

## **SOME FACTORES AFFECTING ON PROTEIN CONTENT IN QUEENS AND DRONES OF HONEY BEE( *Apis mellifera carnica*.)**

**Mustafa, Madeha A.A.\*; Sawsan S.Saleh\*\* and A.M.DIAB\*\*\***

\* Entomology Dept., Faculty of Science, Cairo Univ., Giza, Egypt.

\*\* Plant Protection Research Institute, Giza, Egypt.

\*\*\* Faculty Of Agriculture, El-Azhar Univ., Cairo, Egypt.

### **ABSTRACT**

The present work aimed to study the effect of incubation type on protein contents of the ovaries of queens (different ages) and effect of locality on protein contents in drone stages . Protein contents of newly emerged queens (0-day old) which incubated in an incubator ( artificial incubation )were higher than in those incubated in a colony (natural incubation ), and decreased with age . But queens which kept in a colony, the protein contents increased according to queen age . Also , there were significant differences between protein content in the haemolymph of mature drones produced in two localities Giza and Alexandria . This difference might have been caused by the supply of pollen by nurse bee in the colony .

### **INTRODUCTION**

Proteins play an important role in the haemolymph of insects. Synthesis and utilization of haemolymph proteins are conditioned by heredity and hormonal control (Schmidt et al., 1976; and Hurliman and Chen , 1974). Blood protein also play a major role in oocyte development ( Nielsen and Mills ,1968).Since oogenesis requires proteins , a lack of proteins may cause retarded ovary development (Harris and Harbo, 1990).

Some variations in the protein patterns were observed dependent on the age of bees, and also on the season of the year (Martin,1967). It is also well known that development of the spermatocytes is completed during the pupal stage. The sexual products consist of high concentrations of proteins from which small amounts of proteins can be utilized without weakening the drone,s vitality (Weinberg and Madel, 1985).

This study was directed to investigate the effects of incubation of the queen cells in a colony (natural incubation ) and in an incubator (artificial incubation ) on the total protein content in the ovaries of virgin queens at different ages. And to study the total protein content in mature drones haemolymph in two localities .

### **MATERIALS AND METHODS**

The present study was carried out at the apiary of the queen - rearing stations of the Ministry of Agriculture, both in Giza and in Alexandria region ., while the laboratory experiments were carried out in the Beekeeping Department of plant protection Research Institute during (1989-1990).

**A-Protein content in the ovaries of virgin queens :**

Virgin queens had been obtained by using Doolittle method in queen-rearing.

The total protein content in the ovary of queens was determined according to the method described by Caraway (1960). This method was based on turbidity formed due to the interaction of such proteins with a precipitation agent in dilute solution , using a “Shimadzu” spectrophotometer (at 420 nm wave length ).

A specimen mixture was prepared by mixing a 0.5 ml sample of the queen’s ovary homogenate with 2.5ml 5% trichloroacetic acid . Such mixture was shaken well and left to stand for 5to 10 minutes at room temperature. Before picking up the protein content reading , the mixture was shaken again. Then , the value of protein was calculated using the following equation :

$$\text{Total protein content} = \frac{\text{Au} \times 100}{\text{As}} \quad (\text{mg/dl})$$

Where: Au = Absorbency of the specimen .

As = Absorbency of the standard

“100” Concentration of the standard ( mg/dl )

Albumin (Bovine Fraction V) in 0.85% Sodium Chloride . Dividing by 100 converts mg/dl to mg/ml

**B-Specimen collection and preparation:**

The protein content was determined in the ovaries of virgin queens at different ages (0- day without eating , 1-day, and 5-days were honey fed ). The virgin queens were divided into two groups. The first one contained queens which were reared and incubated in a honey bee colony . The second group contained queens which were reared in a honey bee colony until sealing the queen cells , and then transferred to an incubator at 32c and 70%R.H. until emergence without nursing bees. The emergent queens were preserved in a refrigerator until dissection in cold distilled water under a stereoscopic microscope, and cleaned from any adhering tissues. Every three ovaries were homogenized in 2.5ml distilled water in a glass homogenizer placed in ice .

This procedure was repeated in order to get 15 samples for each age.

**C- Protein content in the Haemolymph of Drones;**

The total protein content in the haemolymph of immature and mature drones (10-days old ) were determined in both Giza and Alexandria.

Drones were reared according to the method that used by Hegazy (1974).

Haemolymph of the drones was withdrawn by a micropipette from the base of the antenna. Then it was transferred into clean tubes. Few crystals of Phenylthiourea were added to prevent melanization . Unclear haemolymph was discarded. Then the haemolymph samples were collected and stored in a refrigerator until investigation . Then the total protein was determined in the

haemolymph of drones (percentage of haemolymph dilution was 6.7) according to the method described before (Caraway,1960).

## **RESULTS**

### **A-effects of incubation on the protein content in the ovaries of virgin queens**

The results indicated that the virgin queens (incubated in an incubator) was significantly (only at 0.05 level) higher than those incubated in a honeybee colony after emerge without eating (0-day old ). Table 1 illustrate the incubation effect on the content of total protein in the ovaries of virgin queens .

Slight differences were found between the mean values of protein contents in ovaries of virgin queens incubated in a colony for 24 hours and those incubated in an incubator .

The mean value of protein content in virgin queens (incubated for 5-days) in a honeybee colony was almost similar to that virgin queens incubated in an incubator.

### **B-Effects of virgin Queens' Age on protein content**

Protein content in the ovaries of virgin queens for the selected ages (0-day, 1-day and 5-days) changed slightly with age. It increased with the increase in age in the case of queens incubated in a colony, while in those kept in an incubator it decreased with the increase in age.

It was also observed that protein content in the ovaries of virgin queens incubated in an incubator was high after emergence (without eating). Then it decreased with the increase in age . while in queens incubated in a colony the mean protein content in the ovaries increased with increase in age. Those results agree with those of Schneider (1991)who stated that the amount of protein increased with age in queens between 0 and 10 days old.

### **C-Effect of locality on protein content in the haemolymph of drones;**

Results (Table 2) indicated that there was no significant effect of locality on the protein content in the haemolymph of immature drones. In Giza and Alexandria there were no significant differences between them.

However, protein contents in the haemolymph of mature drones in Giza and Alexandria, showed a significant difference between them , only at 0.05 level

**Table (1): Mean Protein contents (mg/gm ovary) in ovaries of Queens (incubated in a colony and in incubator) at different Ages .**

Queen Age -----	Mean Protein contents (mg/gm ovary)		T- statistic
	In colony	in incubator	
0-day	128.95*	175.78*	3.281
1-day	145.07	155.60	0.697
5-day	151.79	149.79	0.332

0-day= After emergence without eating .

1-day= After 24 hours emergence with eating .

5-day=After 5 days emergence with eating .

**Table (2): Mean protein contents in the haemolymph (mg/ml) of immature and mature drones in Giza and Alexandria**

Locality	Mean Protein contents (mg/ml)	
	immature drone	mature drone
Giza	19.93	20.78*
Alexandria	20.83	24.29*

\*T-statistic=2.098

These results agree with those of Weinberg and Madel (1985). These authors recorded the average protein contents in the haemolymph of drones to be 17.55 mg/ml.

In Giza and Alexandria the protein content in the haemolymph of the drones increased with the increase in age. There were no significant differences in protein content between immature and mature drones in Giza and Alexandria. Protein contents in the haemolymph of drones were higher in Alexandria than in Giza.

## DISCUSSION

### 1-protein content in the ovaries of Queens;

The results demonstrated that there were significant differences between the mean protein content of ovaries of queens incubated either in a colony or in an incubator after emergence without being fed. There were no significant differences in both one - day and 5-days old virgin queens.

Also , the protein content changed as the age of the queen changed. These results agreed with what was reported by Lensky and Alumot (1969), Gilliam and Jackson (1972),Engels and Engels (1977). These authors stated that changes in the amount of proteins could be correlated with age.

Data also showed that the protein content increased with the increase of age of virgin queens kept in a colony , but it decreased when using an incubator.

Tripathi and Dixon (1968) stated that the quantity of food received from nurse bees during the larval stage led to some changes in reproductive activity . That is , not only the royal jelly had its effects on the development of the female organs , but also other factors affected this development after sealing the queen cells . It was suggested that it might be due to direct contact between nurse bees and the queen cells. It could also be suggested that there was something produced by nurse workers which could diffuse through the queens cell to affect its development .

Smith (1956) assumed that royal jelly alone was not sufficient for queen differentiation , but that some direct contact between nurse bees and queen larvae was necessary. This agreed with the work of Rutz and Lusher (1974) who demonstrated that the hypopharyngeal glands, mandibular glands, salivary glands, postgenal glands ,and honey stomach organs of workers might be involved in protein transmission . Thus , the natural incubation of queen bee cell could be advised for bee-keeping task in Egypt.

## **2-Protein content in the Haemolymph of Drones ;**

From data presented in this study it was clear that protein content in the haemolymph of drones was higher in those of Alexandria than those of Giza .The difference might be due to the presence of certain factors influencing the rearing and maturing of drones . These results agreed with those of Parker(1971).He reported that the low content of haemolymph proteins in newly emerged drones might be attributed to no feeding during pupation .He also found that feeding of the newly emerged adults helped to raise the haemolymph protein content. Also, this difference might have been caused by the supply of pollen by nurse bees in colony . This agreed with Fluri et al . (1982), who stated that the difference of haemolymph level of total proteins might have been caused by the supply of pollen . This supply was better in summer .

It is well known that development of the spermatocyte becomes completed during the pupal stage . The sexual products consist of high quantities of proteins

from which small amounts could be used without affecting vitality of drones (weinberg and Madel, 1985). The results agreed with those of Hill (1962)who reported that protein metabolism might play an important role in reproductive development. Leonhard and Crailsheim (1999) stated that the overall concentration of free amino acids reached its highest level at the fifth day after adult drone emergence , and after the ninth day only minor changes in the concentration and distribution of free amino acids were observed. This coincides with the age when drones reach sexual maturity and change there feeding behavior .

In conclusion , it could mentioned that protein content in virgin queens and in mature and immature stages of drones affect with different factors such as incubation type ( in colony or in an incubator ) , sort of food (natural or artificial) , nursing bees (absence or presence )and virgin queen age ,

therefore, Protein content increased in virgin queen kept in a colony , but it decreased when using an incubator . pollen sources plays an important factor affect on protein content in stages of drones according to locality , therefore, protein content in the haemolymph of drones were higher in Alexandria than in Giza and this due to pollen content form amino acids , flavonoids and minerals.

## REFERENCES

- Caraway, W.T. (1960). Micromethods for blood analysis. cc. Thomas, spring filed (IL).45-47.
- Engels, W. and E. Engels(1977).Vitellogenin and fertility in Stingless bees. *Insectes sociaux*. 24 (1):71-94.
- Fluri,p.M,Luscher, H.Wille, and L. Gerig,,(1982). Changes in weight of the pharyngeal gland and haemolymph Titers of Juvenile hormone, protein and vitellogenin In worker honeybees. *J. Insect Physiol*.28(1):61-68.
- Gilliam, M. and K.K, Jackson,(1972).Proteins of developing worker honeybees *Apis mellifera*. *Ann.ent. Soc. Am.* 65(2):516-517.
- Harris, J.W. and J.R.Harbo, (1990). Suppression of ovary development of worker honeybees by association with workers treated with carbon Dioxide . *J.Apic.Res*.29(4):187-193.
- Hegazy,G.E.M.,(1974).Factor affecting sexual maturity of the drone and queen bees .M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Cairo.
- Hill, L. (1962). Neurosecretory control of haemolymph protein Concentration during ovarian development in the desert Locust . *J. Insect Physiol* .8 :609-619.
- Hurliman ,R.F. and P.S.M. Chen (1974). Ontogenetische veränderungen des enzymmusters in der Hamolymphe von *Phormia regina*. *Revue Suisse zool*, 81:648-654.
- Lensky ,Y . and Alumot,E., (1969). Protein in the spermatheca and haemolymph of the queen bee (*Apis mellifere* var. *Ligustico* spin .). *Comp. Biochem. Physiol* . 30: 569-575.
- Leonhard ,B. and.K. Crailsheim (1999).Amino acids and qsmolarity in honeybee drone haemolymph . *Amino Acids*, 17(2):195-205.
- Martin , P. (1967). Die eiweissfraktionen der bienen Hamolymphe in abhângigkeit von lebensalter und Nosemainfektion . *zeitsch . F. Bienenforsch* . 9: 91-92.
- Nielsen , J.D. and R.R. Mills (1968).Changes in electrophoretic properties of haemolymph and Terminal oocyte proteins during vitellogenesis in the American cockroach.*J.insect Physiol*. 14:163-177.
- Parker, R.(1971). A comparison of haemolymph proteins in two species of leptinotarsa beetles. *J. Insect Physiol*.17:1689-1698.
- Rutz,W.and M., Lusher, (1974). The occurrence of Vitellogenin in workers and queens of *Apis* and the possibility of its transmission to The queens .*J.Insect Physiol*,20:897-909.

- Schmidt, G.h.; B. Wirth and W. Schwankl 1976). Dehydrogenasen-und estersen-aktivitat in der Hamolymphe von metamorphosestadien von Formica-arten nach polyacrylamid-elektrophorese. Insect Biochem . 6:297-301.
- Schneider, H. (1991). Protein pattern of the spermatheca. Apidologie. 22(4):441-443.
- Smith, M. (1956). what makes a queen. Glean. Bee cult., 89 (3):137-138.
- Tripathi, R.k. and S.E.Dixon (1968). Haemolymph esterase in the female larval honeybee, *Apis mellifera* L., during caste development Can.J. Zool. 46(5):1013-1017.
- Weinberg, K.P. and G. Madel (1985). The influence of the Mite *Varroa Jacobsoni* oud. On the protein Concentration and the haemolymph volume of the brood of worker bees and drones of the honeybee *Apis mellifera* L. Apidologie. 16(4):421-436.

**بعض العوامل المؤثرة على المحتوى البروتيني لملكات و ذكور نحل العسل .**  
مديحة عبد العزيز مصطفى \* ، سوسن سعيد صالح \*\* ، عادل دياب محمد\*\*\*  
\*قسم الحشرات - كلية العلوم - جامعة القاهرة.  
\*\*معهد بحوث وقاية النبات - الجيزة.  
\*\*\*كلية الزراعة - جامعة الأزهر.

يهدف البحث الى دراسة بعض العوامل المؤثرة على المحتوى البروتيني لملكات و ذكور نحل العسل حيث درس تأثير نوع التحضين على المحتوى البروتيني لمبايض الملكات في أعمار مختلفة كما درس تأثير اختلاف المكان على المحتوى البروتيني لدماء الذكور .

و أظهرت النتائج ما يلي :

١ - عند دراسة تأثير نوع التحضين على المحتوى البروتيني لمبايض الملكات ذات الاعمار المختلفة وجد أن المحتوى البروتيني للملكات المنتجة فور فقسها مباشرة في حالة التحضين في الحضان كان أعلى من التي حضنت في الخلية و يقل مع العمر ، و لكن في حالة الملكات التي حفظت في الخلية يزيد المحتوى البروتيني مع العمر.

٢ - عند دراسة تأثير اختلاف المكان على المحتوى البروتيني في الذكور وجدت فروق معنوية بين المحتوى البروتيني لدماء الذكور البالغه والمنتجه في منطقتي الجيزة والاسكندرية ، و يرجع هذا الاختلاف ربما الى أمداد الشغالات ليرقات الذكور بحبوب اللقاح .