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**EFFECT OF FEEDING HONEYBEE COLONIES WITH  
SOME POLLEN SUBSTITUTES ON DEVELOPMENT OF  
HYPOPHARYNGEAL GLANDS IN ASSIUT  
GOVERNORATE**

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**ABSTRACT**

The present work was conducted to study the possible development that may take place to the hypopharyngeal gland of honey bee workers at different ages under the feeding of some diets pollen substitutes of diet A (broad bean 30% + sorghum 20% + powdered sugar 50%), diet B (soybean 30% + maize 20% + powdered sugar 50%) and diet C (brewer's yeast 15% + chickpea 15% + Soybean 20%+ powdered sugar 50%) . Data showed clearly that there were significant differences in the mean amount of pollen substitutes consumed by honeybee colonies according to the type of pollen substitutes. The highest mean amounts of food consumption were obtained from colonies fed on diet C during all the experimental period. The highest mean numbers of lobules/1mm of the hypopharyngeal glands obtained recorded from colonies fed on diet A followed by those fed on diet B then diet C, while the lowest ones were recorded of (control) group during the experimental period. Concerning the average acini surface area of the HPG of workers fed on the different diets depended upon the type of diet, and the age of workers. The highest average acinal surface area was obtained from control colonies fed on bee bread followed by the colonies fed on diet

C then diet B, while the lowest level were recorded on those fed diet A.

**Key words:** Honeybee – pollen, substitutes, feeding, hypopharyngeal, glands

### **INTRODUCTION:**

Honeybees like other insects have usual nutritional requirements of carbohydrates, proteins, fats, minerals, water and vitamins. Their chief food consists of pollen and nectar or honey. Nectar and honey are the main sources of carbohydrates and water. Pollen furnishes all the other nutrients. Pollen is an indispensable food to honeybee colonies and is necessary for growth and development of young bees, rearing larvae and reproduction (Keller *et al.*, 2005). HPG are located in the head of a honeybee worker and queen but only develop in workers.

The hypopharyngeal glands in worker bees in special are of great important and interest due to their secretion (royal jelly) that is the main food for raising honeybee brood and queens. These glands are the backbone of the development of honeybee colony (Silva de Moraes, and Bowen, 2000). Honeybee nurses possess large HGs, which enlarge their volume until about day 10 after adult eclosion and shrink after the onset of foraging day 15 (Deseyn and Billen, 2005). In normal condition they are well developed when bees are nursed and they degenerate when bees become foragers. It depends on age of workers, the colony conditions and the time of the year. The effect of worker age on the hypopharyngeal glands development was examined by (Huang

and Otis, 1989). (Alqarni 2006) evaluated some proteinic diets for supplementary feeding of honeybee, the highest rate of food consumption was recorded with improved traditional substitute followed by mixture from date palm pollen and soybean flour, the bee bread or date palm pollen was the best sources for hypopharyngeal gland activation. Seasonal food consumption rates of honeybees fed different artificial diets were evaluated by (DeGrandi-Hoffman *et al.* 2008) and (Al-Ghamdi *et al.* 2011). The diameters of HPG are often used to describe the physiological status of worker honeybees. The normal course of development of these glands (size of acini) is well known (Crailsheim and Stolberg, 1989).

This study focuses on the effects of different pollen substitutes diets on honeybee workers, food consumption rates mean number of lobules the hypopharyngeal gland and acinal surface area.

### **MATERIALS AND METHODS**

The present work was conducted in a private apiary located at Dairut district, Assiut Governorate during August and September of 2014, to study the possible development that may take place to the hypopharyngeal gland of honey bee workers at different ages

under the feeding with some pollen substitutes diets.

**Experimental honey bee colonies:**

Twelve colonies of the hybrid Carniolan bees (*Apis mellifera carnica*) were selected among the colonies of the apiary. The experimental colonies were nearly equalized in strength and having

sister queens. The tested colonies were divided into 4 groups, three colonies of each group. The colonies of the groups were provided with pollen traps and each colony provided with different artificial diet which presented in Table (1), while the fourth group without pollen trap served as control.

Table (1): The protein composition of the tested diets

Diet code	Constituents	Protein %
Diet (A)	Broad bean 30% + sorghum 20% + powdered sugar 50%.	6.68
Diet (B)	Soybean 30% + maize 20% + powdered sugar 50%.	9.19
Diet (C)	Brewer's yeast 15% + chickpea 15% + Soybean 20%+ powdered sugar 50%.	11.00

**Determination of protein content:**

The protein content was determined by using Kieldahl method according to (Ashton 1936) . The results were presented in Table (1).

**Diet consumption rate (gm. /week):**

Each colony received 100 gm./week, of each diet described above in Table (1) for 36 days .The diet was supplied in a cake formed directly above the brood nest of the tested colonies and covered with plastic sheets to avoid drying, after one week the supplied diets were weighted again and the difference between the original weight and the current weight was calculated as the consumed portion .

**Hypopharyngeal gland measurements:**

Sixty newly emerged workers from each group were marked by certain color and left in their colonies until 3 to 15 days old and used to determine the mean number of lobules

/1mm of the hypopharyngeal glands and acinal surface area.

According to the methods of Wang and Moeller (1969) and Yousef-Khalil (1992) , the hypopharyngeal gland was laid bare with a level cut through the head using a razor blade, and placed in a glass slide into a droplet of sodium chloride solution 0.85% (isotonic to the heamolymph) the glands were not covered by cover glass , the gland was taken out from the front region of the head, and lined up in drops of saline solution on a glass slide to investigate under the microscope . The mean numbers of lobules/1mm of the hypopharyngeal gland for each worker were counted under a binocular by the utilization of microscopic micrometer slide. The mean numbers of lobules /1mm were calculated. Hypopharyngeal gland was dissected to determine the HPG development degree and acini surface at 3, 6, 9, 12, 15, days old.

The maximum length and width of ten acini were measured in mm for each worker under stereomicroscope using a micrometer eye piece. Stereoscope microscope provided with ocular micrometer lens was used for this purpose. The recorded measurements were modified according to power of ocular and objective lenses used. Acinal surface area was calculated according to Maurizio (1954) formula:

$$\text{Acinal surface area} = \pi \frac{a \times b}{2}$$

Where a = maximum length,      b =  
maximum width,       $\pi = 3.14$ .

#### **Statistical Analysis:**

The obtained data were subjected to one way analysis of variance and difference among treatments mean were tested through using least significant difference test according to method of (Mead *et al.* 2002).

#### **RESULTS AND DISCUSSION:**

Data tabulated in Table (2) showed clearly that there were significant differences in the mean amounts of pollen substitutes consumed by honeybee colonies.

It is obvious that total consumption during the experiment was significantly different between the diets A, B and C where the current results (283.67, 315.33 and 356.00 gm./colony), respectively. The mean amount of food consumption was higher significantly in colonies fed on diet C (71.20 gm./colony/week) compared with colonies fed on diet B and diet A (63.07 & 56.73 gm./colony/week), respectively. These results were related with the type of pollen substitutes.

The present result was in agreement with Hrasnigg and Crailsheim (1998) which showed that the highest percentage of pollen consumption by honeybee workers was recorded at 3-10 days old. This percentage decreased sharply at 12 days old. Stace and Hayter (1994) indicated that a fat content of 6-10 % in protein substitutes could increase consumption. Alqarni (2006) found that some proteinic diets for supplementary feeding of honeybee. The highest rate of food consumption was recorded with improved traditional substitute followed by mixture from date palm pollen and soybean flour. The bee bread or date palm pollen was the best sources for hypopharyngeal gland activation. Hossein and Rahim (2013) evaluated eight different protein sources in honey bee nutrition i.e. lentil flour, soybean flour, soybean meal, bread yeast, wheat gluten, skim milk powder, fish meal and Pollen. Field experiments showed a significant difference between feed intake in experimental colonies. Honey bees consumed the most amounts of pollen cakes, while bread yeast and soybean cakes were consumed more than soybean meal and wheat gluten; however, fish meal cakes were not accepted by bees. Overall, the present results showed that soybean flour and bread yeast can be used as pollen supplements and substitutes cakes. Hebert and Shumanuki (1980) stated that the food consumption was clearly stimulated by adding the pollen to the mixed diets for honeybee. The results indicated that the type of basic material used in diet greatly affected the food

consumption rate. To ensure suitability of proteinic diets for developing hypopharyngeal glands during period of pollen scarcity, the glands development evaluated by measuring the degree of

numbers lobules the hypopharyngeal gland and acinal surface after the honeybee workers had been fed on some proteinic diets.

Table (2): Mean amounts of pollen substitutes consumed by honeybee colonies (gm/colony/week).

Times of the feeding	Diet consumption (gm. /colony) /week			
	Diet (A)	Diet (B)	Diet (C)	L.S.D
17/8/2014	52.67 <sup>B</sup>	56.33 <sup>AB</sup>	61.33 <sup>A</sup>	5.65
24/8/2014	54.67 <sup>C</sup>	62.00 <sup>B</sup>	71.67 <sup>A</sup>	5.37
31/8/2014	56.67 <sup>B</sup>	62.67 <sup>B</sup>	71.67 <sup>A</sup>	6.10
07/9/2014	60.33 <sup>C</sup>	67.00 <sup>B</sup>	75.00 <sup>A</sup>	5.45
14/9/2014	59.33 <sup>C</sup>	67.33 <sup>B</sup>	76.33 <sup>A</sup>	6.63
Total	283.67 <sup>C</sup>	315.33 <sup>B</sup>	356.00 <sup>A</sup>	16.26
Mean	56.73 <sup>C</sup>	63.07 <sup>B</sup>	71.20 <sup>A</sup>	3.25

Notes: For diets composition see table (1) . Means within the same letter are insignificantly different ( $P \leq 0.05$ ) according to LSD test

Concerning the effect of feeding of pollen substitutes on the mean numbers of the lobules/1mm of the hypopharyngeal glands data presented in Table (3) revealed that the mean numbers of the lobules /1mm were 32.33, 31.78, 29.06, 26.19 lobules/Imm of the hypopharyngeal glands in worker fed on diets A , B , C and control, respectively at the age 3-days, and were

27.94, 26.47, 23.72 and 22.94 of diets A, B, C and control, respectively, where at the age 9 diet B recorded the highest number (24.11) lobules /1mm, followed by diet A (24.08) then diet C (21.64), while control recorded the lowest number (20.78), whoever at 12 and 15 days diet A recorded the highest one then diet B then diet C and control recorded the lowest number.

Table (3): Effect of feeding honeybee colonies with some pollen substitutes on average numbers of lobules of hypopharyngeal gland

Diet code	Average numbers of the lobules the hypopharyngeal gland in (1mm)					L.S.D
	3-days old	6-days old	9-days old	12-days old	15-days old	
Diet (A)	32.33 <sup>aA</sup>	27.94 <sup>abB</sup>	24.08 <sup>acC</sup>	24.81 <sup>acC</sup>	33.14 <sup>aA</sup>	1.66
Diet (B)	31.78 <sup>aA</sup>	26.47 <sup>bbB</sup>	24.11 <sup>acC</sup>	24.44 <sup>acC</sup>	31.11 <sup>baA</sup>	1.18
Diet (C)	29.06 <sup>baA</sup>	23.72 <sup>cbB</sup>	21.64 <sup>bcC</sup>	21.25 <sup>bcC</sup>	28.94 <sup>caA</sup>	1.53
Control	26.19 <sup>caA</sup>	22.94 <sup>cbB</sup>	20.78 <sup>bcC</sup>	20.17 <sup>bcC</sup>	26.89 <sup>daA</sup>	1.16
<i>L.S.D</i>	1.81	1.45	1.33	1.12	1.43	-

Notes: For diets composition see table(1) .Within the same column (small letter) and rows (capital letter) are insignificantly different ( $P \leq 0.05$ ) according to LSD test.

These results were supported by those obtained by El-Barbary (1980) who found that the degree of development of hypopharyngeal gland was nearly about equal in workers fed fresh or stored bee collected pollen, soybean meal and brewer's dried yeast at any level of concentration. Hassan and Khater (2006) noticed that, the maximum development of hypopharyngeal glands was achieved between 6-12 days for all diets used as pollen substitutes.

Data obtained in Table (4) showed that the average acinal surface area of HPG were 0.0255, 0.0257, 0.0294 and 0.0383 mm<sup>2</sup> in diets A, B, C, and control, respectively at 3 days-old .The same table showed that the average

acinal surface area at 6 days-old were 0.0379, 0.0408, 0.0518, and 0.0542 mm<sup>2</sup> of diets A , B , C and control, respectively, where at 9 days-old was 0.0449, 0.0494, 0.0669 and 0.0711 mm<sup>2</sup> of diets A , B , C and control, respectively. The highest the average of acinal surface area of 12 days-old were at workers fed on control 0.0769 mm<sup>2</sup>, followed by diet C 0.0696 mm<sup>2</sup>, then diet B 0.0575 mm<sup>2</sup> ,while the lowest average was on diet A 0.0505 mm<sup>2</sup>. The same results was at 15 days-old when the control recorded the highest area 0.0504 mm<sup>2</sup> , followed by diet C 0.0465 mm<sup>2</sup>, then diet B 0.0362 mm<sup>2</sup> ,while the lowest average was on diet A 0.0297 mm<sup>2</sup>.

Table (4): Effect of feeding honeybee colonies with some pollen substitutes on average of hypopharyngeal glands acinal surface area.

Diet code	Average acinal surface area (mm <sup>2</sup> ) of hypopharyngeal glands					L.S.D
	3-days old	6-days old	9-days old	12-days old	15-days old	
Diet (A)	0.0255 <sup>bE</sup>	0.0379 <sup>bC</sup>	0.0449 <sup>bB</sup>	0.0505 <sup>dA</sup>	0.0297 <sup>cD</sup>	0.004
Diet (B)	0.0257 <sup>bD</sup>	0.0408 <sup>bC</sup>	0.0494 <sup>bB</sup>	0.0575 <sup>cA</sup>	0.0362 <sup>bC</sup>	0.005
Diet (C)	0.0294 <sup>bD</sup>	0.0518 <sup>aB</sup>	0.0669 <sup>aA</sup>	0.0696 <sup>bA</sup>	0.0465 <sup>aC</sup>	0.005
Control	0.0383 <sup>aD</sup>	0.0542 <sup>aC</sup>	0.0711 <sup>aB</sup>	0.0769 <sup>aA</sup>	0.0504 <sup>aC</sup>	0.0057
L.S.D	0.0048	0.006	0.0048	0.0039	0.0042	-

Notes: For diets composition see table(1) .Within the same column (small letter) and rows (capital letter) are insignificantly different ( $P \leq 0.05$ ) according to LSD test.

Statistical analysis showed highly significant differences between acinal surface area of hypopharyngeal glands in fed colonies on different diets and control. These differences may be due to the variation in protein content of the diets, which affected acinal surface area of hypopharyngeal glands. In this concern, Maeicka (1987), Darhous

(1990) and Attallah *et al.* (1995) reported that the rate or hypopharyngeal gland development seemed to be related to the protein content of the diet . Wrigley *et al.* (2006), Crailsheim and Stolberg (1989) reported that workers fed on diets containing protein additives had longer acini in HPGs lobules. The acinal surface increased with age until a

maximum of 12-15 days post emergence. The present results were in agreement with many authors.

They described that soybean flour; dried yeast and skim milk were the most suitable substance used for supplementary feeding of honey bee colonies after pollen Standifer *et al.* (1973). Szymas and Przyby (1996) found that supplementary feeding with pollen substitute contains soybean flour and yeast activated the hypopharyngeal gland as bee bread.

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## الملخص العربي

### تأثير تغذية طوائف نحل العسل على بعض بدائل حبوب اللقاح على تطوير غدة الغذاء الملكي في محافظة أسيوط

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أجريت هذه التجربة لدراسة التطوير المحتمل الذي قد يحدث للغدة التحت بلعومية لشغالات نحل العسل في أعمار مختلفة تحت تأثير التغذية ببعض بدائل حبوب اللقاح المختلفة. تم دراسة ثلاث أنواع من وجبات بدائل حبوب اللقاح والتي تتكون كل منها على النحو التالي : الوجبة الأولى: دقيق فول بلدى 30% - دقيق ذرة رفيعة 20% - سكر بودرة 50% . الوجبة الثانية: دقيق فول صويا 30% - دقيق ذرة شامية 20% - سكر بودرة 50% . الوجبة الثالثة: خميرة بيرة غير نشطة 15% - دقيق حمص 15% - دقيق فول صويا 20% - سكر بودرة 50% .

#### وأظهرت النتائج ما يلي :

كان محتوى الوجبات الغذائية من البروتين في الوجبة الثالثة أعلى نسبة ، تليها الوجبة الثانية بينما كانت الوجبة الأولى تحتوى على أقل نسبة من البروتين. وفيما يخص استهلاك الوجبات فكان هناك اختلافات معنوية في متوسط كمية بدائل حبوب اللقاح المستهلكة من قبل طوائف نحل العسل حيث كانت الوجبة الثالثة أعلى وجبة من حيث الاستهلاك ، تليها الوجبة الثانية ، بينما كانت الوجبة الأولى أقل وجبة تم استهلاكها على مدار فترة الدراسة.

وعند التحدث عن تأثير التغذية بالوجبات السالفة الذكر للشغالات ذات عمر 3-6-9-12-15 يوم على عدد فصوص غدة الغذاء الملكي لكل 1 ملليمتر ، فسجلت الطوائف التي تغذت على الوجبة الأولى أكبر عدد من الفصوص ، في جميع الأعمار تليها الوجبة الثانية ، ثم الوجبة الثالثة ، بينما سجلت الطوائف الكنترول التي تتغذى على خبز النحل أقل عدد من الفصوص. أما مساحة الفص الواحد من غدة الغذاء الملكي فسجل أعلى نسبة في الطوائف الكنترول تليها الطوائف التي تغذت على الوجبة الثالثة ثم الوجبة الثانية بينما سجلت الطوائف التي تغذت على الوجبة الأولى أقل مساحة للفص الواحد في غدة الغذاء الملكي في الأعمار السالفة الذكر.

**كلمات مفتاحية:** نحل العسل – حبوب اللقاح – البدائل – التغذية – غدد الغذاء الملكي.