

## Testing some Feeding Substances and its Protein and Lipid Contents on Rearing Brood of Honeybee Colonies

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### ABSTRACT

Twenty seven of the first hybrid local Carniolan bee race (*Apis mellifera carnica*) of about equal strength, were chosen to start the planned experiments, bee colonies containing at least four frames of brood covered with bees in the Beekeeping Res. Sec. at Dokki., Giza during 2004,2005. The colonies were arranged in nine groups of three colonies each. Groups from 1 to 8 were tested experimentally, while the group nine was left as a control. Several food stuffs containing considerable amounts of protein available in cheap prices in the local market were chosen for this study. These stuffs were used alone or mixed as follows: Bean pollen grains + sugar (1:2), Palm pollen + sugar (1+2), drone larvae + sugar + pollen grains (3:6:1), dried medical yeast + sugar + water (3:3:1), soybean flour + sugar (1:2), fish powder + sugar + pollen grains (3:6:1), Lintels + sugar (1:2) and Crud protein + sugar (1:2). These diets were mixed with sucrose solution (1:1 v/v) making a semi dried paste. The paste was divided into small pieces (100g each) to provide each of treated colony weekly. Consumption of these diets percentage by treated colonies was estimated, and the brood rearing areas were measured. Also, the total protein and total lipid contents in applied diets were determined. The protein contents in fifth instar larvae, newly emerged workers and nurse worker haemolymph were determined. The highly consumption percentage obtained from colonies fed an bean pollen (100%) with the highly area of brood (440.52 inch), while the highly protein percentage was found in fish powder (64.08%) and highly lipid percentage was found in drone larvae diet (41-17%). The obtained results indicated that the highly protein content in fifth instar larvae was found after feeding with drone larvae (6.9%), in newly emerged workers was found in after fed on fish powder (2.32%), while in nurse worker bees was found after fed on drone larvae (4.11%). It could be concluded that feeding honeybee colonies on diets containing bean brood pollen or drone larvae as fish powder is beneficial to give great areas of brood reared which increased consequently the colony strength to gain an increasing in all commercial products produced from honeybee colonies.

### INTRODUCTION

Feeding may have been one of the first manipulations of bees, as evidenced by directions for feeding found in the earliest writings. Bees do not need to be fed regularly as other livestock does, but there are occasions when supplementary or emergency feeding is required, such as a late spring or bad weather which prevents foraging during a nectar flow,

periods of dearth in the active seasons, or a generally poor season, newly established natural swarms, divisions, nuclei, or package bees which need food for making comb and rearing brood and manipulations such as inspecting bees, uniting, queen rearing and many other reasons (Johanson and Johanson, 1977).

In nature, honeybees depend mainly on pollen as source of protein, lipids, minerals and vitamin, necessary for normal growth and activity of different castes (Wahl, 1963; and Kleinschmidt & Kondas, 1978). The protein content of pollen ranges from 7 to 40% (Johanson and Johanson, 1977 and Dietz, 1979). The amino acids constituting the protein are also very variable (Auclair and Jamison, 1948). Taber and Poole, 1974, mentioned that during dearth periods and when pollen is not available or short, it is of advantage to provide essential nutrients in the form of pollen substitutes or supplements.

Much effort has been made in the field of honeybee nutrition towards finding some food materials which could substitute or supplement pollen during dearth periods. In Egypt, Atallah (1968) used pressed dried dates either alone or fortified with brewer's yeast in dearth period of pollen and nectar El-Banby and Gourgi (1970) recommended maize flour, and Abedel-Latif *et al.* (1971) tested feeder and medical yeasts.

The present investigation was conducted to determine the nutritional value of some diets tested by measuring the consumption percentages, brood rearing activity, chemical analysis of pollen substitutes and supplements tested protein and lipids content in haemolymph of different bee workers stages.

## **MATERIALS AND METHODS**

**Bees:** The first hybrid of local Carniolan bee race *Apis mellifera carnica* was used to start the planned experiments. Twenty seven honeybee colonies of about equal strength containing at least four frames of brood covered with bees were chosen in the apiary at Dokki, Giza during 2004 and 2005 seasons. The colonies were arranged in nine groups of three colonies each. Groups from 1 to 8 were tested experimentally, while the group 9 was left as a control.

### **Preparing the diets:**

Several food stuffs containing considerable amounts of protein available in cheap prices in the local market were chosen for this study.

These stuffs were alone or mixed as follows:

- 1- Bean pollen grain + sugar (1: 2) (Huang *et al.*, 1986)
- 2- Palm pollen + sugar (1: 2) (Huang *et al.*, 1986 ; Konopacka *et al.*, 1987)
- 3- Drone larvae + sugar + pollen grain (3 : 6 : 1) (Weiss, 1984)
- 4- Dried medical yeast + sugar + water (3: 3: 1) (Macicka, 1987)
- 5- Soybean flour + sugar (1: 2) (Alves *et al.*, 1997)
- 6- Fish powder + sugar + pollen grain (3: 6: 1) (Chalmers, 1980; Szymas *et al.* 1993)
- 7- Lintels + sugar (1: 2)
- 8- Crude protein + sugar (1: 2)

Flours of these stuffs were sifted using different sets of sifts with different mesh. Thereafter they were mixed with sucrose solution (1: 1 v/v) making a semi dried paste. The paste was spread on a plastic sheet and was left for 24 hours to be little bit dried. The paste was divided into small pieces (100g) by means of a sharp knife. Experimental colonies were provided with one piece of prepared paste each at 7 day interval all over the experimental period (two seasons 2004-2005). These pastes were placed directly over the brood combs covered with plastic sheets to avoid drying. Sucrose syrup (2 Sugar: 1 water) was continuously provided to every colony under investigation. These diets were compared to determine the following criteria.

#### **1- Estimation of pollen supplements and pollen substitutes consumption:**

Each group (3 colonies) colony received weekly 100 g of each the tested diets during the experimental period. The unconsumed diets were collected and weighted to determine the rate of consumption of each diet. The selected colonies including the control were fed with 500 ml sucrose solution.

#### **2- Brood rearing measurements:**

A typical Langstroth frame with dimensions of 17 X 8 inches was divided into square inches to evaluate the quantity of sealed brood. The frame was laid against 3 each side of brood comb and the area occupied by sealed brood was measured. The counts of worker brood were done at 12 day intervals. (Moller, 1967).

### **3- Determination of total protein in the tested diets:**

Total nitrogen was determined by the usual method according to AOAC (1995) as follows:

The dried sample (0.5-1g.) was digested with concentrated sulfuric acid in the presence of digestion catalysts Ca mixture of copper sulfate and anhydrous sodium sulfate, (1:10) the crude protein was calculated by multiplying the total organic nitrogen by 6.25.

### **4- Determination of total lipid in the tested diets:**

One gram of the substance was accurately weighted, and the lipid content was extracted by 100 ml. of methanol-chloroform mixture (2:1) in a soxhlet apparatus for 8 hours, according to the procedure described by Folch *et al.* (1957). The solvent was distilled off under vacuum at room temperature, and the weight of lipid was then measured.

### **5- Determination of protein content in haemolymph of different worker bee stages from treated colonies:**

#### **a) Preparation of haemolymph samples for analysis:**

Three stages of honey bee individual were tested. The first was larvae at the 5<sup>th</sup> instar, the second was newly emerged bees and the third was from the nurse bees found in the colony.

Haemolymph samples from the tested worker larvae were collected throughout a fine puncture in the dorsal side of the larvae. The haemolymph samples from the tested adult workers were collected by withdrawing the head. The exuded haemolymph from the tow stages were sucking into a 10 ml capillary tube. The haemolymph placed in 1.5 ml ice-cold microcenterfuge tubes which contain a few crystals of phenylthiourea to prevent melanization of the haemolymph. The pooled haemolymph was centrifuged using an Eppendorf microcenterifuge at 10.000 r.p.m for 5 min. at room temperature. The supernatant was transferred to a new Eppendorf tube and kept frozen at -20°c till required.

#### **b) Protein determination:**

Protein content was determined by biuret reagent according to the method described by Gorwal *et al.* (1949).

#### **Biuret reagent contains:**

Copper sulfate (6mmol/l), sodium potassium tartarate (21mmol/l), sodium hydroxide (750mmol/l) and potassium iodide (6 mmol).

Standard solution was albumin (5 g/dl).

A known volume of the filtrate (0.05ml) of either haemolymph and the standard were mixed with (2.0ml) of the biuret reagent in a test tube and

incubate for 10 min. at 37°C. Reading the absorbances of the sample (A sample) and the standard (A standard) against the blank at 550nm.

Calculation :

$$\text{Total protein in sample (g/dl)} = \frac{\text{A sample}}{\text{A standard}} \times 5$$

c) Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan multiple test and the L.S.D. values were calculated for the variance according to the method of Snedecor and Cochran 1980.

## RESULTS AND DISCUSSION

Data presented in Table (1) show that bees fed Bean pollen (Bp) reared more brood (440.5 Inch<sup>2</sup>), than those fed on other diets. Bees fed with Lintels (L), Yeast (Y) or Soya bean (Sb) were showed in initiating brood rearing (236.96, 242.10 or 293.97 Inch<sup>2</sup>, respectively).

Colonies fed Fish powder (Fb) and Drone larvae (DI) were nearly equal in brood rearing (392.69 and 393.02 Inch<sup>2</sup>, respectively).

Bean pollen diets was notably consumed, while that containing yeast, Lintels and Soya bean seemed less acceptable to the bees.

The ratio of brood area (Inch<sup>2</sup>) diet consumption (g.) was higher in colonies fed yeast followed by those fed Soya bean and Fish powder.

The obtained data were in agreement with those of Vinogradova (1958) who mentioned that bees can obtain their protein from pollen, Moller (1967) concluded that bees have a distinct preference for supplements made with pollen over those with other materials only, Chalmers (1980) indicated that fish meal diluted to the 23% protein level considered optimal for honey brood rearing by adding sugar cane, using small patties placed on the brood area, Stace (1992) confirmed that crude protein level (23-24%) and amino acids present in canola (rape seed) pollen are adequate for honeybee nutrition.

**Table (1): Brood rearing of honeybee colonies fed different diets and their consumption of tested diets.**

Diets	Mean sealed brood/12 days (inch <sup>2</sup> )	Mean consumed diet (g.)	Ratio of brood area (inch <sup>2</sup> ) / consumed diet (g.)
Yeast(Y)	242.10(D)	7.84(f)	30.88
Drone Larvae(DL)	393.02(b)	96.95(a)	4.05
Palm pollen (PP)	310.83(c)	92.38(b)	3.36
Bean pollen (BP)	440.52(a)	100.00(a)	4.41
Lintels(L)	236.96(D)	49.89(e)	4.75
Crude protein (CP)	378.42(b)	92.06(b)	4.11
Fish powder (Fp)	392.69(b)	77.09(c)	5.05
Soybean (Sb)	293.97(c)	57.22(D)	5.14

L.S.D<sub>0.05</sub> = 3.32 (for consumption)L.S.D<sub>0.05</sub> = 37.27 (for brood area)**Total protein and total lipid in tested diets:**

Results in table (2) show total protein and total lipid percentages obtained from tested diets. The higher total protein percentage were found in (Fp) (64.08%) followed by (Cp) (53.66%), while the lowest were found in (L), (Bp) and (Pp) (21.92, 24.98 and 30.56% respectively).

On the other hand, the higher total lipid percentage were obtained from (DI) (41.17%) followed by (Bp) (23.42%) while the lowest were obtained from (L) and (Y), (1.60 and 2.00%, respectively).

The obtained results was in agreement with that of Atallah *et al.* (1979) when they concluded that bees fed powdered milk (27% protein) reared more brood than other areas that were small in colonies fed supramine (a child food supplement containing 20% protein), brewer's yeast (40.5% protein) or sugar cane yeast (42.0% protein). Popescu *et al.* (1980) indicated that production of honey, brood and amount of pollen collected were greater in colonies receiving the supplement, Lehner (1983) reported that there is no statistical difference was found in brood production at different protein level (from 5% to 30%), the 5% level of protein may not be as good as the others, Stace (1992) concluded that the crude protein

level (23-24%) and amino acids present in Canola (rape seed) pollen are adequate for honeybee nutrition.

**Table (2): Determination of total protein and total lipid in the tested diets.**

Diets	Mean total protein (%)	Mean total lipid (%)
Yeast(Y)	42.00	2.00
Drone Larvae(DL)	32.25	41.17
Palm pollen (PP)	30.56	15.15
Bean pollen (BP)	24.98	23.42
Lintels(L)	21.92	1.06
Crude protein (CP)	53.66	8.19
Fish powder (Fp)	64.08	16.37
Soybean (Sb)	39.96	12.70

**Total protein content in haemolymph of different stages of bee worker from tested colonies:**

Tabulated data in table (3) show the measurements of total protein contents in haemolymph of 5<sup>th</sup> instar larvae, newly emerged bee workers and nurse bees obtained from colonies with different diets. The higher protein contents in 5<sup>th</sup> instar larvae were found in those fed (Fp) (7.14%), while the lowest ones were found in those fed on (Y) and (L) (5.42 and 5.47%, respectively).

The total protein contents in haemolymph of newly emerged workers of treated colonies recorded the higher level with that fed (Fp) (2.32%) and (Di) (2.025), while the lowest ones were found with that fed (L) (1.25%) and (Y) (1.43%).

On the other hand, the total protein contents in haemolymph of nurse bees of treated colonies gave the higher levels (4.11, 4.05 and 4.05%) with that fed (Di), (Pp) and (Fp) respectively), while the lowest one was (3.51%) from nurse bees fed (L).

The obtained data are in agreement with the findings of Haydak (1934) who reported that protein comprise 13% of the fresh body weight of

emerging bees and 15.5% of the fresh weight of 5-day-old bees, Haydak (1935) indicated that the weight of emerged bees and their nitrogen content are directly influenced by pollen consumption of the nurse bees, Groot (1953) stated that the longevity of old bees is increased by supplementing the carbohydrate diet with various protein containing foods.

On the other hand Lensky (1971) found slight changes in the worker haemolymph during larval stages and found also that the main pattern of haemolymph proteins larvae, adults and all stages.

From the above mentioned data, it could be concluded that feeding colonies on Bean pollen (Bp), Drone larvae(Dl) or Fish powder (Fp) mixed with sugar solution (1: 1 V/V) is beneficial for giving large areas of brood rearing which increase the strength of colonies enough to gain more yield of honey and all other commercial products of honeybee colonies.

**Table (3): Measurements of the total protein contents in haemolymph of different honeybee worker stages in colonies fed with different tested diets.**

Diets	Mean total protein in haemolymph		
	(1) 5 <sup>th</sup> instar larvae	(2) Newly emerged worker	(3) Nurse workers
Yeast (Y)	5.42	1.43	3.57
Drone Larvae (DL)	6.90	2.02	4.11
Palm pollen (PP)	6.73	1.96	4.05
Bean pollen (BP)	6.67	1.90	3.87
Lintels (L)	5.47	1.25	3.51
Crude protein (CP)	6.72	1.78	3.87
Fish powder (Fp)	7.14	2.32	4.05
Soya bean (Sb)	6.36	1.90	3.63
Control (C)	5.18	1.25	3.21

(1) L.S.D<sub>0.05</sub> = 0.708

(2) L.S.D<sub>0.05</sub> = 0.382

(3) L.S.D<sub>0.05</sub> = 0.407



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## الملخص العربي

### اختبار بعض مواد التغذية ومحتواها من البروتين والليبيدات على تربية الحضنة لطوائف نحل العسل

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أجرى هذا البحث على الهجين الأول الكرنولي حيث تم اختيار ٢٧ طائفة متساوية في القوة تقريبا كل منها تحتوي على أربعة أقراص من الحضنة مغطاة بالنحل وذلك بمنحل قسم بحوث النحل بالدقى - الحيزة . قسمت هذه الطوائف الى تسعة مجموعات كل منها من ٣ طوائف وتم تغذية ٨ مجموعات منها على ثمانية أنواع من التغذية بينما كانت المجموعة التاسعة عبارة عن المقارنة - أنواع التغذية التي طبقت في هذه الدراسة تكونت من : حبوب لقاح الفول البلدي + السكر ( ١ : ٢ ) ، حبوب لقاح نخيل البلح + السكر ( ١ : ٢ ) ، يرقات الذكور + السكر + حبوب اللقاح ( ١ : ٣ : ٦ ) ، الخميرة الطبية الجافة + السكر + الماء ( ٣ : ٣ : ١ ) ، دقيق فول الصويا + السكر ( ١ : ٢ ) ، بودرة السمك + السكر + حبوب اللقاح ( ٣ : ٦ : ١ ) ، العدس الاصفر + السكر ( ١ : ٢ ) ، بروتين خام + سكر ( ١ : ٢ ) قدمت هذه التغذية بمقدار ١٠٠ جم لكل طائفة معاملة وذلك بعد إعداد جميع أنواع التغذية للمختبرة في صورة عجائن وتم تغذية طوائف المقارنة بواسطة المحلول السكري (سكر : ١ ماء) فقط. وتمت دراسة تأثير كل من أنواع التغذية على الطوائف المعاملة من حيث كمية المستهلك من التغذية المقدمة في كل معاملة ومساحة الحضنة المرياة في كل معاملة وتقدير نسبة البروتين والليبيدات في أنواع التغذية المستخدمة وكذلك تقدير المحتوى البروتيني في هيوليمف الاعمار المختلفة من الشغالات الناتجة في الطوائف المعاملة وقد اوضحت النتائج أن أكبر مساحة من الحضنة وجدت في الطوائف المعاملة المغذاة بالتغذية المحتوية على حبوب لقاح الفول (٢٠٥٢ر ٤٤٠ بوص مربعه) وكانت نسبة المستهلك من التغذية ١٠٠% وكانت أعلى نسبة من البروتين وجدت في بودرة السمك (٦٤ر ٠٨%) بينما كانت أعلى لبيدات موجودة في يرقات الذكور

(١٧ر٤١%) . وأتضح من الدراسة أن أعلى نسبة بروتين توجد في دم يرقات العمر الخامس للشغالات (١١ر٤١%) في حالة التغذية على بودرة السمك وأعلى نسبة للبروتين في دم الشغالات السارحة (١١ر٤١%) في حالة التغذية على يرقات الذكور. من تلك النتائج المتحصل عليها يمكن استنتاج أن تغذية طوائف النحل على أنواع تغذية محتوية على حبوب لقاح الفول أو يرقات الذكور أو بودرة السمك يمكن أن تكون مفيدة لاعطاء مساحات أكبر من الحضنة المرباة والتي تزيد بالتالي من قوة الطوائف للدرجة التي تسمح بزيادة جميع المنتجات التجارية الناتجة من طوائف النحل.