey bee colonies and their shortage intermittent periods cause several problems for the colonies. Completely absence of protein sources in the hive can cause starvation for honey bees and consider among potential reasons for colony collapse disorder (CCD) (Seitz *et al.*, 2015). So, providing colonies with protein source all over the year, especially during dearth periods of pollen is a critical matter. Recently, several studies have given more attention to formulate supplementary diets or substitutes to compensate the lack of the natural protein source (pollen) (Zheng *et al.*, 2014; Amro *et al.*, 2016; Negri *et al.*, 2017; Gamal Eldin *et al.*, 2018; Gregorc *et al.*, 2019; Younis, 2019). Moreover, different pollen types from different plant origin differently effects on the physiological conditions of worker honey bee (Amro *et al.*, 2015).

Pollen substitutes should have some specific characteristics to be suitable for honey bees: palatable (Saffari *et al.*, 2010), can be consumed by bees (Al-Ghamdi *et al.* 2011), attractive to bees (Abd El-Wahab *et al.*, 2016), and have good physiological effects (Amro *et al.*, 2016). Also, they should be able to stimulate colony growth and support aspects of worker quality especially brood rearing activity and worker longevity (Manning *et al.*, 2007). There are several materials that can be used during the preparation of pollen substitutes including: soybean meal (Abbasian and Ebadi, 2002), yeast (Abd El-Wahab and Gomaa, 2005), skimmed milk (Amro *et al.*, 2016), wheat gluten (Nutter *et al.*, 2017) and chickpea flour (Younis, 2019). Such materials have high content of protein, which encourages the development of hypopharyngeal glands (HPG), hence stimulating the secretion of royal jelly and promoting brood rearing activity. However, the role of these materials to enhance physiological characteristics of bee workers is not fully known.

Some pollen substitutes were tested for feeding honey bee workers by Amro *et al.* (2016) and Gamal Eldin *et al.* (2018). They have considered the consumption of pollen substitute by newly emerged bee workers, development of HPG, longevity of bee workers, and weight of rectal content as important criteria for estimating the suitability of diets for bee workers. These criteria are impacted by the type of diets. For example, Al-Ghamdi *et al.* (2011) have found differences in the development degree of HPG when the bees were fed on different diets (bee bread, pollen loads, and a mixture of 1 yeast: 1 gluten and 2: sugar). The longevity of honey bee workers appears to be directly associated with level of body protein (Sagili *et al.*, 2005), newly emerged bees fed on protein diet showed increase in longevity compared with those fed on sugar candies only (Škerl and Gregorc, 2014). Weight of rectal content indicates the utilization of food by bees, and has been considered as directly reflects the food suitability to bees (Al-Qarni, 2006). Also, the weight of rectal content can be varied according to feeding type (Amro *et al.*, 2016). This study aimed to evaluate the physiological effects of some proteinaceous diets prepared using soybean meal, chickpea flour, brewer's dried

yeast, skimmed milk powder, date syrup, turmeric, and fenugreek powders on caged honey bee workers. Then, some parameters: diet consumption, longevity, HPG development, and weight of rectal content were utilized to evaluate the nutritional values and physiological effects of the tested diets.

## Materials and methods

The experiments were done in the laboratory at Bee Research Department, Plant Protection Research institute, Agricultural Research Center, Egypt, during summer from 10 July to 30 August 2018.

### *The Proteinaceous materials*

Seven materials (Table 1) which are rich in their protein content were selected to prepare the pollen substitutes. Total protein percentage of these raw materials was determined by Kjeldahl method (Kirk, 1950). Four proteinaceous mixtures were prepared from the raw materials (Table 2). All diets are new except diet 2 which was prepared according to Abd El-Wahab *et al.* (2016) and consisting of (10g brewer's yeast + 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A, D and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup). Bee bread was used as a control diet. Pollen substitutes were kept in the refrigerator at 4 °C until use.

**Table 1:** Total protein percentage of raw materials used for pollen substitutes.

Raw materials for pollen substitutes	Total protein %
Soybean meal ( <i>Glycine max</i> (L.) Merr.)	39.88 ± 0.13* ab**
Brewer's dried yeast ( <i>Candida tropicalis</i> )	40.57 ± 0.19 a
Skimmed milk powder	28.82 ± 0.19 c
Date syrup ( <i>Phoenix dactylifera</i> L.)	7.55 ± 0.26 f
Wheat germ ( <i>Triticum aestivum</i> L.)	31.58 ± 0.27 b
Chickpea flour ( <i>Cicer arietinum</i> L.)	22.24 ± 0.20 d
Fenugreek powder ( <i>Trigonella foenum-graecum</i> L.)	23.02 ± 0.21 cd
Turmeric powder ( <i>Curcuma longa</i> L.)	9.40 ± 0.15 e

\* ± Standard deviation

\*\*Means followed by the same letter do not differ significantly at the 5% level of probability.

**Table 2:** Description of mixed proteinaceous diets administrated to honey bee workers.

Materials	Composition of the diets / 1 Kg.			
	Diet 1	Diet 2	Diet 3	Diet 4
<b>Soybean meal</b> ( <i>Glycine max</i> )				252
<b>Chickpea flour</b> ( <i>Cicer arietinum</i> L.)			258	
<b>Date syrup</b> ( <i>Phoenix dactylifera</i> L.)	280			
<b>Fenugreek powders*</b> ( <i>Trigonella foenum-graecum</i> L.)		250		
<b>Wheat germ</b> ( <i>Triticum aestivum</i> L.)			86	
<b>Dried skim milk</b>	91			84
<b>Brewer's yeast</b> ( <i>Candida tropicalis</i> )	91	100	86	84
<b>Sugar powder</b>	457	457	429	420
<b>Honey (ml)</b>	17	17	17	17
<b>Water (ml)</b>	64	176	124	143
<b>Total</b>	1000	1000	1000	1000

\* Pollen substitute tested by Abd El-Wahab *et al.* (2016)

### Honey bees

The present study was carried out in experimental cages using newly emerged honey bee (*Apis mellifera carnica* poll.) workers. To obtain the newly emerged bees (0-12 hours), sealed brood combs empty from bee bread were placed in an incubator at  $32 \pm 1$  °C,  $65 \pm 5$  RH in screen cages, and any pollen or honey stored in these combs were covered with aluminium foil or wax (Standifer *et al.*, 1960) to prevent the emerged bees from consuming them.

### Experimental cages

Wooden cages were used in the experiments with dimensions of 15×15×5 cm and with two sides: one was a glass side and the other one was covered with black muslin. Every cage was provided with two vials: one for tap water and other one for sugar solution 1:1 (w/v), and a piece of wax comb was attached to the top of each cage to imitate natural conditions experienced by honey bees (Williams *et al.*, 2013). Eight cages (100 workers per each cage) were devoted to each treatment. The pollen substitutes mentioned above were placed in a small plastic feeder (1 cm. height and 3 cm. diameter) covered with a small sheets of polyethylene and then introduced into each cage. The polyethylene sheets were used to avoid water evaporation from diets as well as any loss due to the contact between caged bees and the diets (i.e. sticking of diets on the legs, wings etc.). Five grams of each diet was placed in the feeders, and the diets were changed every 3 days. All cages were kept in the dark inside incubator (the same conditions inside the hive) at  $32 \pm 1$ °C and  $65 \pm 5$  RH. Additionally, a sample of each diet was placed in cages empty from bees inside the incubator to screen the weight loss, and

then to calculate the daily evaporation rate from each diet type. Then, the daily evaporation rate was subtracted from the consumed amount recorded to each diet to obtain accurate calculations according to Pernal and Currie (2000).

#### *Measurements*

Two experiments were performed: the first one using 20 cages (4 per each group) to estimate daily food consumption by caged bees and worker longevity, and the second experiment using 20 cages (4 per each group) to study the degree of HPG development and the weight of rectal content.

#### *Food consumption*

Food consumption was calculated every 3 days and represented as mg./bee/3 days over 15 days. The number of survived bees was taken into consideration when the amount of diet/cage was calculated according to Schmidt *et al.* (1987).

#### *Workers longevity*

Dead bees in each cage were counted and removed at 3 days interval until 50% mortality of caged bees according to a method of Standifer *et al.* (1960) which results from inadequate protein in the diets. The LT<sub>50</sub> (the time at which 50% mortality occurred) was estimated.

#### *HPG development*

Development of HPG was determined in honey bee workers at age 3, 6, 9, 12 and 15 days old. Ten bee workers were used to represent each age from each treatment. The heads of bee workers were dissected under a binocular in physiological saline solution. Then, the degree of gland development was determined according to Maurizio (1954), using an arbitrary scale (I to IV) to determine the degree of development: grade I (undeveloped gland) and IV (complete development).

#### *Weight of rectal content*

The ten bees from each group per age used for measuring HPG development were also used to determine the weight of rectal content. This was done by pulling the rectum with a fine forceps from each bee worker. Then, placing it on a cover glass and weighting the rectum using an analytical balance according to Al-Qarni (2006).

#### *Statistical analysis*

All the experiments were of completely randomized design (CRD). The analysis of Variance (ANOVA) was performed, and subsequently means were compared using Duncan's multiple range test at 5% level of probability (Duncan, 1955). The probit analysis was used to analyse longevity data. All the analyses were accomplished utilizing the SAS 9.1.3 (SAS institute, 2004).

## Results and discussion

### Food consumption

An ANOVA indicated significant differences ( $P < 0.05$ ) in food consumption for all tested diets. The consumption rates of the tested pollen substitutes are illustrated in Fig. 1. Data revealed that consumption of bee bread in control groups ranked the first ( $P < 0.05$ ). While, the lowest consumption rate was recorded in caged provided with diet 4. The consumption patterns were nearly similar to all the tested diets. The greatest rate of consumption was recorded during the first six days especially during the period from day 4 to day 6, then the consumption decreased sharply and stopped at low level by day 15. These results are concordant with Amro *et al.* (2016) that protein diets were mainly consumed by caged honey bees from ages 1 to 8 days, which is the same age during which bees in a colony perform brood care behaviour, with the highest consumption observed on day 3. The total amount of food consumption per bee during the 15 days was 10.5, 8.3, 7.8, 6.3 and 4.2 mg./bee/3days for bees fed on bee bread, diets 2, 1, 3, 4, respectively. Our results not compatible with Younis (2019), in spite of, he used diet formula based on chickpea flour, wheat germ, dried brewer's yeast as a rich protein source for honey bee colonies. This may be due to the different in application method which reflects the highly consumption rate of the diet in field in comparison to laboratory. Although the variations between the tested diets in their protein percentages, the consumption rates by caged bees were not linked to protein percentages in the diets. Accordingly, Altaye *et al.* (2010) indicated to the nutrient intake of broodless workers was directly related to their own physiological requirements and not based on the protein level in the presented diet. Additionally, bees do not collect pollens based on their protein content (Liolios *et al.*, 2016).

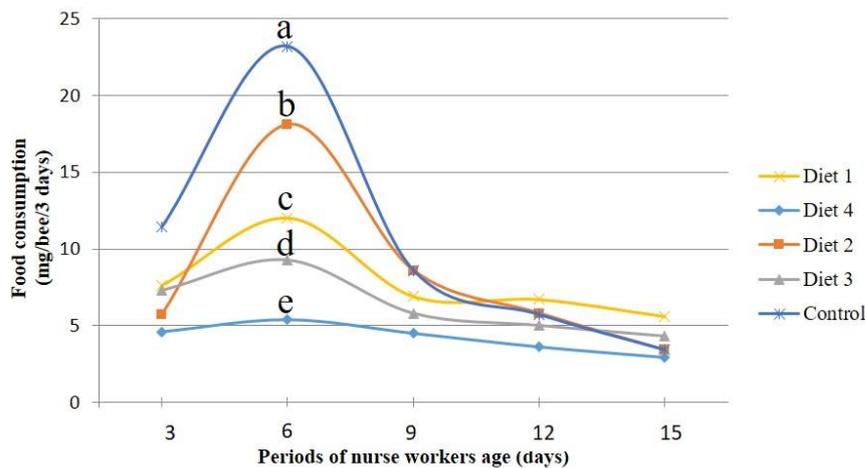


Fig.1: Rate of food consumption by carniolan honey bee workers fed with tested pollen substitutes in cages placed in an incubator at  $32 \pm 1$  °C,  $65 \pm 5$  RH. The letters indicate to the significant differences between means according to Duncan's multiple range test 0.05.

### Workers longevity

The mortality percentages and  $LT_{50}$  are shown in Fig. (2). Data showed that feeding bees on pollen substitutes (diet 4) based on soybean showed the lowest longevity of bee workers ( $LT_{50}$  =21.0 days) in comparison with those fed on the other pollen substitutes. The results indicate that the longevity of bee workers fed on diet 1 (date syrup) and diet 2 (fenugreek powders) was mostly similar with 24.2 and 25.1 days, respectively. When bees fed on diet 3 (chickpea flour) their  $LT_{50}$  extended to 27 days. Chickpea flour contain 21g protein, 53g carbohydrates, 10g crude fibre, 6g fat and 356 calories (Wallace *et al.*, 2016), which may explain the high longevity of workers fed on diet 3. The mortality rates of newly emerged workers fed on different protein sources was found to be related with the material type and their contents of protein (Rinderer *et al.*, 2012). From the obtained results, it appears that the commonly accepted protein sources used as pollen substitute for bees is diet 3. It can be used as protein source for feeding honey bee colonies. The lowest survival was observed in caged bees which fed with soybean meal. In line with Manning *et al.*, (2007), they found low longevity of bees feed on soybean than pollen diets which reflect that protein content of soybean meal is not completely accepted, useful and reflect bad physiological condition for bee workers.

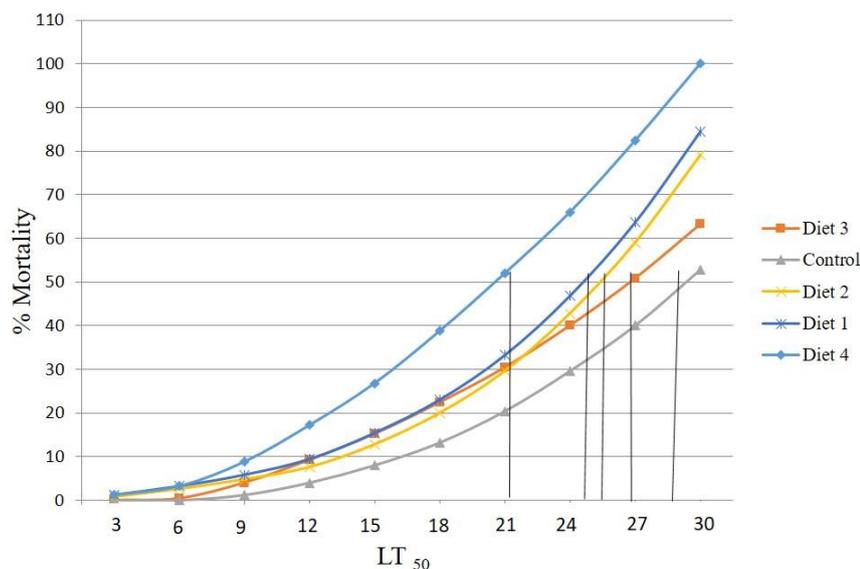


Fig.2: Cumulative mortality percentage and  $LT_{50}$  of Carniolan honey bee workers after feeding with tested pollen substitutes in cages placed in an incubator at  $32 \pm 1$  °C,  $65 \pm 5$  RH.

### HPG development

HPG development of worker bees fed on different pollen substitutes are illustrated in Fig. (3). The results clearly showed the significant differences ( $P < 0.05$ ) in the development degree of HPG between the workers

fed on the tested diets. The acini reached their maximum size when the bees were at age of 2 to 9 days old and then became smaller. It was found that the activation of the HPG was maximal after about eight days in colony conditions but was reduced in the absence of brood and in caged bees (Omar *et al.*, 2017). Our results confirm the positive effect of a high-protein diet on HPG size for bees sampled at day 8, which corresponds to the age of maximum development of HPG in worker bees (Al-Ghamdi *et al.*, 2011). The highest development of HPG was recorded in bees received bee bread (control) with HPG development degree of 3.78 and the lowest one was recorded to diet 4 which based on soybean meal (2.14). The present results indicated that the general means of HPG development degree were 3.24, 2.70, 2.36 and 2.14 HPG development degree for bees fed with diets 3, 2, 1 and 4, respectively. The HPG organs secrete enzymes, royal jelly and respond quickly to any changes in the nutritional value of protein food available for bees. Moreover, De Grandi-Hoffman *et al.* (2010) found that bees fed sugar syrup alone had lower protein concentrations and smaller HPGs compared with the other diets especially as the bees aged. Additionally, the development of HPG is strongly correlated with the amount of protein consumed from diets by bee workers (Pernal and Currie, 2000). Therefore, crude protein (nitrogen content) is an essential dietary component for the development and prosperity of bee colony. In accordance with the present study, Sagili *et al.* (2005) recorded that bees fed 1% soybean trypsin inhibitor (SBTI) had significantly reduced HPG protein content. This confirms the low feeding benefits of diet 4 (soybean meal as the main component).

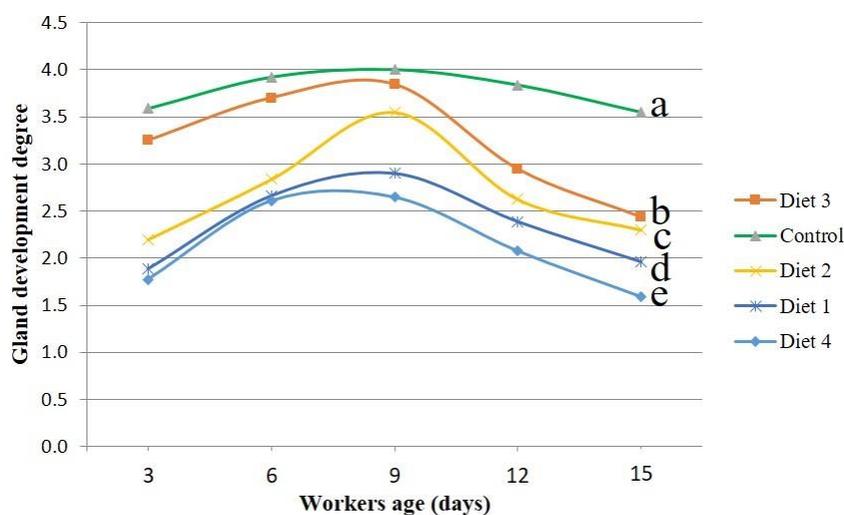


Fig. 3: Hypopharyngeal gland development of Carniolan honey bee workers fed with different pollen substitutes in cages placed in an incubator at  $32 \pm 1$  °C,  $65 \pm 5$  RH. The letters indicate to the significant differences between means according to Duncan's multiple range test 0.05.

### Weight of rectal content

Fig. (4) shows an increase in the weight of rectal contents from bees with age of 3 days up to the highest weight in bees aged 15 days, and this trend was for most tested diets. The lowest weight means of rectal contents were to control group (bee bread) followed by diet 3, suggesting the highest suitability of these diets to bees. The highest weight means of rectal contents were to diet 4 (soybean meal) 18.25 mg/bee and to diet 2 (fenugreek powders) 16.12 mg/bee with significant differences ( $P < 0.05$ ) than the other diets. Honey bees do not tend to defecate inside cages, resulting in the accumulation of waste material in the rectum (Škerl and Gregorc, 2014). Therefore, the accumulation of wastes in the rectum of caged bees is a good indication for the digestion of diets by bees. In the same line De Grandi-Hoffman *et al.* (2016) recorded that nurse bees digested less of the protein in pollen substitutes than the pollen. These findings insured ours, that digestion was high to control diet and diet 3 over the other tested diets, especially diet 4. Accordingly, Amro *et al.* (2016) recorded the highest rectal content weight in bees fed with soybean meal. That reflects unsuitability of soybean protein for honey bees as a pollen substitute.

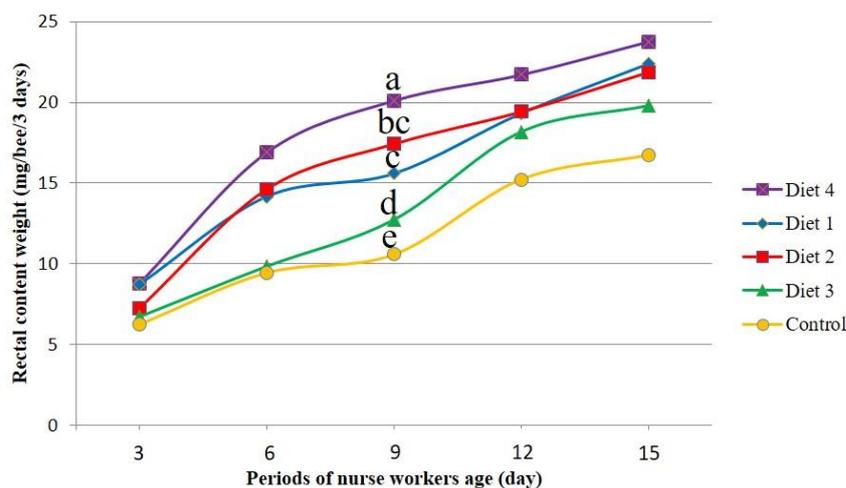


Fig.4: Variation of rectal contents of Carniolan honey bee workers fed with tested pollen substitutes in cages placed in an incubator at  $32 \pm 1$  °C,  $65 \pm 5$  RH. The letters indicate to the significant differences between means according to Duncan's multiple range test 0.05.

### Conclusion

Providing honey bee colonies with protein is very important, especially when no natural protein sources (pollens) are available for them. Among the tested diets, diet 2 was most rapidly consumed by caged bees. Also, this diet enhanced some physiological characteristics of caged bees better than diet 1 (date syrup). In spite of the low consumption rate of diet 3, it had the highest HPG development, the best longevity results,

and the lowest weight of rectal content compared to the other tested diets. Therefore, these two types of diets are recommended to be used by beekeepers when no or few natural pollen sources are available for their bee colonies.

### **Conflict of interest**

It is hereby declared that no competing interest exists among the authors and the authors declare no potential conflict of interest.

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