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Physiological effects of some pollen substitutes diets on caged honey bee workers *Apis mellifera* (Hymenoptera: Apidae)

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Abstract:

Nutritional value of four proteinaceous diets and its physiological effect on honey bee workers were evaluated under laboratory conditions. The tested diets were as follows, diet 1 (Date syrup, skimmed milk powder and dried brewer's yeast), diet 2 (Turmeric, fenugreek powders and dried brewer's yeast), diet 3 (Chick pea flour, wheat germ and dried brewer's yeast) and diet 4 (Soybean meal, skimmed milk powder and dried brewer's yeast). *Apis mellifera* L.(Hymenoptera: Apidae) carnica was used in small groups of caged honey bee workers. The consumption rate, longevity, hypopharyngeal glands development degree (HPG) and rectal contents were determined. The greatest consumption rate was recorded for the control group which fed with bee bread and the lowest one recorded for diet 4. Feeding bees on diet 3 gave the longest LT_{50} (27.0 days) after the controlled bees, which gave LT_{50} (29.0 days). On the other hand, the honey bee workers that fed on diet 4 gave the lowest LT_{50} (20.5 days). The highest degree (3.78 HPG degree) of gland development was recorded at 9 days old in bees fed on bee bread followed by those fed on diet 3 (3.24 HPG degree). Whereas, the lowest developed HPG (2.14HPG degree) was obtained in bees fed on diet 4. The results of rectal content weight of honey bee workers reflect the suitability of diet 3, diet 1 and diet 2 for honey bee workers which recorded 13.43, 16.03 and 16.12 mg/bee/3 days, respectively.Accordingly, the diet 3 had good nutritive value for honey bee colonies which help bees to establish healthy colonies with good production.

Introduction

Pollens are indispensable food for honey bee colonies and their shortage intermittent periods cause several problems for the colonies. Moreover, different pollen types collected from different plant origins could be had differently effects on the physiological conditions of worker honey bee (Amro *et al*., 2015). Completely absence of protein sources in the hive cause starvation for honey bees and consider main reason for colony collapse disorder (CCD) (Seitz *et al*., 2015). So, provide colonies with protein source all over the year, especially during dearth periods of pollen is critical

matter.Recently, several studies gave more attention to formulate supplementary diets or substitutes to compensate the lake ofthe natural protein source (pollen) (Zheng *et al*., 2014; Amro *et al*., 2016; Negri *et al*., 2017; GamalEldin *et al*., 2018 and Gregorc *et al*., 2019).

The presented pollen substitute showed have special specification, i.e. Palatable (Saffari *et al*., 2010), consumption (Doull, 1973), attractiveness (Abd El-Wahab *et al*., 2016) and have a good physiological effectson honey bees (Amro *et al*., 2016). Also, the useful pollen substitute should be stimulating colony growth and support aspects of worker quality, such as large brood areas and the long length of adult stage (Winston *et al*., 1983). Several studies documented many food materials as a suitable pollen substitutes for honey bees. However, Soybean meal (Abbasian and Ebadi, 2002), yeast (Abd El-Wahab and Gomaa, 2005), skimmed milk (Amro *et al*., 2016) and wheat gluten (Nutter *et al*., 2017) can be used as a basic material for preparing pollen substitute cakes. Its high content of protein encourages HPG to stimulate royal jelly secretion and promote honey bee workers to rearing moor brood. However, the roll of these materials to enhance workers physiological condition still lacking. Some pollen substitutes were tested for feeding honey bee workers by Amro *et al*. (2016) and GamalEldin *et al*. (2018). They considered consumption of pollen substitute in the period after worker emergence, development of HPG, longevity and rectal content weight are important criteria for estimating the suitability of proteins diets for honey bee workers.Younis (2006) found that, the Wheat Germ is the best pollen substitute, as it increases the bee's activities, especially in the lack of pollen grains sources. Followed by Dried Brewer's yeast, then Soybean flour and the Palm Date come last. Al-Ghamdi *et al.* (2011) found significant differences in the degree of HPG development when the bees were fed on bee bread, followed by pollen loads and a mixture of yeast, gluten and sugar (1:1:2). The longevity of honey bee workers appears to be directly associated with level of body protein (Sagili *et al*., 2005). De Groot (1953) concluded that protein feeding increases the length of life emerged bees in cages, compared with those fed sugar syrup only. Al-Qarni (2006) consider honey bee workers rectal content weight directly reflects the food suitability. It used to test the variability of food demonstrated to honey bee and its utilization by honey bee colonies. Also, Amro *et al.* (2016) found that, the heist rectal content weight was recorded in caged honey bee fed with corn gluten, while the lowest one was recorded for FeedBee®. These finding reflect the good digestion and full benefits reward from FeedBee® to the workers.

Recently, some commercial pollen substitutes as Feedbee® and Bee-pro® were offered for application. The suitability ofFeedbee® was tested by Omar *et al*. (2017) and recorded that it promotes HPG development better than a protein-free diet but not as good as the pollen mixture or mono-floral pollen from *Asparagus*sp. or *Castanea* sp. Also, Amro *et al.* (2016) concluded that Feedbee® was able to enhance brood rearing activity in despite of present it under isolation condition in full absence of pollen. However, its high price consider barrieragainst its application.

This study was done to evaluate the physiological effects ofsomeproteiounes diet based on soybean meal, chick pea flour wheat germ, dried brewer's yeast, skimmed milk powder, date syrup, turmeric and fenugreek powders. Diet consumption, longevity, HPG development and rectal content weight of caged honey bee workers were used to evaluate nutritive values of the tested pollen substitute diets.

Materails and methods

The experimental research was carried out under laboratory conditions at Department of Apiculture Research, Plant Protection Research institute, Agriculture

research Center, Egypt, during summer season of 2018.

1.Proteinaceous materials :

Seven materials (Table, 1) that are rich in their protein content and are available in local area were selected for testing them as pollen substitute. Total protein% of these raw materials was determined by Kjeldahl method (Kirk, 1950). Four proteinaceous mixtures were prepared from the raw materials (Table, 2). Diet 2 is a pollen substitute tested by 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).Beebread was used as a control diet in the tested cages.

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

Raw materials for pollen substitutes*	Total protein %
Soybean meal (*Glycine max*	39.88± 0.13 b
Brewer's dried yeast	40.57 ± 0.19 a
Skimmed milk powder	28.82 ± 0.19 d
Date syrup (*Phoenix dactylifera*)	7.55 ± 0.26 g
Wheat germ (*Triticum aestivum*)	31.58 ± 0.27 c
Chick pea flour (*Cicer arietinum*)	22.24 ± 0.20 f
Fenugreek powder	23.02 ± 0.21 e

*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
Soybean meal (*Glycine max*)				252
Chick pea flour (*Cicer arietinum*)			154	
Date syrup (*Phoenix dactylifera*)	280			
Diet 2*		1000		
Wheat germ (*Triticum aestivum*)			154	
Dried skim milk	91			84
Brewer's yeast	91		62	84
Sugar powder	457		315	420
Honey (ml)	17		251	17
Water (ml)	64		64	143
Total	1000	1000	1000	1000

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

2. Honey bees:

The present study was carried out in experimental cages using newly emerged honey bee (*Apis mellifera carnica* poll.) workers. Sealed brood combs free from bee bread were placed in an incubator at 32 ± 1 °C, 65 ± 5 RH. in screen cages to obtain newly emerged bees (0-12 hours) to prevent the emerging bees from consuming pollen or honey in the brood comb, all areas of comb containing these materials were covered with aluminium foil wax or both (Standifer *et al*., 1960).

3.Experimental cages:

Experimental wooden cages of 15×15×5 cm. dimensions with a glass side and other side were covered with black muslin. Every cage was provided with a vial of tap water and other vial of 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). Four cages were used for every treatment; each contains 100 workers.The above mentioned pollen substitutes were introduced to each cage, into a small plastic feeder (1 cm. height and 3 cm.

diameter) covered with a small sheets of polyethylene to avoid water evaporation and any food loss which could be occurred at free access of honey bees to the whole (Sticking on the legs, wings, the small hairs on the body, etc.). Each feeder contains an average amount of 5 g. Pollen substitutes stock are kept into the refrigerator at 4 °C until administration. The diets were changed in each cage every 3 days. All cages were kept in the dark in an incubator at 32 ± 1 °C and 65 ± 5 RH. Additionally, the weight loss by one sample of diet without bees was measured to determine the daily evaporation rate. For this calculation, the percentage of evaporation was subtracted from the recorded amount of the protein diet consumed (Pernal and Currie, 2000).

4. Measurements:

The experiment was carried out in two groups, each one consists of twenty cages, daily food consumption of nurse bees and worker longevity were measured in the first group. However, the degree of HPG development and rectal content were measured in the second group.

5. Food consumption:

Daily food consumption was calculated every 3 days by mg./bee/3days until 15 days old. The amount of diet/cage was compared with the number of live bees existing in each cage during the investigation (Schmidt *et al*., 1987).

6. Workers longevity:

Dead bees in each cage were counted and removed every 3 days interval until half of the initial number of honey bee were died (Standife *et al*., 1960). The LT_{50} was estimated. Values in days of bee workers fed with different diets were determined by a computerized probit analysis program using SAS 9.1.3 programme (SAS Institute, 2004).

7. Hypopharyngeal glands development:

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

8.Weight of rectal content:

The same ten bees, used for measuring HPG development were used to determine the weight of rectal content by extracting the rectum with a fine forceps, and placing it on a cover glass, previously weighted and then reweighed on an analytical balance **(Al-Qarni, 2006)**.

9. Statistical analysis:

The experimental design for the all experiments mentioned above were completely randomized design (CRD). ANOVA was performed and means were compared by using Duncan's multiple range tests at 5% level of probability (Duncan, 1955) with the SAS 9.1.3 programme (SAS Institute, 2004).

Results and discussion

1.Food consumption:

The consumption rate of tested pollen substitutes is illustrated in Figure (1). The consumption patterns were quietly similar in all tested diets. During the first six days measurements recorded the greatest rate of consumption especially during the second period (4-6 days).The consumption rate decreased sharply till the appearance of the of the minimum value after 15 days at low level by the 15^{th} days.These results confirmed the findings of Crailsheim *et al*. (1992) who's reported that protein diets were mainly consumed by caged honey bees aged between 1 and 8 days old.Wherever, the same age bees in a colony performed the same brood care behaviour, with the highest consumption observed during day 3. The total amount of pollen substitute consumed per bee throughout 15 days were 10.5 > 8.3 > 7.8 > 6.3 > 4.2mg./bee/3days for bees fed on bee bread, diets 2, 1, 3 and 4respectively.The obtained results revealed significant

differences between all treatments under the experimental condition. Although, the main row material of pollen substitutes was differed in the protein percentages, the pollen substitute consumption did not affect by the protein content of these materials. Schmidt and Johnson (1984) found weak correlation between bees feeding preference and/or the protein level of pollen diets. These findings agreed with present results whereas bees do not increase consumption to compensate the reduction in dietary protein. Also, they suggested that consumption may be influenced by physical or chemical factors that are unrelated to diet quality.

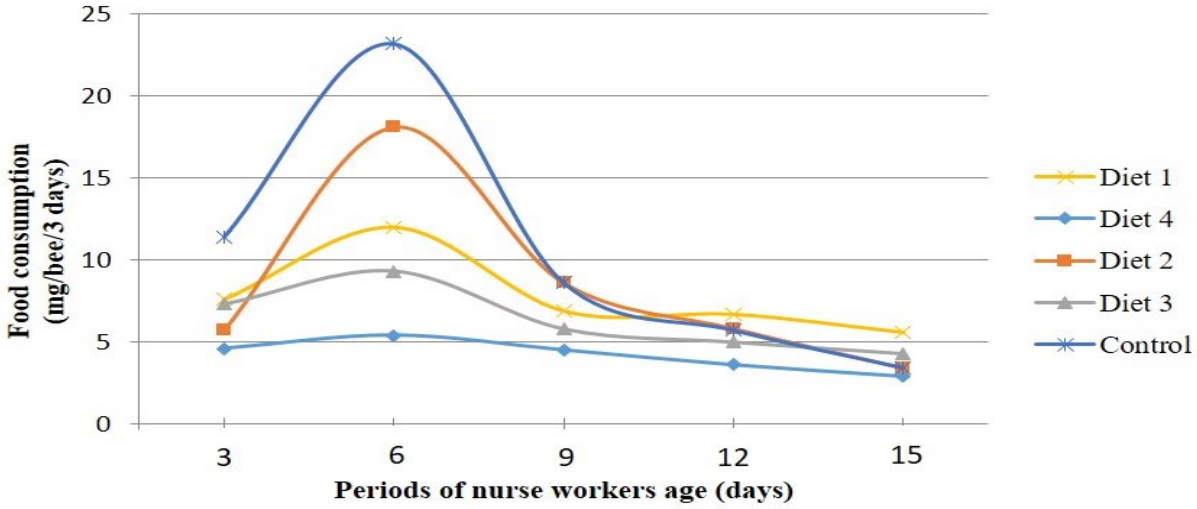

Figure (1) : Rate of food consumption by Carniolan honey bee workers fed on tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

2. Workers longevity:

The mortality percentages and LT_{50}were illustrated in Figure (2). Data revealed that feeding bees on pollen substitutes contained soybean fell short the longevity of honey bee workers (LT_{50}, 21.0 days) in comparison with those fed on other pollen substitutes. The results indicated that the life span of honey bee workers fed on diet 1 (date syrup) and diet 2 (Fenugreek powders) was quietly similar and recording 24.2 and 25.1 days respectively. Bees fed on diet 3 (chick pea flour+ wheat germ) presented LT_{50}reached to 27 days. Finding of Younis (2006) which proved that, feeding with Wheat Germ gave the longest bees average life. While the bees fed on Soybean flour gave the shortest average life, Wallace *et al*. (2016) showed that, chick pea flour contains 21g protein, 53g carbohydrates, 10g crude fibre, 6g fat and 356 calories, may be reflect the high longevity of workers fed on diet 3 during the present work. 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. From the obtained results, it appeared that the commonly accepted protein sources used as pollen substitute for bees is diet 2. This diet can be used as protein source for feeding honey bee colonies mixed with other ingredients such as yeast, skimmed milk and pollen. On the other hand, the lowest survival was observed in caged bees which fed on soybean meal.In this approach, Manning *et al*. (2007) studied soybean effect on bees longevity. They reported that, soya bean flour had the lowest oleic acid concentration and was the best in giving bees greater longevity, but it was still worse than pollen diets.

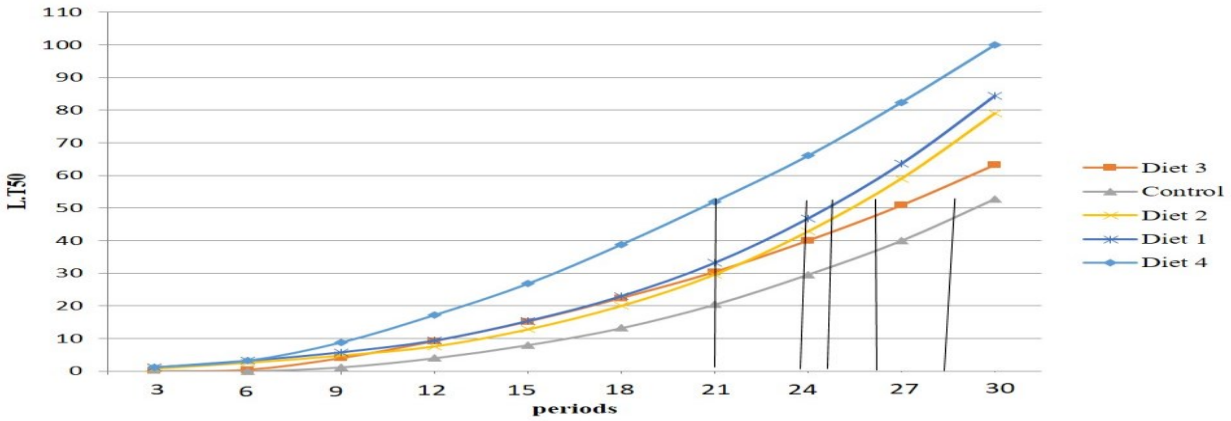

Figure (2): Cumulative mortality percentage and LT50 of Carniolan honey bee workers after feeding on tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

3. Hypopharyngeal glands development:

Hypopharyngeal glands (HPG) development of worker bees fed on different pollen substitutes are illustrated in Figure (3). The result clearly showed significant differences in the development of HPG between the workers fedon different Proteinaceous diets.The acini reached their maximum size when the bees are 2 to 9 days old and then became smaller. This confirms previous findings in colonies or cages (Altaye *et al.* , 2010). The highest development of HPG was recorded in bees received bee bread (control) (3.78 HPG development degree) and the lowest one was recorded by bees received diet 4 which containedsoybean meal (2.14 degree). The present results indicated that the general means of HPG development degree were 3.24>2.70> 2.36>2.14HPG development degree for bees fed diets 3, 2, 1 and 4 respectively.According to Huang (1990) the HPG organs secreting enzymes and royal jelly, quickly respond to changes in the nutritional value of feed protein. This means that the development of HPG was strongly correlated with amount of protein consumed by honey bee workers from presented diets (Pernal and Currie, 2000). Therefore, crude protein is an essential dietary component for the development and will being of bee colony. In the same line, Sagili *et al*. (2005) recorded that bees fed 1% soybean trypsin inhibitor (SBTI) had significantly reduced HPG protein content. They also concluded that nurse bees fed a pollen diet containing at least 1% SBTI would be poor producers of larval food, potentially threatening colony growth and maintenance. The supply of soybean meal caused an abrupt reduction in pupae emergence, attributing this to the lack of niacin amino acid in soybean meal (Haydak, 1949).

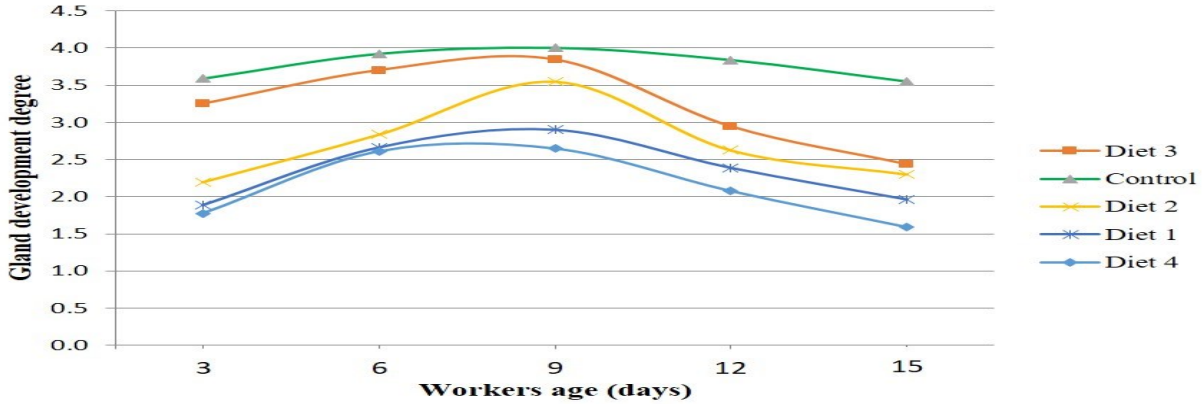

Figure (3): Hypopharyngeal gland development of Carniolan honey bee workers fed on different pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

4.Weight of rectal content:

Figure (4) showed that for most of the tested diets the rectal contents started with low weight on the first inspected age (3-days), then grew up to reach the highest weight at the eldest age (15 days). This result explain the progress of workers appetite with the age progressing. The lowest means of workers rectal contents weight showed that values of bee bread as a most suitable food for honey bees. The highest general mean was presented by the bees fed on diet 4 (soybean meal) with an average of 18.25mg./bee/3 daysand on diet 2 (Fenugreek powders) 16.12mg./bee/3 days with significant differences from the means of honey bee workers fed on other diets. Diets 3 contains chick pea flour+ wheat germ ranked the second after control cages followed by date syrup diet recording 13.43 and 16.03 mg./bee/3 days, respectively. As honey bees do not defecate in the cage, the accumulation of waste material in the rectum, depending on other components present in each protein source, may lead to the premature death of caged workers (Maurizio and Hodges, 1950). This may contribute to the higher rectal content weigh in bees fed diet (4). These results were in the same line with those obtained by Amro *et al.* (2016) which recorded the highest rectal content weight in bees fed with soybean meal.

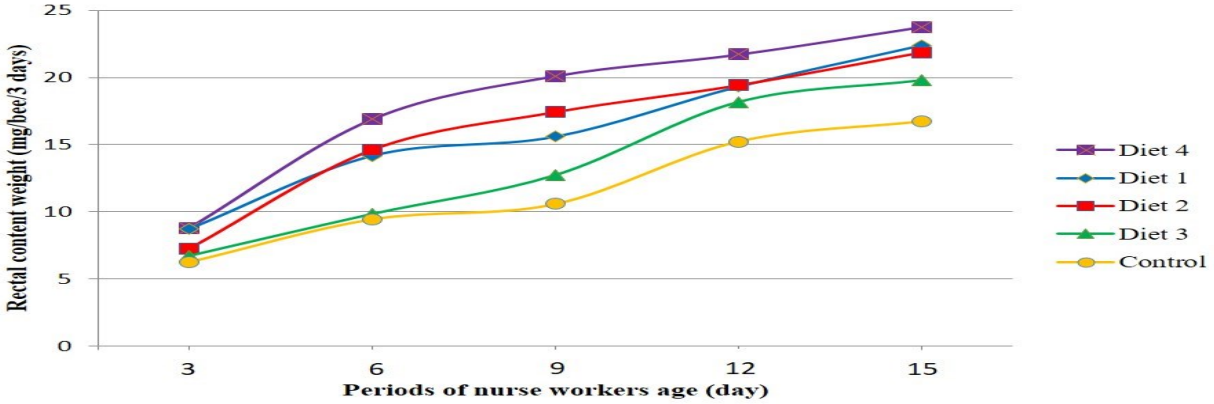

Figure (4):Variation of rectal contents of Carniolan honey bee workers fed on different tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

Since providing honey bee colonies with protein is very important, especially when no natural protein sources (pollens) are available for them. Using diet 2 was found tobe consumed rapidly other than tested diets. Also, itis successful to enhance some physiological characteristics of honey bees better than date syrup. Although, diet 3 was not consumed rapidly by the bees, it had the highest HPG development, pest longevity and the lowest rectal content weight compared to the other tested diets. While,diet 4 was not good in regard to the investigated parameters, and this diet is not recommended. Beekeepers are advised and diet 3 when no or few natural pollen sources are available for their bee colonies.

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