J. Plant Prot. and Path., Mansoura Univ., Vol. 4 (2): 177 - 187, 2013

VIRGIN QUEENS OVARIES PROTEINS AND NITROGEN CONTENT OF DIFFERENT VIRGIN QUEENS BODY PARTS

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ABSTRACT

This investigation was conducted at the apiary of Sakha Agricultural Research Station to evaluate certain factors affecting protein content in the ovaries of virgin queens and nitrogen content in different body regions (head, thoray and abdomen) of virgin queens. Major factors affecting protein in queen ovaries were type of cups used in rearing the larvae , with plastic cups are better than wax ones . The highest nitrogen value was recorded in soybean diet with plastic cups , with a mean value of 3.372 %, while the lowest value was recorded in natural pollen grains in wax cups with a queen head value of 0.521 %. The highest nitrogen value was recorded in soybean diet offered in plastic cups with a queen thorax value of 3.146., The lowest value was recorded in natural pollen grains offered in wax queen head with mean value of 0.446. The effects of larval ages and different seasons during queen rearing were studied.

INTRODUCTION

Many attempts have been made to study the factors affecting contents of queen ovaries of protein and nitrogen, especially in commercial queen-rearing operations with *Apis mellifera*. Although a number of factors control queen ovaries protein and nitrogen contents, it is particularly necessary to understand the feeding behavior of nurse bees and the factors influencing the queen ovaries protein and nitrogen.

Hill (1962) measured the changes in the haemolymph protein concentration during ovarian development. A high haemolymph protein concentration was found to be correlated with an active system and developing ovaries. Protein concentration vary not only among species but also with the development within a species.

Harris and Harbo (1990) mentioned that since ogenes require protein, a lack of protein might retard ovary development.

El-Mohandes (1993) studied the effect of type of incubation on protein contents of the ovaries of the queens of different ages. The results showed that protein contents of newly emerged queens (0-day old) incubated in an incubator (175.78mg/g ovary) were higher than those incubated in a colony (128.95mg/g ovary), and decreased with age. Whereas, the protein contents in queens kept in a colony increased with age.

It is known that the quality of the queen economic characteristics depend mainly on the honeybee colony. The queen quality, may affect contents of protein and nitrogen in queen ovaries. Quality of the queen is not only genetically controlled, but also depends on the conditions in which it grows as larvae, the size and vigor of a colony of honey bees are a direct reflection of the genotype of the queen, and also of her individual size and vigor. For example, as her body weight increases, the number of ovarioles

increases. Also, a part of variations, that are frequently observed among many queens which inherit similar size and body conformations, is a results of variations in environmental factors during rearing.

The present work aimed to study factors affecting queen ovaries protein and nitrogen. These factors include larval age, type of queen cups, diets offered to the larvae and rearing season.

MATERIALS AND METHODS

queenless colonies :experimental design was completely

The present study was carried out during 2007 and 2008 seasons in the apiary of Sakha Agricultural Research Station and Laboratory of Bee Keeping Research, Ministry of Agriculture, El-Dokki, Giza.

Twelve honey bee colonies were prepared for the current study. All queens and brood combs containing unsealed broods were removed from the brood chamber with a suitable space between the rest combs to insert a frame holding the grafted queen wax cups. The queenless colony was provided with sealed brood combs continuously. The queenless colonies were fed on sugar syrup for 3 days prior to grafting and throughout the cell building period. The colonies were examined before inserting the grafted cells and all the normal queen cells were destroyed.

Preparation of young larvae for grafting:

In order to obtain larvae at the proper age (24, 48 hours), a prolific queen of a selected colonies was confined with a marked empty worker wax comb in a special cage provided with queen excluders on both sides. The cage was placed in the center of the brood nest of the colony. It was found better to use a comb from which worker bees have just emerged, because the queen prefers such comb to deposit its eggs. After 24 hours, the comb with newly laid eggs was removed and placed in the same colony. By using this technique, the queen found no place for egg laying other than the comb provided for this purpose. Therefore, the age of the larvae used for grafting did not exceed 24 hours. Queen cell cups were attached to a wooden stick batch. This process was repeated for the queenless colony and the queen-rearing unit.

Preparation of start rearing colonies:

In order to obtain a sufficient number of nursing bees of the proper age, a special technique was applied. Sealed brood combs taken from twelve colonies of the hybrids (four from the first hybrid) of the Italian strain were prepared by the following methods:

When over stocking with young bees was insured in the queenlees colony and; unsealed brood combs were transferred to a nucleus box and left in the original place to house the foraging bees. The queenless portions with the rest of the bees and the sealed brood combs were added. The queenless colonies were fed on sugar syrup, and on the next day, the grafted cells were introduced.

Preparation of finishing rearing colonies:

To obtain a sufficient number of field workers for rearing queen cells, four strong healthy colonies were selected. Each hive was moved to a new place in the apiary during the midday when most field bees were foraging. Another hive supplied with two newly sealed brood combs and two combs of honey and pollen were placed in the new hive body and situated in the original location. All field workers returning to the original site of their hive automatically entered the prepared hive. On the other hand, any field workers of the parent colony will also return to the accustomed site and enter the new hive, and only the young workers remain with the queen and the brood in the parent colony. The new colony and fed on sugar syrup and during the following day, the grafted cells were introduced.

Grafting technique:

The method of Laidlaw (1979) was followed by chosing larvae for transferring from those laid on abundant royal jelly in their worker cells. These' queen cell cups were prepared from bee wax tier (Doolittle 1909). Fifteen cell cups were fixed on a wooden bar, using melted wax. The bars were fitted singly into a frame

(two wooden bars were provided to the cell building colony) at each time for few hours before grafting in order to let the bees clean the queen cell cups.

The tip of the grafting tool was slipped under the larva; lifting it out with small portion of its surrounding jelly, and then quickly deposited in the cell cups. Thirty grafted cells were fitted into the frame, and into the middle part of the queen-rearing introduced unit, or the center of the sealed brood combs of the queenless colony. The bars were fixed in three positions upper ;middle and lower of each frame.

Determination of total protein:

Colorimetric determination of total protein in serum based on the principle of the Biurtet reaction (Copper salts in an alkaline medium), according to Henry, (1964).

Reagents:

Reagent 1(Standard)	**Protein standard**	**6 g/dl**
Reagent 2	Biuret Reagent	
	NaOH	0.2 N
	K-Na-tartarate	18 mmoN
	Potassium iodide	12 mmoN
	Cupric sulfate	6 mmoN

Stability

All reagents are stable up to expiration date given on label when stored at 2-8^{o}C.

Samples:
Serum or heparinized plasma:
Procedure:

	Blank	Standard	Specimen
Standard	-------	20 µl	------
Specimen	-------	------	20 µl
Reagent 2	1 ml	1 ml	1 ml

The color intensity is stable… 5 minutes wave length: 546 nm.
OD = optical density
Calculation:

$$\text{g/L} = \frac{\text{OD sample- OD plank}}{\text{OD stander- OD plank}} \times \frac{\text{Sample volume}}{\text{Stander total volume}} \times \frac{\text{Stander weight}}{\text{Sample weight}} \times 100$$

1. Samples of thirty five queens were taken from each colony for chemical analysis to determine nitrogen content of head, thorax and abdomen of the tested queens according to Kjeldahl method (Vogel., 1961).
2. The organic nitrogen was digested to convert the organic nitrogen into inorganic in the form of ammonium sulphate. This was achieved by heating the sample (1.5-2.0 hrs) with 10 ml of concentrated sulphuric acid (95%) and 1-2 ml of digestion solution hydrogen-peroxided solution (30%) w/v for 30 minutes.
3. Determination of nitrogen in the form of ammonium hydroxice after its liberation using excess amount of sodium hydroxide 40%. Liberated ammonia was received in a conical flask containing boric acid 4%.The boric acid was titrated with mixed indicator (0.2 gm bromocresol green + 0.1 gm methyl red) dissolved in 100 ml ethanol with a standard acid.At the end point, the blue colour just disappears. One drop in excess turns solution pink.

The nitrogen content was calculated as follows:

$$\text{Nitrogen content (\%)} = \frac{\text{Read of sample}}{\text{S.S x 5}} \times \frac{\text{Sample volume}}{\text{S.S}} \times \frac{\text{S.S.W}}{\text{S.W}} \times 100$$

Where:

S.S = standard solution.
S.W = sample weight.
S.S.W = standard solution weight.
Digestion of the samples:

1- Sulphuric acid M.W: 98.7% ($4H_2So_4$).
2- Hydrogen peroxide solution 30% (H_2).

Extraction of nitrogen from that samples:

1- Sodium hydroxide scales M.W 40% (NaoH).
2- Boric Acid P. Powder (H_3Bo_3).
3- Ethyl alcohol absolute M.W. % (C_2H_5-oH).

(Indicator :)
a. Bromocresol green.
b. Methyl red.

4- Hydrochloric acid(30.34%) (2 Hcl).
5- Ammonium sulphate (98.5%) [WH_4) So_4].

All data were organized and programmed using a computer for statistical analysis according to Factorial randomized complete block design (Duncan.,1955).

Field experiments:

Twenty four honey bee colonies of first hybrid Italian bees, *Apis mellifera logastica* of about equal strength containing at least three frames of brood covered with bees were randomly chosen in an apiary at Sakha Agricultural Research Station, Kafr El-Sheikh governorate. In late summer of 2006 and 2007/2008, the colonies were arranged in two groups of twelve colonies each, which were divided into two groups each group represent two treatments, three replicates for each.

Diets:

Several food stuffs containing considerable amounts of protein and sources available in cheap prices in the local market were chosen for this study. These stuffs were as follows:

1. Soybean + sugar powder + agwa (4:3:1 w/w).
2. Chick-pea + sugar powder + Brower's yeast + agwa (4:3:1:1 w/w)

For all diet treatments, the following stuffs were added (orange peels + carrot peels + apple peals + canations oil (1:1:1 w/w) or anise oil (5 cm^3) or 5 cm^3 flours of these stuffs were sifted using different sets of siftes with different mesh. Thereafter, they were mixed with sucrose solution (1:1 v/v) making pastes and offered to the bees in the cake form placed directly over the brood nests covered with plastic sheets to avoid drying.

RESULTS AND DISCUSSION

Protein content of ovaries in virgin queens:

Date in Table (1) show the effect of different factors on the ovaries protein content (mg/g) of virgin queens during different seasons (2007) The highest value was recorded in one day larval age reared in late summer in plastic cups with a mean value of 17.96 (mg/g). The lowest value was recorded in two days larval age reared in wax cups with a mean value of 14.907 (mg/g). The differences among means of different treatments are significant .

Date in Table (2) show the effect of different factors on the protein (mg/g) of ovaries of virgin queens during different seasons (2008). The highest value was recorded in one day larval age reared in summer in plastic cups with a mean value of 16.909 (mg/g), while the lowest value was recorded in two days larval age reared in summer in plastic cups (13.093 mg/g).

Table (1): Effect of some factors on the ovaries protein content (mg/g) of virgin queens during different seasons (2007).

Larval age	Season	Type of queen cup	Mean ± S.E(mg/g)
One day	Spring	Wax	16.639±0.642 abcd
		Plastic	17.805±0.678 ab
	Summer	Wax	16.639±0.578 abcd
		Plastic	17.244±0.234abc
	Late summer	Wax	16.311±0.494 abcdef
		Plastic	17.96±0.616a
Two days	Spring	Wax	16.149±0.991 bcdef
		Plastic	16.089±0.111 cdef
	Summer	Wax	15.122±0.279 ef
		Plastic	15.462±0.784 def
	Late summer	Wax	14.907±0.481 f
		Plastic	15.462±0.344 def

L.SD at 0.05 = 1.669 Value F = 3.142*

Means marked with different letters are significantly different at 0.05 level of probability.

Table (2): Effect of different factors on the protein (mg/g) of ovaries of virgin queens during different seasons (2008).

Larval ages	Season	Type of queen cup	Mean ± S.E(mg/g)
One day	Spring	Wax	15.880±0.29a
		Plastic	16.367±0.49ab
	Summer	Wax	14.318±1.47abc
		Plastic	16.909±0.65a
	Late summer	Wax	15.199±0.71abc
		Plastic	16.306±0.40ab
Two days	Spring	Wax	14.984±0.97abc
		Plastic	14.016±0.31
	Summer	Wax	14.925±1.32abc
		Plastic	14.633±0.68abc
	Late summer	Wax	14.656±0.48abc
		Plastic	13.093±1.20c

L.SD at 0.05 = 2.452 Value F = 1.631ns

Means marked with different **letters were significantly differ at 0.05 level of probability.**

Date in Table (3) show the effect of different factors on the protein content (mg/g) of ovaries in virgin queens reared on different diets during late summer year (2007). The highest value was recorded in one day larval age reared in soybean plastic, wax cups with a mean value of 17.233 (mg/g), while the lowest value was recorded in two days, soybean in wax cups with a mean value of 14.619 (mg/g).

Table (3): Effect of factors on the protein content (mg/g) of ovaries in virgin queens reared on different diets during (2007).

Larval age	Diet	Type of queen cup	Mean ± S.E(mg/g)
One day	Natural pollen grains	Wax	16.854±0.359ab
		Plastic	16.622±0.264ab
	Soybean	Wax	17.233±0.437a
		Plastic	17.233±0.306a
	Chick pea	Wax	15.188±1.923ab
		Plastic	17.096±0.550a
Two days	Natural pollen grains	Wax	15.462±0.727ab
		Plastic	16.494±0.427ab
	Soybean	Wax	14.619±0.869b
		Plastic	16.089±0.638ab
	Chick pea	Wax	15.188±0.903ab
		Plastic	16.254±0.560ab

L.SD at 0.05 = 2.306 **Value F = 1.243ns**
Means marked with different letters significantly at 0.05 level of probability.

Date in Table (4) show the effect of different factors on ovaries protein content of virgin queens reared on different diets during late summer year (2008). The highest value was recorded in one day larval age reared in soybean plastic cups with a mean value of 17.233 (mg/g), but the lowest value was recorded in two days larval age feden chick pea plastic cups with a mean value of 14.619.

Table (4): Effect of factors on the ovaries protein content of virgin queens reared on different diets during late summer (2008).

Larval ages	Different diets	Type of queen cups	Mean ± S.E
One day	Natural pollen grains	Wax	16.854±0.425a
		Plastic	17.016±0.564a
	Soybean	Wax	16.700±0.416ab
		Plastic	17.233±0.303a
	Chick pea	Wax	16.494±0.227ab
		Plastic	17.025±0.490a
Two days	Natural pollen grains	Wax	16.318±0.367ab
		Plastic	15.880±0.295ab
	Soybean	Wax	16.306±0.165ab
		Plastic	16.254±0.473
	Chick pea	Wax	15.188±0.782ab
		Plastic	14.619±2.182b

L.SD at 0.05 = 2.214 **Value F = 1.049ns**
Means marked with different letters significantly at 0.05 level of probability.

From Tables (1, 2, 3, 4), factors affecting ovaries protein were the queen cups type, and different diets with the larval age had no effect on the ovaries protein

El-Bahnasswy (1998) , found significant difference among different races in foraging honeybee workers during winter and summer but no significant differences were found during autumn and spring. It was clear that

the blood protein levels were significantly higher in the Egyptian race than in the others.

Mahmoud (2005), in Egypt, studied the effect of different wet grafting methods of queen rearing (a fresh royal jelly diet, royal jelly+ 10% honey, royal jelly+ 10% stored pollen and royal jelly+ 5% stored pollen+ 5% honey) on total protein in the ovary of virgin queen and found that the greatest rate of protein was found in ovaries of virgin queens resulting from using pure royal jelly. The lowest level of protein was found in ovaries when feeding on royal jelly+ 10% stored pollen. He added that pure royal jelly could be advised for bee-keeping task in Egypt..

Nitrogen content of queen body regions :

Date in Table (5) showed the effect of different diets on nitrogen content of queens body region nitrogen content during late summer, year (2007). The highest nitrogen value was recorded in soybean plastic cups queen thorax with a mean value of 3.146, while the lowest value was recorded in natural pollen grains wax queen head with mean value of 0.446. The differences between means were significant .

Table (5): Effect of different diets on nitrogen content of queens body during late summer, (2007).

Diet	Queen body part	Cup type	Means
Natural pollen grains	Head	Wax	0.446f
		Plastic	0.873def
	Thorax	Wax	2.903ab
		Plastic	2.463abc
	Abdomen	Wax	2.238bc
		Plastic	3.013ab
Soybean	Head	Wax	0.468ef
		Plastic	0.729ef
	Thorax	Wax	2.198bc
		Plastic	3.146a
	Abdomen	Wax	2.451abc
		Plastic	2.480abc
Chick pea	Head	Wax	0.624ef
		Plastic	0.546ef
	Thorax	Wax	2.194bc
		Plastic	2.962ab
	Abdomen	Wax	1.680cd
		Plastic	1.284de

L.SD at 0.05 = 0.834 **Value F = 10.942****

Means marked with different letters are significantly different at 0.05 level of probability.

Virgin queens storage.

The differences between means as in Tables (5&6), may be due to different queens body regions and cups type in season 2008, while in season 2007 all factors have a clear effect. Many authors discussed the factors affect queens body regions and found that

Ivannov and Spasov. (1990) stated that nitrogen was highest in summer. Body weight and head weight were highest in summer and

decreased in winter. Compared to seasonal alterations, differences between colonies managed for in various ways between weak and strong colonies, and between colonies with or without on seam disease were smaller and insignificant.

Salem (2002) found that the percentage of nitrogen content in the body ash of newly emerged queens increased through different months. April was the highest month (6.95%) in this respect. The nitrogen content varied with different queen rearing methods. The author found that the highest mean was 5.92 ± 0.78% in miller method while the lowest one observed with Doolittle.

Date in Table (6) show the effect of different diets on nitrogen content for different region of queens during late summer, year (2008). The highest nitrogen value was recorded in soybean plastic cups queen thorax with a mean value of 3.372. While the lowest value was recorded in natural pollen grains wax queen head with a mean value of 0.521. The differences between means were significant.

Table (6): Effect of diets on nitrogen content of queens body parts during late summer, (2008).

Different diets	Different body parts queens	Cups type	Means
Natural pollen grains	Head	Wax	0.521e
		Plastic	0.664de
	Thorax	Wax	3.023ab
		Plastic	2.955ab
	Abdomen	Wax	2.955ab
		Plastic	2.200bc
Soybean	Head	Wax	0.603e
		Plastic	0.880de
	Thorax	Wax	2.922abc
		Plastic	3.372a
	Abdomen	Wax	2.320abc
		Plastic	2.591abc
Chick pea	Head	Wax	0.546e
		Plastic	0.635de
	Thorax	Wax	2.291bc
		Plastic	2.722abc
	Abdomen	Wax	2.015bc
		Plastic	1.675cd

L.SD at 0.05 = 1.052 **Value F = 7.572****

Means marked with different letters significantly at 0.05 level of probability.

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تـأثير بعض العوامـل المختلفـة علـى المحتـوى البروتينـي لمبـايض عـذارى الملكـات والمحتوى النتروجيني لمناطق الجسم المختلفة لعذارى الملكات

أشرف شريف فتحي و قطب ابراهيم محمد هلالي
مركز البحوث الزراعية معهد وقاية النبات قسم بحوث النحل

تمت هذه الدراسة في منحل محطـة البحـوث الزراعيـة بسـخا لمعرفـة العوامـل التـي تـؤثر علـى المحتـوى البروتينـي لمبـايض الملكـات والمحتـوى النتروجينـي فـي رأس وصـدر وبطـن الملكـات العـذارى . بينـت النتـائج أن الملكـات التـي تربـت فـي كـؤوس مـن البلاسـتك أحتـوي علـى نسـبة بـروتين أعلـى مـن التـي تربـت فـي كـؤوس شـمعية . سـجلت أعلـى نسـبة للنتـروجين فـي البطن والصـدر والـرأس فـي الملكـات التـي ربيـت فـي كـؤوس بلاسـتيكية مـع التغذيـة بفـول الصـويا بمتوسـط ٣.٣٧٢ ملليجرام/جـرام بينمـا سـجلت أقـل قيمـة فـي رؤوس الملكـات التـي تربـت فـي كـؤوس شـمعية مـع التغذيـة بحبـوب اللقـاح بمتوسـط ٠.٥٢١ ملليجرام/جرام . أعلى نسبة نيتروجين سجلت في صدور الملكات التي تربت في كؤوس بلاستيكية مع التغذية بفول الصويا بمتوسط ٣.١٤٦ ملليجرام/جرام بينما سجلت أقل قيمة فـي الملكـات التـي تربـت فـي كـؤوس شـمعية مـع التغذيـة بحبـوب اللقـاح بمتوسـط ٠.٤٤٦ ملليجرام/جرام.

قام بتحكيم البحث

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