J. Agric. Sci. Mansoura Univ., 32 (4): 3161 - 3167, 2007

BIOCHEMICAL STUDIES ON THE LIQUID YEAST DIET (*Candida tropicalis*) AS A NEW POLLEN SUBSTITUTE IN HONEY BEE COLONIES

Hamad, Hoda M.*; M.E. Nour*; M.E. Zakaria **and Azza T. Ashor *

*** Dept. of Economic Entomology and Pesticides, Fac.. of Agric. Cairo Univ., Giza, Egypt.,**

**** Dept. of Apiculture, Plant Protection Res. Inst. Agric. Res. Center, Dokki, Giza, Egypt.**

ABSTRACT

The liquid yeast diet **(***Candida tropicalis)* was used as a new pollen substitute in queenless builder colonies at 25% concentration, to study their effects on some biochemical changes in the haemolymph of nurse worker bees (unknown age) collected from open queen cells.

The obtained results indicated higher levels of total protein, ALT, AST enzymes and protein bands in the haemolymph of the tested bees. The liquid yeast diet can be used as effective pollen substitute in honeybee colonies to produce high quality of royal jelly and virgin queens.

INTRODUCTION

The development of body tissue, muscles and glands such as the hypopharyngeal glands depends upon adequate amounts of protein in the honey bee's diet (Graham,1992). Beekeepers often feed their colonies with supplemental protein diets during periods of pollen & nectar dearth. Colonies are normally fed on supplemental protein foods to produce strong colonies for package production, to develop colonies with optimum population for pollination of crops, to build up colony population for autumn and spring divisions and for queen rearing. The protein foods are either fed as pollen substitutes or pollen supplements (Standifer *et al.,* 1977). Royal jelly production from worker honey bees, *Apis mellifera* had potential physiological effects on honey bee colonies reflects on bees productivity (Szymas and Przyby, 1995). The artificial protein diet which simulates royal jelly can be used for the rearing of honeybee larvae is involving variations from the standard formulation indicated rather specific requirements for some constituents (Shuel and Dixon, 1986). Worker hypopharyngeal glands has two separate phases of secretion: first, it produces larval food, then enzymes for honey elaboration (Kaatz and Takenaka, 1986). The protein secretion of the hypopharyngeal glands of nurse bees using the 2-D gel electrophoresis demonstrated this protein complement is constituted of 61 different polypeptides, 5 proteins were related to the metabolism of carbohydrates and to the oxido-reduction metabolism of energetic substrates, 1 protein was related to the accumulation of iron in honeybee bodies and 1 protein may be a regulator of MRJP-1 oligomerization (Santos *et al.,* 2005). Balanced of the Amino acids pool in insects is the result of various biochemical reactions carried out by the group of enzymes called amino-transferases (Meister, 1957).The changes of the AST&ALT were parallel with the changes in protein and Amino acids concentration (Abdel Hafez *et al.* 1987). The aim of this

work is to study the effect of liquid yeast diet (*Candida tropicalis*) as a new pollen substitute on some biochemical changes of the haemolymph of the nurse bees collected from queenless builder colonies.

MATERIALS AND METHODS

This work was carried out at the Apiary yard of the experimental station, Faculty of Agriculture, Cairo university, Egypt during summer season, 2006.

I- Tested bee colonies: Six queenless builder honey bee colonies of Carniolian hybrids were conducted for this study and divided into two groups (Three colonies for each). Artificially queen rearing method was performed to collect the nurse bee of unknown age from the reared unsealed queen cells. The first group of bee colonies was fed daily on liquid yeast diet (*Candida tropicalis*) at 25% concentration as a new pollen substitute in a rate of ½ liter/colony. The second group was fed daily only on sugar syrup (1w:1v) in a rate of ½ liter/colony, as control. Not less than 1000 of nurse bees of unknown ages were collected directly from the reared unsea
led queen cells from the tested two groups colonies.

II- Collection the haemolymph samples: The technique for collecting the haemolymph samples from the tested bees was based on the method of **Gilliam and Shimanuki ,(1971**).

III- Determination of total protein: The haemolymph total protein of tested bee workers represented the queenless builder colonies fed on liquid yeast diet as well as sugar syrup was determined according to the method of Lowery et *al.*, (1951).

IV-Determination of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) concentration: Aspartate Amino Transferase (AST) & Alanine Amino Transferase (ALT) were determined in the haemolymph samples of the two tested bee groups of colonies using colorimetric method according to the technique of Retiman and Frankel, (1957) .

V- Polyacrylamide Gel Electrophoresis (PAGE): The haemolymph proteins separated was carried out using the electrophoretic analysis technique (Polyacrylamide Gel Electrophoresis- PAGE) to identify how long changes occurred in the protein structure in the haemolymph of the worker bees collected from colonies fed on liquid yeast diet and those fed sugar syrup. The technique used for this study was carried out according to the method of Laemmli, (1970). Analysis of protein fractions and molecular weights of electrophoretic separated serum proteins were made by the computerized Gel. using Gel Pro Analyzer V.3.0 report program (Mass. Comp., Cairo, Egypt) .

RESULTS AND DISCUSSIONS

I- Determination of total haemolymph protein:

As shown in Table (1), marked differences in the total haemolymph protein between worker bees collected from queenless builder colonies fed with liquid yeast diet (*Candida tropicalis*) at 25% concentration as a new pollen substitute and other workers collected from colonies fed only on

sugar syrup were recorded.

Worker bees fed on liquid yeast diet recorded higher levels of the total protein (mg/ml) than the control one. The level was 54 mg/ml, while with the control it was 42 (mg/ml).

II– Determination of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) concentration:

Data presented in Table (1) indicated clear differences in the (AST) and (ALT) enzymes concentration in the haemolymph of honey bee workers collected from the queenless builder colonies fed on liquid yeast diet (*Candida tropicalis*) at 25% concentration in comparison with those recoded with worker bees collected from the bee colonies fed only on sugar syrup. (AST) & (ALT) enzymes concentration in the haemolymph of honeybee workers fed on the liquid yeast recorded exceed higher levels than the control one.

It could be suggested that queenless builder colonies fed on liquid yeast diet (25% concentration) as a new pollen substitute resulted higher activities of the (ALT) and (AST) enzymes as well as in the total protein concentration in haemolymph of feeding bee workers in comparison with those fed only on sugar syrup. It could be concluded that the increase of haemolymph enzymes of worker bees fed on liquid yeast diet, may be attributed to the ability of these bees to produce more amino acids or proteins with higher molecular weights. These components are very important to produce high quantity and quality of royal jelly secreted from food glands of worker bees. The reared queen larvae were fed on high quantity and quality of royal jelly during their developmental period. Thus, high quality of virgin queens may be produced.

Table (1): Effect of feeding honeybee workers on the liquid yeast diet (*Candida tropicalis*) on the haemolymph total protein, (ALT) & (AST) enzymes concentration.

Diet	Total protein (mg/ml)	Enzymes activity	
		ALT (U /L)	AST (U /L)
Sugar syrup (1:1w)	42	92	169
Liquid yeast *Candida tropicalis*	54	179	278

ALT: Alanine Amino Transferase. **AST: Aspartate Amino Transferase.**

III- The haemolymph protein differentiation

As shown in Table (2) and Fig. (1), the electrophoretic haemolymph protein analysis of worker honey bees collected from queenless builder colonies fed on liquid yeast diet (*Candida tropicalis*) at 25% concentration showed higher number of protein bands (23 bands) with molecular weights ranged between 33-195 (kDa.) than those recorded with worker bees collected from queenless builder colonies fed only on sugar syrup which recorded 21 protein bands with same ranged molecular weights.

It could be suggested that feeding queenless builder colonies with liquid yeast diet (*Candida tropicalis*) at 25% concentration as pollen substitute caused some biochemical changes in the haemolymph particularly with the high levels of enzymes (ALT& AST) which are responsible for producing

proteins and amino acids. This may reflect high quantity and quality of royal jelly production from the specific secretion glands (Hypopharyngeal and mandible glands) which introduced to the developmental queen larvae during their development. This may lead to produce high quality of virgin queens.

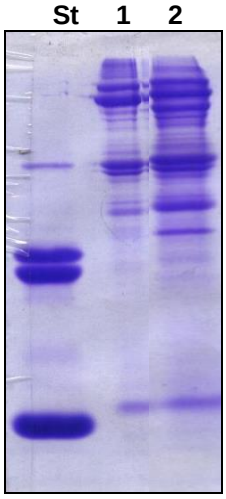
Table (2): The haemolymph protein differentiation of nurse honey bee workers collected from queenless builder colonies fed on liquid yeast diet.

Band No.	Protein standard	Haemolymph proteins (KDa.)	
		Sugar syrup	Liquid yeast diet
1		195	195
2			190
3		176	177
5			160
6		156	158
7		151	152
8		145	143
10		135	135
12			126
13		120	
14	116	116	115
15		112	109
16			105
17		102	101
18		97	97
20		92	92
23	97	78	78
24		69	68
25	66	65	
26	45		
27			56
29		48	
30		45	
31		41	41
32			40
33		37	
35		35	35
36	36	33	33
Total protein bands		21	23

It could be recommended that feeding honeybee colonies specially queenless builder colonies with the liquid yeast diet as pollen substitute resulted worker bees with high physiological status, particularly their glands which are able to produce protein components including more amino acids. Such bees are able to do their duties in rearing and producing high quality of virgin queens.

Standifer *et al.*, (1977) and El Shemy,(1997) recorded that the supplemental protein diets may help bee colony survival or help brood rearing and colony development. Zhelyazkova and Nenchev, (1997) reported

that the several types of the protein diets may be used as stimulating factors on character of haemolymph and bee venom proteins. Suzuki *et al.,* (2003) reported that royal jelly from honey bee, *Apis mellifera* had several nutritional functions. The protein complement of the hypopharyngeal gland secretion of nurse-bees (*Apis mellifera* L.) partially identified by using a combination of 2D-PAGE containing 5 proteins were related to the metabolism of carbohydrates, and one protein was related to the accumulation of iron in honeybee bodies(Santos *et al.,* 2005). Bursell, (1963) pointed out that amino acid transferase especially (ALT) considered one of the components of oxidative metabolism of protein which is certain insects is utilized during the initial periods of flight activities. Salem *et al*., (2001) indicated that protein finger prints of tolerant healthy worker bees to varroa mites contained discrete protein did not occur in non- tolerant healthy one. In addition the higher levels of (GOT) and (GPT) were detected in tolerant worker bees.



1*0% Polyacrylamide Gel (SDS*)
Molecular weights (kDa.)

Fig.(1): Effect of liquid yeast diet (*Candida tropicalis*) at 25% concentration on the haemolymph Protein of worker bees in queenless builder colonies. St: Protein standard.
1- Sugar syrup as control.
2- Liquid yeast diet as pollen substitute.

They assumed that the ability of these bees to produce more proteins or amino acids with the higher molecular weights. Nour *et al.,* (2004) attributed the differences between bee venom protein bands of tested honey bee races and hybrids to the race and bees age. Zakaria, (2002) found decrease in the total protein concentration (g/dl) and both of (GOT) & (GPT) enzymes in the haemolymph of infested bee workers by Varroa mites. (Ursu *et al.,* 1984) found that increasing the activity of Aspartate aminotransferase (ASP) and Alanine aminotransferase (ALA) was correlated with an increase in free amino acids from 5.36-5.95 mg/g body weight in November to 6.73-6.82 mg/g in February, in addition wintering is an active process, and those bees need

to accumulate a large amount of protein in order to winter successfully and to be able to start breeding early in spring before pollen is being available. Reham El Wassef, (2002) found an increase in the quantity of the total protein in bees of in/out door groups and increasing in the (ALT) & (AST)and protein bands in honey bees colonies fed on some diets as pollen substitutes than others fed with sugar syrup only. Abdel-Hafez *et al.,* (1987) concluded that the changes in (AST) and (ALT) enzymes activities were parallel with these changes occurred in protein and amino acids concentration.

REFERENCES

Abdel -Hafez, M. M.;El-Mala, M. A. and Shaban, M. N. , (1987): Changes in transaminases in relation to protein and amino acids concentration during developmental stages of American bollworm *Heliothis armigera* (HBN): *Bulletin Society Of Entomology, Egypt, 67:231 – 242.*

Bursell, E. (1963): Aspects of the metabolism of amino acids in the teste fly Glossina (Diptera) . *Journal of insect physiology, 9:439-452.*

El-Shemy, A.A.M. (1997): Effect of two pollen substitutes on brood rearing and some activities of honeybee colonies. *Bulletin of Society of Entomology, Egypt,75: 1-11.*

Gilliam, M. and H. Shimanuki (1971): Blood cells of the worker honeybee. *J. Abic. Res.,10(2): 79-85.*

Graham, J.M. (1992): The hive and the honeybee. Dandant & Sons, Library of Congress Catalog Card Number 92-81904.

Kaatz-H.H; Takenaka-T. (1986): Protein synthesis activity of hypopharyngeal glands of worker honeybees. *Apidologie. 1987, 18: 4, 374-376* .

Laemmli, U.K. (1970): Cleavage of structural protein during assembly of head of bacteriophage T4. *Nature, lond., 277: 680-685.*

Lowery, O.H.; Rosen- brouch, N.J.; Farr, A.L. and R.J. Randll (1951): Protein measurement with the phenol reagent. *Journal of Biology Chemistry, 93:265-275.*

Meister, A.(1957): Biochemistry of the amino acid. Academic press, New York, 175-196.

Nour, M.E.; Zakaria, M.E. and Abd El-wahab, T.E. (2004): Electrophoretic studies on venom proteins of the honeybee. *Bull. Ent. Soc. Egypt, 81:43-51.*

Reham, El-Waseef. A. I.,(2002): Ecological and physiological studies on honeybee colonies under different environmental conditions . *M. SC. Thesis Fac. Agric. Cairo Univ*

Retiman, S. and Frankel, S. (1957): Colormetric method for aspartate and alanine transaminases*. American Journal of Clinical Pathology. 28-56.*

Salem, M.S.; Nour, M.E.; Dimetry, N.Z. and Abd El. Wahab (2001): Biochemical changes in the haemolymph of honeybee *Apis mellifera* L. affecting tolerance to varroa infestation. 1st Cong. Pest Manag., 53-59.

Santos, K S; Santos, L D dos; Mendes, M A; Souza, B M de; Malaspina, O; Palma, M. S. (2005): Profiling the protein complement of the secretion

from hypopharyngeal gland of Africanized nurse-honeybees (*Apis mellifera* l.):*Insect Biochemistry and Molecular Biology. 35(1): 85-91*

Shuel R.W. and Dixon S.E. (1986): An artificial diet for laboratory rearing of honeybees.*Journal of Apicultural Research.1986, 25: 1, 35-43; B*

Standifer ,L.N.; F.E. Moeller ; N.M. Kauffeld ; E.W. Herbert Jr. and H. Shimanuki ,(1977): Supplemental feeding of honeybee colonies. *USDA Agriculture Information Bulletin No. 413,8pages.*

Suzuki, K.M.; C. Yoshida, K. Tokunaga; H. Maruyama; Y. Futamura; Y. Araki and S. Mishima, (2003): Inhibition of angiotensin I-converting enzyme by protease digests from royal jelly. Nippon Shokuhin Kagaku Kogaku Kaishi. *Journal of the Japanes Society for Food Scienc and Technology. 50(6): 286-288*

Szyams, B. and Pizbyl, A. (1995): Application of potato protein in the feeding of honeybees (*Apis mellifera* L.). *Pszczelnicze Zeszyty Naukowe, 39:1,49-53; (C.F. CAB Abst).*

Ursu, N. A. and Eremiya, N. G. (1984): Changes in Aminotransferase activity in honeybees. Pchelovodstvo. 1984, No. (10): 8-9.

Zakaria, M.E. (2002): physiological studies on honeybee *Abis mellifera* L. under varroa parasitism *Varroa Jacobsoni* oud. *Ph.D. thesis, Fac. Agric.Cairo univ. Egypt.*

Zhelyazkova, I. and Nenchev, P.(1997): Influence of additional feeding with pollen substitute on the fresh weight of queens and worker bees (*Abis mellifera* L). Zhivotnov"dni Nauki,1997, No.Supplement, PP. 110-112,13.(C.F. CAB Abst).

دراسات بيوكيميائية على الخميرة الحية السائلة (*Candida tropicalis*) كبديل جديد لحبوب اللقاح في طوائف نحل العسل

هدى حماد محمد على حماد*، محمود السيد نور* , محمود عزت زكريا و عزة توفيق عاشور***

*** قسم الحشرات الاقتصادية والمبيدات – كلية الزراعة – جامعة القاهرة - الجيزة**

**** قسم بحوث النحل - معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة**

تم استخدام الخميرة الحية السائلة (*Candida tropicalis*) كبديل جديد لحبوب اللقاح بتركيز ٢٥% في طوائف تربية الملكات وذلك لدراسة بعض التغيرات البيوكيميائية التي تحدث في دم شغالات النحل التي تغذي البيوت الملكية المفتوحة.

أشارت النتائج إلى حدوث ارتفاع واضح في معدلات البروتين الكلي, الإنزيمات (AST & ALT) و التفريد الكهربي للبروتين وذلك في الشغالات التي تم الحصول عليها من طوائف تربية الملكات المغذاه على الخميرة عن تلك المأخوذة من طوائف تربية الملكات المغذاه تغذية سكرية فقط (كنترول) .

من النتائج المتحصل عليها، يمكن التوصية بإمكانية إستخدام الخميرة الحية السائلة في طوائف نحل العسل لإنتاج كميات كبيرة من الغذاء الملكي بمواصفات جودة مرتفعة وملكات عذارى بمواصفات انتاجية عالية.

3162 3163 3164 3165 3166 3167

3162 3163 3164 3165 3166 3167