

## INFLUENCE OF Pollen Substitutes ON LONGEVITY AND HYPOPHARYNGEAL GLANDS OF CAGED HONEYBEE WORKERS ( *Apis mellifera* L. )

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### ABSTRACT

The present study was carried out to evaluate the affect of some supplementary feeding on longevity and hypopharyngeal glands of caged honey bee workers . The experiment was performed in the apiary and laboratories of Agricultur Faculty of Assiut, EL – Azhar University , during 2005 season .

**The tested diets were:** tow parts corn flour + one part + defatted soya bean flour

tow parts bean flour- one part + date paste : one part skimmed milk

10 % Prewer,s :three parts chick pea flour + one part skimmed milk bee - bread

**The data could be summarized as follows :**

The full time for the tested diets was the longest (31 days) for the bees fed on bee bread followed by that composed of 10% brewers : three parts chick pea flour : one part skimmed as the survival period recorded (24 days) .

The longevity of caged bees fed on the mixtures consisted of tow parts corn flour+one part deffated Soya been flour was (18 days) while other bees lived shorter periods (15 days) when fed on diet consists of tow parts bean flour : one part dute paste : one part skimmed milk .

The development of the hypo pharyngeal glands increased gradually since emergence of the workers until reaching the maximum measurements on 6-12 th day of worker age .

It was found that the workers fed on bee bread gave the highest mean acinal surface area followed by that composed of 2 parts corn flour : 1 part deffated Soya been flour then that composed of 2 parts bean flour : 1 part date paste : 1part skimmed milk .

While the lowest acinal surface area was detected in caged bees fed on 10 % brewers : 3 part chick pea flour+ 1 part skimmed milk.

Generally , the workers fed on bee bread was the best in both cases (the longevity of caged bees and development of Hypo pharyngeal glands of caged bees ) .

### INTRODUCTION

The natural food of honeybees is composed commonly of two components , i.e. pollen and honey or nectar . pollen is the main source of proteins, amino acids, vitamins etc....., whereas hony and nectar represent the main sources of carbohydrates . In early spring before potlen and nectar are available or in other times of the year when these materials are in short supply , supplementary feeding may help the colony to survive or to make to more populous and productive . As modern land-use practices reduce dependable nectar and pollen supplies, the need for supplemental food becomes more urgent for subsistence and continued brood rearing until nectar and pollen can be collected (Standifer, 1967). when pollen is not available , the colonies are fed pollen supplements or substitutes to develop and maintain colonies with optimum populations for nectar flows, autumn and spring divisions , queen and backage-bee production and wintering .

The present work aimed to evaluate some diets as Pollen Substitutas on Longevity and Development hypopharyngeal glands of caged honeybee workers Collected from carnica hybrid colonias .

## MATERIALS AND METHODS

This study was conducted in Dept. Of plant protection , Faculty of Agric. Assiut, AL-AZHAR University during 2005 seasons.

### 1- Tested workers :

Not less than 200 Newly emerged workers (0-6 hrs) from carnica hybrid colonias were subjected for the present study the tested bees were obtained by incubating sealed brood combs at  $34 \pm 1^\circ \text{C}$  and  $80 \pm 5\% \text{RH}$  for 6 hours .Thereafter , the emerged workers were collected , to prevent the emerging bees from consuming any pollen or honey in the brood combs.

### 2-Tested diets:

The test were offered to each cage of bees in cakes form prepared by mixing the diets with sugar syrup (1:1) .The tested diets were :  
The first diet was tow parts corn flour + one part deffated soya bean flour, while the scond was tow parts been flour : one part date paste : one part skimmed milk, and the, while the third parts defatted soya bean flour + one brewer's dried yeast+ one skimmed milk, and the fourth was bee-bread as control which collected from the bee colonies.

### 3- Experimental cages used :

Experimental wooden cages of 15x15x5 cm dimensions with a glass side and in other side the cage was covered with black muslin .Every cage was provided with a vial of tap water and other vial of sugar solutions 1:1 (w/v) and a pieces of wax comb were attached to the top of the cage . Four cages (each contains fifty workers) were used for every tested material .

The past - like diets were introduced for each cage , into a small plastic feeder (1 cm highest and 3 cm diameter) .Each feeder contains an average amount of 5 gm from each diet.Stock diets were kept in the refrigerator at  $4^\circ \text{C}$ , until used . The diets were changed in each cage every threedays . All cages were kept in the dark in an incubator at  $32 \pm 1^\circ \text{C}$  and  $65 \pm 5\% \text{RH}$  .

### 4- Determenation of Longevity of caged bees :

Fifty newly emerged honeybee workers were confined all together and offered the tested diets in the forementioned cages . All cages (4 cages in each treatment) Were inspected dialy to count and eliminate the dead bees and The counts continued until all bees died.The mortality percentage was calculated from the beginning of the experiments and continued till its end .

### 5- Mesurments of The development of the hypopharyngeal glands of caged bees:

The development of the hypopharyngeal glands was measured after 3,6,9,12,15 and 18 days of feeding bees on the tested diets . The glands were dissected out in saline solution (0.9% na cl) , then maximum length and width of 20 acini were measured for each worker in each experimental cage.



Stereoscope microscope provided with ocular micrometer lens was used for this purpose. Thereafter, the recorded measurements were modified according to the power of ocular and objective lenses used. acinal surface area was then calculated according to Maurizio's (1954) formula .

$$\text{Acinal surface area} = X n \frac{A \times B}{2}$$

Where A= maximum length , B= maximum width , =3.14

## RESULTS AND DISCUSSTION

### I-Effect of the tested diets on the longevity of caged honeybee workers :

Data presented in Table (1) indicated that the mean length of life LT50 (the time passed until 50% of workers died) 10,9,7,12 days for honeybee workers fed on diets were 1,2,3 and 4 respectively .The length of life (full time) of caged honeybee worker was longest (31days) in worker fed on bee bread (diet 4) followed by workers fed on diet 1 (24 days) and diet 2 (20 days), while the workers fed on diet 3 lived for shorter periods of time (19 days) .In general , it could be concluded that the longevity of bees seems to be related to diet constituents , as the larger longevity was observed when

**Table 1 : Longevity of caged honeybee workers fed on tested diets , expressed as LT 50 & LT 100**

Diets	LT 50 (days)	% of control	Full time (days)	% of Control
1*	10	83.33	24	77.42
2**	9	75.00	20	64.52
3***	7	58.33	19	61.29
4****	12	100.00	31	100.00

T 50 = The time passed until 50% of workers died

\* 1- 2 parts corn flour + 1 part defatted soya bean flour .

\*\* 2- 2 parts been flour : 1 part date paste : 1 part skimmed mil

\*\*\* 3- 3 parts defatted soya bean flour + 1 brewer's dried yeast + 1 skimmed milk .

\*\*\*\* 4- bee-bread ( control )

bee bread are used in this respect , Haydak (1937) reported that feeding newly emerged bees on sucrose solution only caused 59% mortality during the21-day period as compared to 12.7% in control bees fed on protein source (fresh pollen) . Louveaux *et al.* . (1977) found that the average half life of bees was significantly increased by adding 1% corn sleep liquor to the syarup. They attributed this increase to the high contant of the liquor from nitrogen (protein and amino acids) , vitamins and minerals . Macicka (1987) reported that honey bees fed with carbohydrate feeds containing protein additives had longer life than bees fed on diets without protein additives. Also, Darhous (1990) found that the corn flour and cooking potato cakes proved to be satisfactory sole sources for the length of life of caged bees . Corn flour with defatted soya bean flour or chickpea flour and finely ground wheat bran gave good results . On the other hand , Mostafa (2000) found that the mortality rates of honeybee workers fed on different proteinic mixture

diets gave the longest LT 50 being 53.89 days , while the honeybee workers fed on bee- bread gave LT 50 29.20 days .

## II- Effect of the tested diets on the development of the hypopharyngeal glands

The development of the hypopharyngeal (hp) glands was studied in caged honeybee workers aging 3,6,9, 12, 15, 18days by measuring the maximum length and width as well as the surface area of 20 glands acini from each worker of the samples ( 50 workers / treatment for each age )

### 1- Three day old workers :

Data presented in Table (2) indicated that the longest significant mean acinal length was recorded for workers fed on bee – bread (0.1259 mm), while the lowest significant one was recorded for workers fed on diet 2 (0.1164 mm) Workers fed on diets 1,4 show similar acinal width being 0.0865mm, while this parameter recorded 0.0708 and 0.0852mm for workers fed on diets 2 and3 ,respectively . The recorded means of acinal surface area of three - day old workers were 0.0166, 0.0129,0.0162 and 0.0171mm<sup>2</sup> for workers fed on diets 1,2,3 and bee – bread , respectively .

### 2- Six – day old workers :

Data presented in Table (2) , indicated that the highest significant mean acinal length was recorded for workers fed on diets 4 (0.1488mm) followed by that of workers fed on diets 1 ( 0.1337 mm )and 2 (0.1310mm). On the other hand, the lowest significant mean acinal length (0.1172mm) was recorded for workers fed on diet three.As shown in Table (2) , it is clear that the highest significant acinal width was recorded for workers fed on diet four (1.01046 mm) , followed by that of workers fed on diet three (0.0976 mm) and diet one (0.0954 mm). On the other hand , workers fed on diet showed the lowest significant mean acinal width. Data presented in Table (1) indicate that the highest significant mean acinal **surface** (0.0244 mm<sup>2</sup>) was recorded for workers fed on diet four , while the lowest significant value (0.0180 mm<sup>2</sup>) was detected in diet three , in addition , the differences between all the treatments were no significant.

### 3- Nine – day old workers :

As shown in Table (2) , the lowest significant acinal length was recorded in workers fed on diet 2 ( 0.1473 mm ) .However , the recorded means at acinal length were 0.1507,0.1563 and 0.1588mm for workers fed on diets 1,3 and 4 , respectively .The recorded means at width of the hypopharyngeal gland acinus induced that diet four was highest significant acinal width , where the workers fed on diet 3 showed the lowest significant one . Data presented in Table (2) indicate that The workers fed on diet four (0.0290 mm<sup>2</sup>) showed the highest significant mean acinal surface area , while the least was recorded for workers fed on diet three (0.0189 mm<sup>2</sup>).

### 4- Twelve-day old workers :

The recorded means of width of the hypopharyngeal gland acinus could be arranged descendingly as follows :0.1128 , 0.1436 , 1553 and 0.1831mm for workers fed on diets 3,2,4 and 1, respectively .Workers fed on diet 1 showed the highest Significant mean acinal length , while those Fed on diet 3 showed the lowest significant valve.



Table 2: Mean Hp gland acinal length L ( mm ) ,width W(mm) and surface aread S ( mm<sup>2</sup>) at different ages of caged workers as influenced by feeding on the test diets

Diets	3			6			9			12			15			18			Total		
	I	W	S	I	W	S	I	W	S	I	W	S	I	W	S	I	W	S	I	W	S
	(days)																				
1*	0.01224	0.0865	0.0166	0.1337	0.0954	0.0200	0.1507	0.1000	0.0237	0.1831	0.0704	0.0202	0.1436	0.0847	0.0191	0.1256	0.0871	0.0172	0.8591	0.5241	0.1168
2**	0.1164	0.0708	0.0129	0.1310	0.0949	0.0195	0.1437	0.0892	0.0206	0.1436	0.0847	0.0191	0.1224	0.0892	0.0171	0.1128	0.0845	0.0150	0.7699	0.5133	0.1042
3***	0.1214	0.0852	0.0167	0.1172	0.0976	0.0180	0.1563	0.0770	0.0189	0.1128	0.0986	0.0175	0.1209	0.0885	0.0168	0.1268	0.0746	0.0149	0.7554	0.5215	0.1028
4****	0.1259	0.0865	0.0171	0.1488	0.1046	0.0244	0.1588	0.1164	0.0290	0.1553	0.1120	0.0273	0.1430	0.1071	0.0240	0.1460	0.0971	0.0223	0.8778	0.6237	0.1441
total	0.4861	0.3290	0.0633	0.5307	0.3925	0.0819	0.6095	0.3826	0.0922	0.5948	0.3657	0.0841	0.5299	0.3695	0.0770	0.5112	0.3433	0.0694	3.2622	2.1826	0.4679
Mean	0.1215	0.0823	0.0158	0.1327	0.0981	0.0205	0.1524	0.0957	0.0231	0.1496	0.0814	0.0210	0.1325	0.0924	0.0193	0.1278	0.0858	0.0174	0.8156	0.5457	0.1170
L.S.D 0.05	0.0061	0.0077	--	0.0111	--	0.0024	0.0114	0.0089	0.0015	0.0101	0.0117	0.0018	0.0064	0.0093	0.031	0.0076	0.0093	0.0019			
0.01	0.0092	0.0117	--	0.0168	--	0.0037	0.0172	0.0135	0.0023	0.0154	0.0179	0.0028	0.0097	0.0142	0.0047	0.0115	0.0141	0.0029			

\* 1-2 parts corn flour + 1 part defatted soya bean flour .

\*\* 2-2 parts been flour : 1 part date paste : 1 part skimmed milk

\*\*\* 3-3 parts defatted soya bean flour + 1 brewer's dried yeast + 1 skimmed milk .

\*\*\*\* 4- bee-bread ( control )

Data presented in Table (2) , indicated that the highest significant mean acinal width was noticed for workers fed on diet 4 ( 0.1120mm ). On the other hand . Workers fed on diet 1 showed the lowest significant mean acinal width (0.0704mm).The recorded means at acinal surface area were 0.0202 , 0.0191 , 0.0175 and 0.0273 mm for workers fed on diets 1,2,3 and 4 , respectively . The highest significant mean acinal surface area was recorded for workers fed on diet four , while lowest was recorded for workers fed on diet three ( Table 2 )

#### 5- Fifteen \_ day old

Data presented in Table (2) , indicated that The highest significant mean acinal length was recorded for workers fed on diet two (0.1436 mm), while the lowest showed for workers fed on diet three (0.1209 mm). As shown in Table (2) , workers fed on diet four showed the highest significant mean acinal width ( 0.1071mm).While the differences between workers fed on diets 1,2and 3 (0.0847 , 0.0892 and 0.0885mm respectively ) were insignificant.The recorded means of acinal surface area arranged descendingly were 0.0240, 0.0191 , 0.0171 and 0.0168mm for workers fed on diets 4,1,2 and 3, respectively Table (2) . The highest significant mean acinal surface area was recorded for workers fed on diet 4 , while the differences between diets 1,2 and 3 were insignificant .

#### 6- Eighteen . day old workers :

The recorded means of length of the hypopharyngeal gland workers fed on diet 4 showed the highest significant mean acinal length , while the differences in the acinal length of worker fed on diets 1,2 and 3 were insignificant ( Table 2 ) .As shown in Table (2) , it is obvious that longest significant acinal width was recorded in workers fed on diet 4 (0.0971mm). However , the recorded mean of acinal width were 0.0871,0.0845 and 0.0746 mm for workers fed on diets 1,2And 4 , respectively .Data presented in Table (2), indicated that the recorded means at acinal surface area were 0.0172, 0.0150 , 0.0149 and 0.0223 mm for workers fed on diets 1,2,3, and 4 respectively. Workers fed on diet 4 showed the highest significant mean acinal surface area , while the least was recorded for workers fed on diet 3 .It could be concluded in general that the highest inducement of the hypopharyngeal gland development was recorded in workers offered diet 4 (bee bread ) Followed by , diets 1 ( 2 parts corn flour + 1part defatted soy bean flour ) and 2 ( 2 parts bean flour +1.part date paste + 1 part skimmed milk) On the other hand , feeding caged workers on diet 3 ( 01 % brewer s + 3 parts chick pea flour ) caused the lowest development of the hypopharyngeal gland . This statement is in agreement with that of Rosca *et al.* (1972 ) and Yousif – khalil (1983 ) .

Different investigating authors had judged the effectiveness of the diet by the degree of the hypopharyngeal gland development such as Szymas (1977). The effect of different pollen supplement and substitutes on the development of hypopharyngeal gland was also studied by many authors . For instance , Macicka ( 1987 ) reported that the development of hypopharyngeal gland was promoted by high protein concentration of the diet , Szymas and Torgowski (1979) stated that soya bean extract induced the hypopharyngeal gland development El – Barbary (1980) stated that the



degree of hypopharyngeal gland development was nearly the same in workers fed on fresh or stored bee – collected pollen , soya been meal and brewers yeast at any level of concentrations

Data of the present investigation indicated that the maximum development of the gland was achieved between 6-12 days for all diets . In this concen , yousif – khalil *et al.* (1988) who reported that the maximum development of the gland was take place between 6-9 days and may extend to the 12-day .Sato (1984) reported that the gland reach the maximum size on the 14<sup>th</sup> days .0

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## تأثير التغذية بدائل حبوب اللقاح علي فترة طول العمر وتطور الغدد الفوق بلعومية لشغالات نحل العسل

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أجريت هذه الدراسة بمنحل ومعامل كلية الزراعة بأسيوط جامعة الأزهر خلال عام ٢٠٠٥ & بهدف دراسة تأثير التغذية الإضافية ببعض بدائل حبوب اللقاح علي فترة طول العمر وتطور الغدد الفوق بلعومية لشغالات نحل العسل المرباه داخل أقفاص بالمعمل: وكانت مكونات الوجبات المقدمة بالاتي .

الوجبة الأولى - ٢ جزء دقيق ذرة + ١ جزء دقيق فول صويا منزوع الدهن  
الوجبة الثانية - ٢ جزء دقيق فول بلدي + ١ جزء بلح عجوة + ١ جزء لبن فرز مجفف  
الوجبة الثالثة - ١٠% خميرة بييرة + ٣ جزء دقيق حمص + ١ جزء لبن فرز مجفف .  
الوجبة الرابعة - خبز النحل (كمقارنة).

ويمكن تلخيص النتائج كالتالي :-

أوضحت نتائج الدراسة العملية أن تغذية نحل الأفاص علي خبز النحل أعطت أطول مدة لحياة الشغالات (٣١ يوما) يليه مخلوط مكون من ١٠% خميرة بييرة + ٣ جزء دقيق حمص : ١ جزء لبن فرز مجفف والتي عاشت لمدة (٢٤ يوما) ثم يليه مخلوط مكون من ٢ جزء دقيق ذرة : ٢ جزء دقيق صويا منزوع الدهن والتي بلغ طول عمر الشغالات علي هذه الوجبة (١٨ يوما) بينما بلغ طول عمر الشغالات في الوجبة المكونة من ٢ جزء دقيق فول بلدي : ١ جزء عجوة : ١ جزء لبن فرز مجفف (١٥ يوما) . وأتضح من الدراسة العملية أيضا أن اعلي تطور لنمو غدة الغذاء الملكي قد سجل في الشغالات عمر من ٦ - ١٢ يوما عموما . وان الوجبة الغذائية الرابعة (خبز النحل) قد أعطت اعلي تطور لنمو غدة الغذاء الملكي ويلبها الوجبة المكونة من ٢ جزء دقيق ذرة : ١ جزء دقيق فول صويا منزوع الدهن ثم الوجبة ٢ المكونة من : ٢ جزء دقيق فول بلدي : ١ جزء بلح عجوة + ١ جزء لبن فرز مجفف .  
في حين أعطت الوجبة ٣ المكونة من ١٠% خميرة بييرة + ٣ جزء دقيق حمص : ١ جزء لبن فرز مجفف اقل معدل لتطور نمو الغدة فوق البلعومية في الشغالات المقصص عليها .  
عموما نجد أن تغذية الشغالات علي خبز النحل كانت الأفضل في الحالتين (طول فترة حياة الشغالات وكذلك تطور الغدد فوق البلعومية في نحل الأفاص)