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Effect of different feeding diets on the haemolymph of the newly emerged honeybee workers *Apis mellifera* L.

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ABSTRACT

Five diets were compared for measuring their effects on the differential haemocytes types of the newly emerged worker bees, and also on their haemolymph proteins, lipids and glucose content. As blood haemocytes cell play a role in defending honeybees against parasites and pathogens. Five groups of newly emerged worker bees were fed with one of the following diets in patty form with sugar: faba bean (*Vicia faba*) pollen, maize (*Zea mays*) pollen, date palm (*Phoenix dactylifera*) pollen, Egyptian clover (*Trifolium alexandrinum*) pollen and soya bean (*Glycine max*) flour (as pollen supplement). Differential haemocyte counts (DHC) were evaluated in smears of their haemolymph. The types of the haemocytes (prohaemocytes, plasmatocytes, oenocytoids, granulocytes, coagulocytes and binucleated cells) were recorded. The most abundant type was plasmtoycte cells (over 90%) followed by granulocyte cells and coagulocyte cells. These blood cells perform phagocytosis and encapsulation of foreign bodies in the honeybee body cavity. Feeding honeybees with these different diets caused significant differences between the haemocyte cells. The highest number of plasmatocyte cells recorded in bees which fed on maize pollen, while the lowest was found in bees fed on bean pollen. The highest protein content was found in the haemolymph of bees fed on date palm and the lowest was found in bees fed on bean pollen and the difference was non-significant. The difference in lipids content was non-significant in worker bees fed on these different diets. There was a significant difference in glucose content between bees fed on maize pollen and date palm pollen.

Keywords: honeybee, haemolymph, beedieds

INTRODUCTION

Insect’s haemolymph plays a very important role in transport and storage of nutrients and is crucial for the recognition and defense against micro-organism (Bogaerts *et al*., 2009). Honeybees possess an open circulatory system and numerous haemocytes are contained in its haemolymph. Prohaemocytes, plasmatocytes, granular cells, cystocytes, sphaerula cells and oenocytoids are the cells that comprise the bee haemocyte population (Gupta, 1991). They are variable in morphology and functions. They are responsible for the defense reactions against foreign agents that penetrate the haemocoel (Tepass *et al*., 1994; Falleiros and Gregorio, 1995; Gliński and Jarosz, 1995; and Inoue *et al*., 2001). They perform phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation, transport of food material, (Patton, 1983). The haemocytes can engulf and destroy smaller foreign objects such as bacteria or fungal spores, but larger parasites, bacterial clumps or fungal hyphae, are encapsulated by several haemocytes and then removed from circulation (Gliński and Jarosz, 2001).

Honey bees require proteins, carbohydrates, lipids, vitamins, minerals, and water in their diets. As protein plays a major role in the life of honey bees (Amdam and Omholt, 2002), colonies that have no access to pollen have reduced capacity to rear brood, quickly decline in population, and may eventually die. Protein deficiency also affects the ability of honey bees to resist diseases (Matilla and Otis, 2006) and causes a major disturbance in the structure and functioning of the haemolymph cellular system (Rogala and Szymas, 2004). Lipids are also important for the functioning of cellular membranes. Carbohydrates form a large part of the diet of the colony and are required by both the larva and adult for normal growth and development. Carbohydrates in the bee's diet are used mainly to generate energy for muscular activity, body heat, and vital functions of certain organs and glands, such as wax production (Standifer *et al*., 1977)

The objective of this study was to evaluate the influence of different feeding diets (pollen grains and pollen substitute) into the differential haemocyte count (DHC) in the haemolymph of the newly emerged worker honeybees, once that the success of the immune response in insects depends on the number and the types of haemocytes (Russo *et al*., 2001). Also the different diets effects on proteins, lipids and glucose content in their haemolymph.

MATERIALS AND METHODES

This study was carried out in the Apiary of Beekeeping Research Department, Plant Protection Research Institute during the late summer of 2009 till summer 2010. There were five experimental groups each group contained three colonies of equal strength. Each group fed with one of the following pollen grain cakes by mixing 50gm of each pollen with 100gm powdered sugar. Distilled water was added to obtain a patty. The pollen grains used in this experiment were collected by honeybees from four plants in different regions of Giza Governorate. These pollen grains were faba bean (*Vicia faba*), maize (*Zea mays*), date palm (*Elaies guineensis*), Egyptian clover (*Trifolium alexandrinum*) and soya bean (*Glycine max*) flour. These pollen grains were collected from pollen traps and stored in a deepfreeze at about -4ºC. The diet cakes were placed directly over the center of the brood combs and the top of the cake was covered with waxed paper to prevent moisture loss. The experimental colonies were subjected to these different feeding treatments every week begin from late summer of 2009 till summer 2010. Investigated bees were collected by placing frames with emerging brood from each colony fed with these feeding treatment in incubators set to the temperature of 33°C and the relative humidity of 65% till the worker honeybee emerged. A drop of the haemolymph of the newly emerged worker was taken over a clean microscope slide and a smear was made. Smears were stained with Giemsa stain and differential haemocyte counts (DHC) were evaluated. One hundred cells were counted per slide under oil immersion and phase contrast using light Leitz microscope. The types of the haemocytes (prohaemocyte, plasmatocytes, granulocytes, coagulocyte and oenocytoid) were recorded using the key of Gupta (1979). About 30 smears of haemolymph for each diet were examined. Chemical analysis was done on the haemolymph of pooled sample collected from newly emerged worker bees and kept in deep freezer till analysis. Total protein content was determined by Biuret reagent according to Gornal, *et al.,* (1949) method. Total lipids were determined using Knight *et al.*, (1972) method while total carbohydrate was determined using Trinder (1969) method. The statisitical analysis including one way ANOVA at P < 0·05 level were done using SPSS 10 software.

RESULTS AND DISCUSSION

Six haemocyte types were observed in the haemolymph of the newly emerged worker bees feeding on different types of diet and were represented in Table (1) and Figure (1). They were classified as prohaemocytes, plasmatocytes, granulocytes, coagulocytes, oenocytoid and binucleated cells.

Table 1: Effect of different feeding pollen diets on different haemocytes count of newly emerged worker of honeybee

Diet	Percentage of haemocyte types (mean ± SE)**					
	PR	PL	GR	CO	OE	Bin
Faba Bean pollen	2.13 ±1.08	90.29* ±3.09	5.38* ±1.30	1.50* ±1.01	0.38* ±0.15	0.33* ±0.21
Maize pollen	0.0	97.05* ±0.83	1.70 ±0.24	0.97* ±0.53	0.08* ±0.003	0.20* ±0.15
Date palm pollen	0.0	93.95 ±0.97	3.39 ±1.30	2.33 ±0.19	0.0	1.33* ±0.33
Clover pollen	1.20 ±0.61	90.27* ±1.52	2.90 ±0.10	5.07* ±2.13	0.33* ±0.12	0.23* ±0.003
Soya bean flour	0.0	93.01 ±0.66	2.72 ±0.73	4.07 ±0.41	0.13 ±0.01	0.08* ±0.01

(PR, prohamocyte; PL, plasmatocyte; GR, granulocyte; CO, coagulocyte, OE, oenocytoid, Bin, binucleated cells)

* indicates significant differences (*P* < 0.05) between the mean values of the haemocytes

** SE= standard error

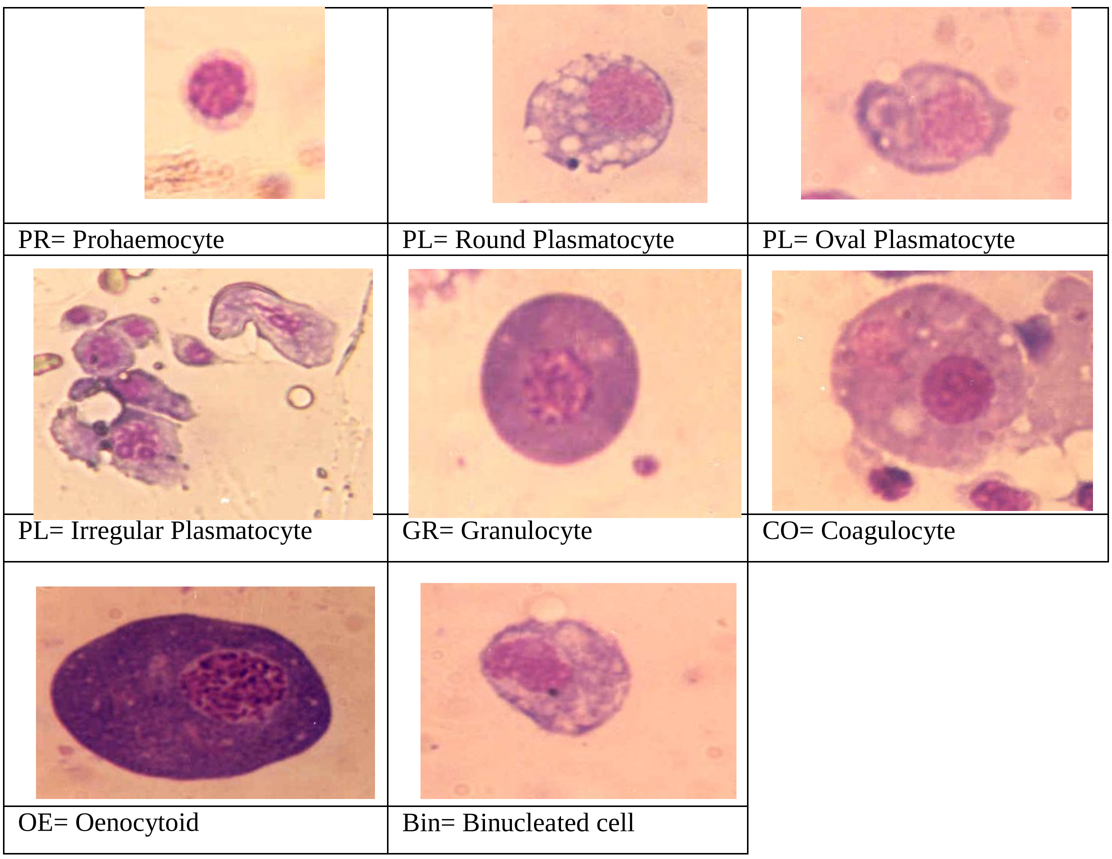

Fig. 1: Haemocyte types in the haemolymph of the newly emerged worker honeybees

Prohaemocytes (PR) are mostly appear rounded in shape but some are oval or spindle – shaped, and small in size. The nucleus fills almost the entire cell and is mostly centrally located. The cytoplasm is restricted to a thin layer around the nucleus showing pronounced basophilia. It contains small granules, droplets or vacuoles. Plasmatocytes (PL) are larger than prohaemocytes with variable sizes, round, oval or spindle in shape and sometimes irregular with projections. The nucleus is round or elongated in shape and is generally centrally located with a distinct nucleolus. The cytoplasm is abundant basophilic and may exhibit small vacuoles. Granulocytes (GR) are rounded to ovoid cells and the nucleus is relatively small, rounded or elongated and is centrally located. The cytoplasm is characteristically granular. Coagulocytes (CO) are spherical or oval cells. The nucleus is relatively small, rounded or elongated and is centrally located. The cytoplasm is characteristically or irregular polygonal. Oenoctoids (OE): The OEs are rounded or oval in shape, and mostly large cells. Compared to cell size, the nucleus is small and is eccentrically located. It exhibits a moderate acidophilia, and reveals a homogenous cytoplasm containing fine and weak acidopholic granulation. Binucleated cells (Bin): It seemed to represent incomplete mitosis of cell division according to Arnold and Hinks (1976).

The predominant type of the haemocytes of bees fed with the five tested diets types was the plasmatocyte cells followed by the granulocyte, and coagulocyte. These results are in agreement with those of previous studies which mentioned that plasmatocytes were the most numerous cells in the haemolymph of young honeybee from free-flying colonies (Jędruszuk, 1998a, b and c and Szymas and Jędruszuk, 2003). Other haemocyte cells like the prohaemocyte, oenocytoid and binnucleated cells were less abundant or not present in all the feeding type. The high percentage in plasmatocytes cells was found in bees fed on maize pollen (97.05), while the lower percentage was found in bees fed on clover pollen (90.27). There were significant differences between the effect of maize pollen and both of faba bean and clover pollen. Vice versa the highest percentage of granular haemocyte was found in worker bees fed on faba bean pollen (5.38) and the lowest percentage was found in bees fed on maize pollen (1.70) with significant difference between them. The high percentage of coagulocyte cells were present in bees fed on clover pollen while the low percentage was found in bees fed on maize pollen. There were significant differences between clover pollen and both of bean and maize pollen. Prohaemocyte cells present only in bees fed on faba bean and clover pollen and were absent in worker bees fed on maize pollen, date palm pollen and soya bean flour. The high percentage of prohaemocyte cells was found in bees fed on faba bean pollen. Oenocytoid cells were observed in the haemocyte of bees except these fed on date palm pollen where it was absent. The higher percentage of oenocytoid cells was found in bees fed on faba bean pollen (0.38%) and a lower percentage was found in bees fed on maize pollen and the difference between them was significant. The highest percentage of binucleated cells was found in date palm pollen and the lower percentage was found in bees fed on soya bean flour. There was a significant difference between the date palm pollen and all the other different diets.

The cellular immune reactions have been shown to be accompanied by changes in both of the number of circulating haemocytes and in the relative proportions of different haemocyte types in the blood (Hink, 1970). From the previous results it was noticed that the plasmatocyte are the most abundant cells followed by granulocyte and coagulocyte cells. These results agreed with those of Szymaś and Jedruszuk, (2003). They mentioned that there was a significantly higher percentage in plasmatocytes and a significant decrease of granular haemocytes in the haemolymph of bees fed with pollen substitute, compared with the haemocyte system of bees fed with pollen. As phagocytosis and encapsulation are two common types of defense reactions in honeybee against invading pathogens. Encapsulation began when granular cells attached to the foreign target and this was followed by attachment of multiple layers of plasmatocytes to form the outer layer of the capsule (Louis and Michael, 1996; Gliński and Jarosz, 2001 and Glinski and Buczek, 2003). The physiological mechanisms effect of phagocytosis, encapsulation, and other related defense mechanisms was due to the availability of circulatory immune cells particularly plasmatocytes and granulocytes (Sanjayan, *et al*, 1996). Bee haemocytes may directly kill bacteria, fungal spores and other small foreign molecules in phagocytic process (Gotz, 1986).

The influence of the different diets on the haemolymph proteins, lipids and glucose of the newly emerged worker honeybee was studied and was presented in Table (2). It was noticed that the total protein content in the haemolymph of the newly emerged worker fed on the five diets were varied in their values but with no significant between them.

Date palm pollen was the highest (10.48%) in haemolymph protein content followed by soya bean flour (9.77%), while faba bean pollen, maize pollen and clover pollen were similar in their haemolymph protein and were the lowest. The natural protein is an important factor in the functioning of the haemolymph cellular system of bees (Szymaś and Jedruszuk, 2003).

Concerning to the total lipid contents in the newly emerged workers, results in table (2) showed no significant differences between the five tested diets. It ranged between 48.36% in date palm pollen and 50.0% in soya bean flour.

Table 2: Protein, lipids and glucose content in the haemolymph of the newly emerged worker bees fed on different diets (mean value ±S.E.).

Type of Diets	Protein mg/ml	Lipid mg/ml	Glucose mg/ml
Faba bean pollen	8.28 ±0.31	49.29 ±0.36	7.06 ±0.19
Maize pollen	8.74 ±0.30	49.84 ±0.16	7.38*±0.01
Date palm pollen	10.48 ±1.17	48.36 ±1.4	6.86*±0.23
Clover pollen	8.97 ±0.58	49.76 ±0.14	7.22 ±0.02
soya bean flour	9.77 ±0.80	50.0 ±0.00	7.23 ±0.01

± S.E. = standard error values
* indicates significant differences ($P < 0.05$) between the mean values

Lipids are used by larvae and young adult bees as sources of energy and for the synthesis of reserve fat and glycogen. For that young adult bees require and utilize some of the lipids in the pollen they consume. Manning (2001) mentioned that pollens with high lipid concentrations and dominated by linoleic, linolenic, myristic and dodecanoic acids probably play a significant role in inhibiting the growth of the spore-forming bacteria, Paenibacillus *larvae* (American foulbrood), *Melissococcus pluton* (Europeanlarvae larvae foulbrood) and other microbes that inhabit the brood combs of bee hive

Table (2) also represents the haemolymph glucose content of the newly emerged worker honeybees. The highest value was found in workers fed on maize pollen (7.38%) while the lowest content was recorded in worker bees fed on date palm pollen (6.86%) with a significant difference between them. Other treatments were arranged in between with no significant differences between them and the glucose content on these fed on maize pollen. The main energy source for social insects was carbohydrates and it provides adult honey bees with the energy to fly and exist. The glucose was taken as example to carbohydrate.

The forementioned results proved the important role of the nutritional value of feeding diets on the haemocyte types, and its proteins, lipids and carbohydrates content. The most abundant haemocyte cells were the plasmtocytes followed by the granular cells. Haemocytes play an essential role in defending insects against invading parasites and pathogens. The effective physiological mechanisms of phagocytosis, encapsulation, and other related defense mechanisms were primarily due to the availability of circulatory immune cells particularly plasmatocytes and granulocytes. Feeding honeybee with these different diets may help enhancing the immunity of the bees due to their positive effects on the types and percentage of the haemocytes. Accordingly, it is premise feeding honeybee with pollen grain or their supplement to increase their defense reaction against different diseases.

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ARABIC SUMMARY

تاثير التغذية المختلفة على دم شغالات النحل حديثة الخروج Apis mellifera L.

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أجريت هذة الدراسة فى منحل قسم النحل بالدقى فى اواخر صيف 2009 حتى نهاية صيف 2010. وقد تم أستخدام خمسة انواع من التغذية ودراسة تاثيرها على انواع خلايا الدم فى النحل حديث الخروج وكذلك تاثيرها على المحتوى البروتينى والدهنى والكربوهيدرات فى دمائها. استخدمت فى هذة التجربة 15 خلية نحل قسمت الى 5 مجموعات كل مجموعة 3 خلايا المجموعة الأولى تم تغذيتها على حبوب لقاح فول (50 جم) مخلوطة مع سكر بودرة (100جم) وقليل من الماء. المجموعة الثانية تم تغذيتها على حبوب لقاح ذرة بنفس النسب السابقة. والمجموعة الثالثة تم تغذيتها على حبوب لقاح طلع النخيل بنفس النسب السابقة. والمجموعة الرابعة تم تغذيتها على حبوب لقاح البرسيم بنفس النسب السابقة. اما المجموعة الخامسة فقد تم تغذيتها على دقيق فول الصويا بنفس النسبة السابقة. عند فحص انواع خلايا دم النحل الخارج :حديثا لكل مجموعة وجدت 6 أنواع من خلايا الدم وهى بروهيموسيت، بلازماتوسيت، اوينوسيتويد، جرانيولوسيت، كواجيولوسيت، خلية ذات نواتين) . وقد وجدت أعلى نسبة مئوية فى خلايا البلازماتوسايت ازيد من 90 % تليها خلايا جرانيولوسيت وخلايا كواجيولوسيت أما الخلايا الأخرى فقد كانت نسبته قليلة أو لم تظهرفى انواع التغذية الأخرى . وقد وجدت فروق معنويه فى خلايا الدم عند تغذيتها بهذة التغذية المختلفه وكانت اعلى نسبة فى خلايا البلازماتوسيت (97.05 %) عند تغذية النحل على حبوب لقاح الذرة وأقل نسبة مئوية وجدت فى النحل الذى غذى على حبوب لقاح البرسيم (90.27 %). وقد وجدت أعلى نسبة فى خلايا الجرانيولوسيت (5.38 %) فى النحل الذى غذى على حبوب لقاح الفول أما أعلى نسبة مئوية فى خلايا كواجيولوسيت فى النحل الذى غذى على حبوب لقاح البرسيم (5.07 %). وحيث أن هذة الخلايا هى الخلايا الدفاعية فى حشرة نحل العسل نجد ان التغذية بحبوب اللقاح المختلفة قد تزيد من مناعة حشرة نحل العسل.

وعند دراسة تاثير التغذية بهذة الأنواع السابقة على المحتوى البروتينى والدهنى فى النحل الخارج حديثا وجد ان الفرق غير معنوى عند التغذية بهذة الأنواع الخمسة . بينما فى حالة الجلوكوز وجد فرق معنوى عند التغذية بكل من حبوب لقاح الذرة وطلع النخيل وقد كان أعلى فى حبوب لقاح الذرة(7.38 %).

ومن هذة الدراسه نجد أن التغذية بحبوب اللقاح أو بدائل حبوب اللقاح يزيد من الخلايا الدفاعية فى حشرة نحل العسل وبالتالى يزيد من مقاومتها للأمراض .