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# EFFECTS OF FEEDING POLLEN SUBSTITUTES ON THE ROYAL JELLY AND HAEMOLYMPH OF HONEYBEES

**Zaitoon, A. A.**
**Department of Economic Entomology, Faculty of Agriculture, Alexandria University, Egypt**

## ABSTRACT

Haemolymph protein and quality of royal jelly produced by honeybee colonies fed only on pollen substitutes were compared with those of natural colonies. These pollen substitutes were check pea-milk and defatted soy flour. The total sugar amounts of crude protein and 10-hydroxy-2-decenoic acid varied slightly but within the reported range for natural royal jelly. No detectable differences occurred in the electrophoretic densitogram of water-soluble protein and haemolymph protein. Idealy no differences were noted in the characters of queen bees reared on the royal jelly.

## INTRODUCTION

Honeybees depend mainly on pollen as a source of protein, lipids, minerals and vitamins, necessary for normal growth and activity of different castes ( Whal, 1963 and Kleinschmidt and Kondos, 1978). Since no single pollen source provides all their nutritional needs, honeybees must have a number of pollens available to them to remain healthy and to produce the royal jelly required to feed the queen and rearing brood. When colony inspections show little or no pollen in the combs, or the anticipated weather is going to prohibit pollen foraging fro more than a couple days, it is time to feed some pollen substitutes (PS) and sugar syrup at the same time. Szymas and Przybyl (1995) found that bees fed PS with some amino acids showed satisfactory development of hypopharyngeal glands fat body and ovaries. Nabors, (2000) demonstrated that feeding PS to colonies in early spring was more productive than those which were not given PS. El-Shemy (1997) found that simulative feeding through autumn with any diet increased the activities of colonies. Also stated that protein feeding is important for queen rearing. Imdorf *et al.* (1988) showed that protein feeding caused an increase in brood rearing. Colonies that have no access of protein during active season have a reduced capacity to rear replacement bees (Cremoner *et al.*, 1998). Pollen or PS is necessary to maintain strong colonies (Herbert, 1992). The effect of PS on the RJ produced from colonies fed only PS has not been completely investigated. Chang *et al.* (1993) reported that feeding bees with some PS increased the 10-HDA content of RJ. Herbert and Shimanuki (1983) found that no significant differences occurred between the pH of worker jelly produced by caged bees fed on PS and the pH of bees fed on pollen. The composition of RJ varies with seasonal climatic and ecological conditions (Karaali *et al.*, 1988). Bitondi and Simoes (1996) and Cremoner *et al.* (1998) measured total protein in the haemolymph in young bees for testing protein diets for honeybees. Barchuk *et al.* (2002) showed that in both castes of honeybees the appearance of vitellogenin in the

haemolymph occurs during the pupal period but the timing was different in the queen and workers.

The objective of this study is to investigate the effect of feeding honeybee with pollen substitutes on the quality of royal jelly, resultant queen differentiations and haemolymph protein.

## MATERIALS AND METHODS

Three experimental colonies of the carniolan hybrid (*Apis mellifera* L.) each including about 2kgs of worker bees of different ages were chosen at the apiary in the North Tahrir region. Each hive was provided with pollen traps to collect pollen before coming inside the colonies. Sealed brood frames were obtained from the apiary of the Department of Economic Entomology, at the Experimental Farm of the Faculty of Agriculture, Alexandria University and other private apiaries. The experimental diets were put in plastic Petri dishes (9 cm diameter, 1 cm depth) placed upside down over the central part of the top bars. The diet was replaced every day and Ca 60 % sucrose solution was offered from the feeder.

**Diets:**

Two pollen substitutes were chosen:

1. Check pea-milk diet, consisting of 25g check, 25g milk powder, 50g sucrose, 5g cellulose powder and 50 ml water.
2. Soy flour diet consisting of 50 g defatted soy flour, (43% protein), 50 g sucrose, 5g cellulose powder and 70 ml water.

A pollen diet was used as one of the two controls, and was composed of 50 g of pollen loads stored for one month at -20°c (18% protein), 50 g sucrose, 10 g cellulose and 20 ml water. In the other control colony was left without pollen traps.

Collection of royal jelly:

Royal jelly (RJ) was collected after [25 days of constructing the colonies. After removing the queen from each colony, young worker larvae not older than 24 hrs from hatching, were grafted into plastic queen cell cups from the mother colony]. They were left in the colony for 48 hrs until collection. The removed queen was confined in an incubator with a few workers during the collection of RJ and released back into the mother colony. Collection of RJ was lyophilized and stored at -20° C until measurement analysis of RJ.

**Crude protein:**

The total nitrogen was determined by the semi-micro Kjeldahl method at the Department of Animal Production, Fac. of Agric., Alexandria and the crude protein content was estimated as total nitrogen × 6.25.

**Sugar:**

The total sugar content was analyzed by the phenol sulfate method using glucose for the standard (Dubois *et al*,. 1956).

**10- hydroxy-2-decenoic acid (10-HDA):**

10-HDA was extracted with diethyl ether (Yoneyama *et al.* 1976) and analyzed by gas chromatography (Shimadzu 4cm) at the Faculty of Science, Alexandria using a 3m x 3mm glass column packed with SE-30 on 80-100 mesh chromosorb w., column temperature was programmed at a rate of 3°C/ min. with the initial temperature of 180°C, and the final temperature was 220°C. The internal standard was heptadecenoic acid.

**pH:**

pH was measured by a pH apparatus.

**Water soluble protein:**

Slub electrophoretic separation was determined at the Department of Dairy science, Fac. of Agric., Alexandria University with polyacrylamide gel. The lower separation gel contained 5.6% acylamide, while the upper stacking gel contained 2.5% acrylamide. The electrophoresis was accomplished at 1.5 m A per column with tris-grisine buffer, running at pH 8.3. The developed gel was stained with amido black and the densitogram was obtained from the optical density at 560 nm.

**Queen rearing:**

The queens were reared in different test colonies provided with pollen traps. Each colony contained 1Kg (ca 10.000) workers of different ages. A mated queen and newly built empty combs and offered the different pollen substitutes. Young worker larvae were grafted into plastic cups after 25 days, and the queens were removed after sealing. Capped queen cell cups were removed and kept in an incubator at 34°C and 75% RH. Morphological characters of emerged queens were evaluated according to the criteria of Rembold (1976).

**Haemolymph studies:**

Newly emerged bees were obtained for the haemolymph studies by placing combs of sealed brood in an incubator at 35°C. Emerged bees were removed from the combs into small cages. Each test diet was fed to four cages, each containing 25 bees.

The haemolymph of bees of different ages was collected from a small incision at the level of the 3 $^{rd}$ dorsal tergite, using microcapillary tubes was led in a 0.1% phenylthiourea solution in water. The haemolymph of workers from different diets and ages was stocked in microcapillary tubes at -20°C for determination of haemolymph protein.

Slub electrophoretic separation was made with polyacrylamide gel as for RJ. Also the densitogram was obtained from the optical density at 560 nm.

## RESULTS AND DISCUSSION

The present investigation aimed to describe the qualitative aspects of the royal jelly produced from the colonies reared only on supplementary

diets without pollen, and also for haemolymph protein. In this respect the diets were rather simple.

The amount of diet consumed, brood reared, RJ stored in queen cells and the degree of hypopharyngeal glands development of nurse bees will not include in this study as fundamental data of the experimental colonies.

**Chemical nature of Royal jelly:**

Crude protein, total sugar, 10-HDA and pH of the RJ collected from the experimental colonies are compared in Table 1.Crude protein content varied from 29.8 to 35.0 % . The range is lower than that reported by Tomoda *et al.* (1974) and Lercker *et al.* 1982. Crude protein content of RJ collected from the pollen diet was rather lower than that from check pea-milk. Herbert *et al.* (1977) reported that the optimum amounts of brood continued to be reared by bees fed on diets containing 23 % to 30 % protein. Thus the low protein content of pollen diet was 9.3 % ob basis of dry weight. The total sugar content was similar to that reported by Takenaka and Echigo (1980) and Zaitoon (2001).

**Table 1: Comparison of three major constituents and pH among RJs collected from colonies fed on pollen substitutes**

| Diet (%protein) | pH | Crude protein (%)* | Total sugar (%)* | 10-HAD (%) |
|---|---|---|---|---|
| Control 1Free | 4.1 | 31.1 | 43.6 | 4.4 |
| Control 2(pollen) (9.3) | 4.0 | 29.8 | 50.7 | 6.4 |
| Check pea Milk (21.8) | 4.0 | 35.0 | 49.1 | 6.5 |
| Soya flour (20.5) | 4.1 | 30.7 | 43.0 | 6.9 |

*Dry weight basis

The 10-HDA content varied from 6.4% to 6.9%, which was similar to the content of normal colonies. The pH values of RJ samples were within the range of 4.0 to 4.1.

No differences were found between control and the experimental diets. The responsible acids were not determined, but the results were attributed to the gluconic and many other kinds of water-soluble acids which have been found in the honey produced by a confined colony fed only on sucrose solution (Echigo and Takenaka, 1974). Electrophoretic densitograms of the water–soluble protein had three main peaks in addition to a small combined peak (Fig.1).

There were no differences between the diets, except for a slight difference in the small combined peak, which is situated towards the cathode side.

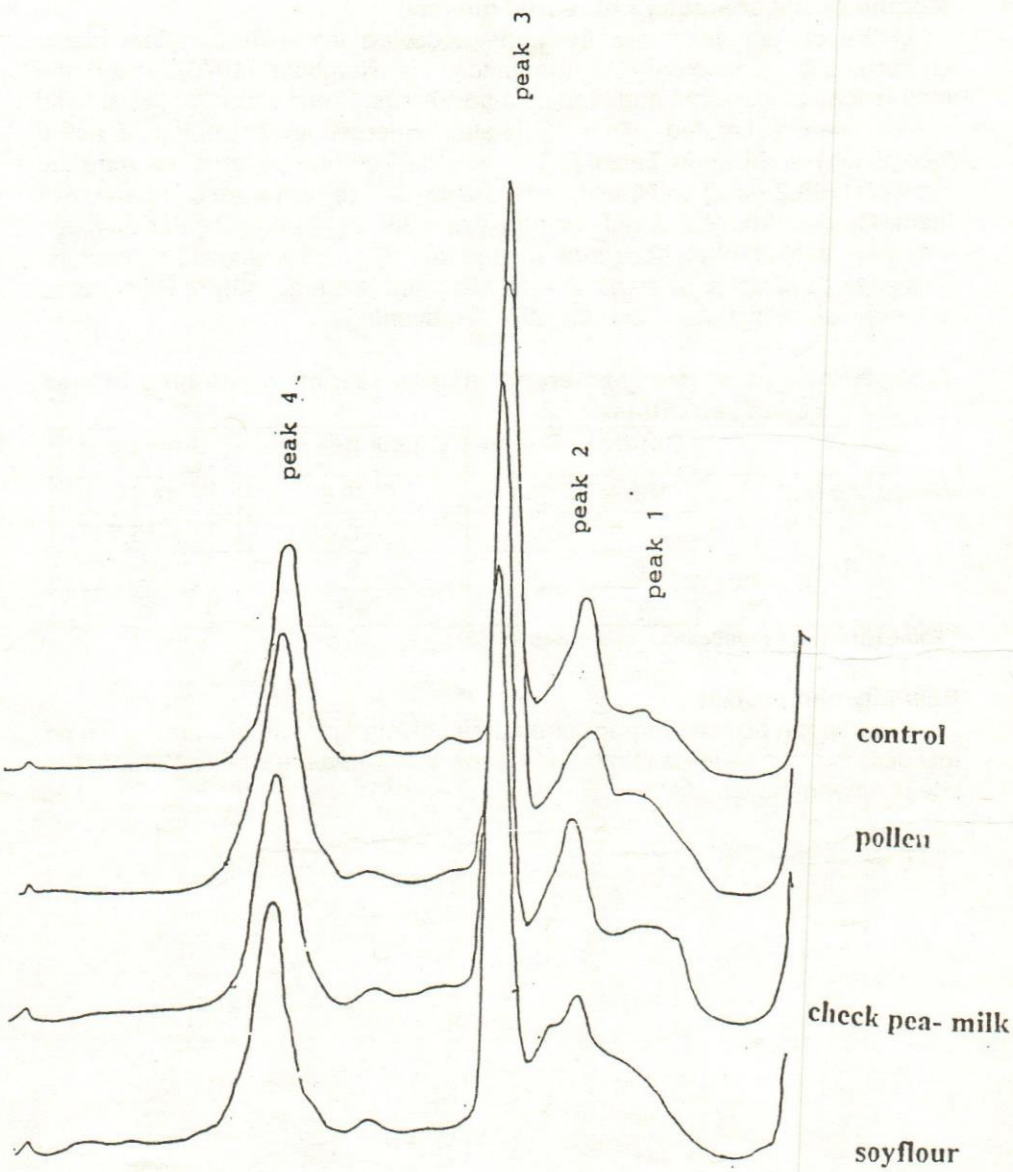


Fig. 1 Electrophoretic patterns of the royal jelly for the colonies fed on test diets.

**Morphological characters of reared queens:**

All the queens produced from the accepted larvae had typical queen characteristics according to the criteria of Rembold (1976), using the morphology of mandible and hind tibia-basitarsus. Fresh weight of the starved queens were estimated within 12 h after emergence (beginning of active locomotion) is shown in Table 2. The queens from the pollen diets were the heaviest (198.2 ± 4.2) and those from the check pea-milk were comparable to them. Queens from the soy flour only diet were significantly lighter although they were evaluated as complete queens from the morphological characters. This adverse effect is probable due to attributed to the quantity of RJ supplied to the queen cells, but not to qualitative disadvantages.

Table 2: **Weight of newly-emerged queens reared in colonies offered pollen substitutes**

| | Control | Pollen | Check pea milk | Soya flour |
|---|---|---|---|---|
| Weight(mg+sd) | 180.8 | 198.2 | 186.0 | 165.9 |
| | ± 2.0 | ± 4.3 | ± 6.3 | ± 15.7 |
| n. | 8 | 7 | 8 | 8 |
| | a* | b | ab | c |

*** Same letters not significantly different ($p < 0.05$)**

**Haemolymph protein:**

Electrophoretic densitogram of the haemolymph protein of workers fed on test diets in small cages is shown in Figures 2-4. There are small differences in peaks among the test diets.

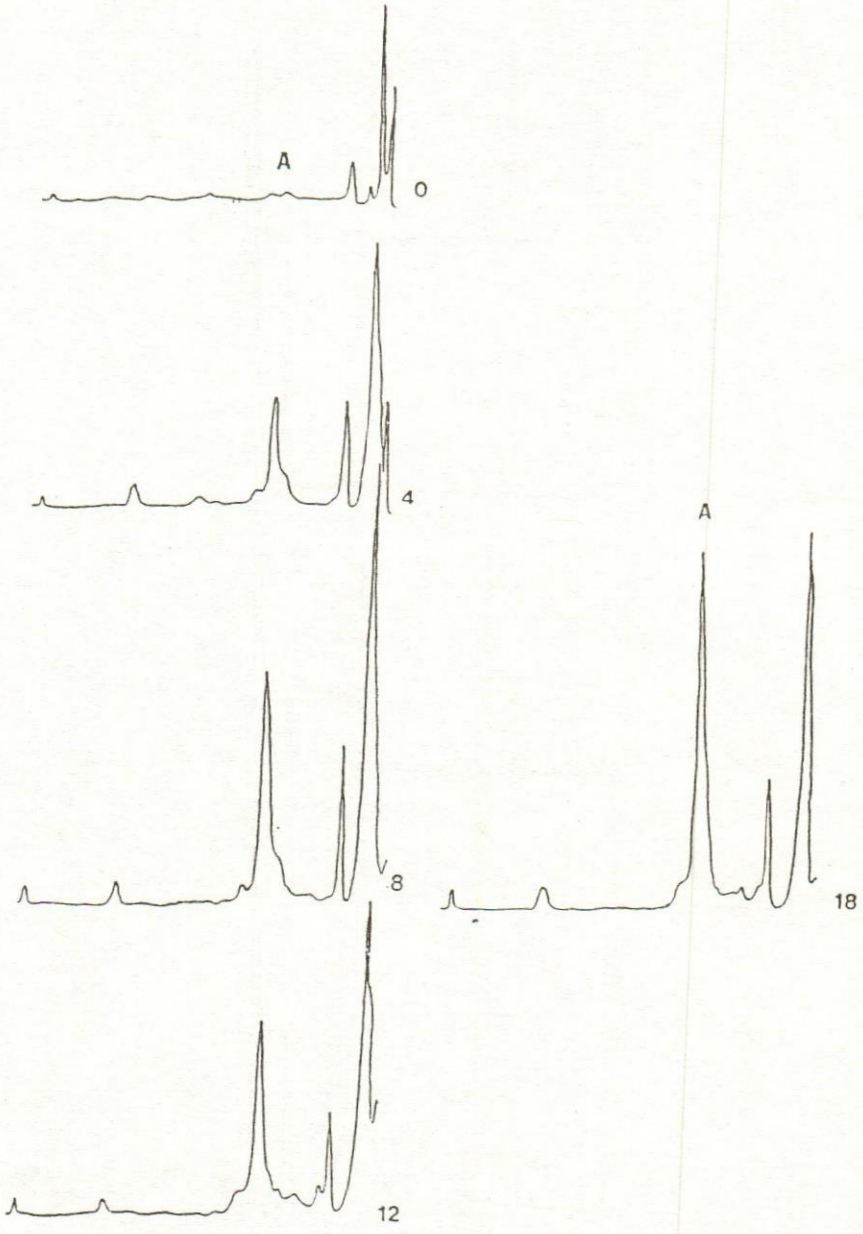


Fig. 2 Electrophoretic patterns of the haemolymph protein of worker bees of different ages.

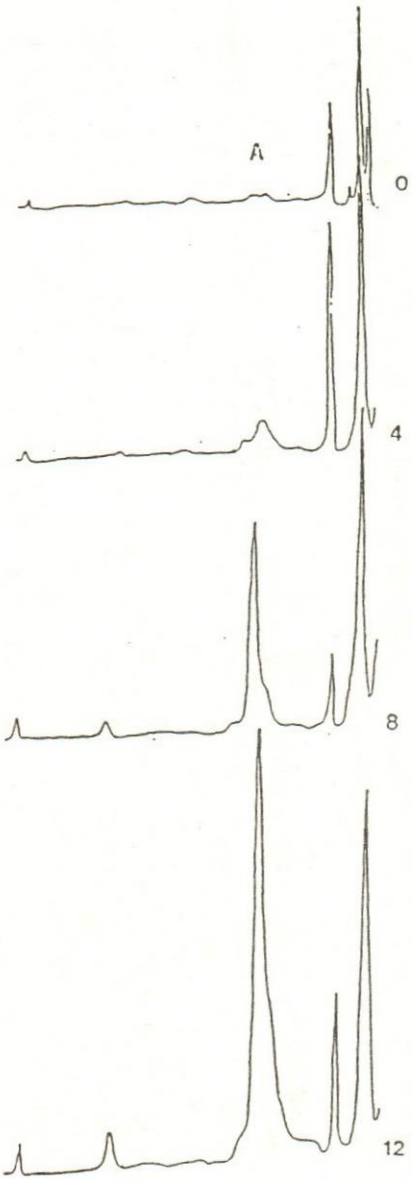


Fig. 3 Electrophoretic patterns of the haemolymph protein of worker bees of different ages.

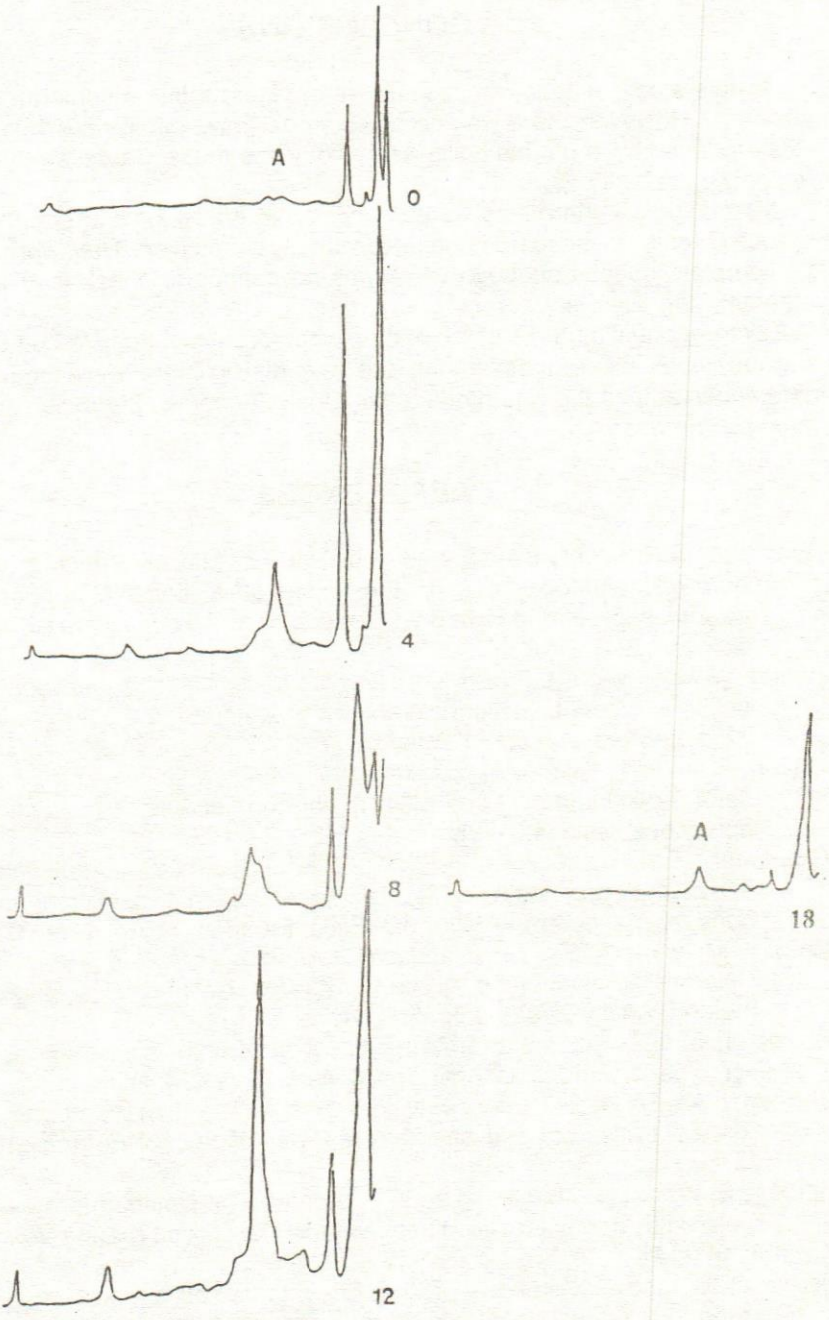


Fig. 4 Electrophoretic patterns of the haemolymph protein of worker bees of different ages.

## CONCLUSIONS

In this study, sugar composition, vitamin and mineral contents were not checked. However, there was no notable qualitative differences between the RJs produced by a natural pollen-fed colony and those produced by colonies fed only on substitutes.

Morphological characters were checked and the weights of queens reared in such colonies isolated from the pollen supply were given. They were found to be complete queens although there was no chance to check their ability as normal laying queens.

Electrophoretic patterns of the haemolymph protein of bees fed on test diets showed small differences among the test diets. There were quantitative differences among the RJs produced but the inferiority of the pollen substitute must be improved.

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## التغذية ببدائل حبوب اللقاح على الغذاءالملكي و الهيموليمف في حشرات نحل العسل

**أحمد علي زيتون**

**كلية الزراعة – جامعة الأسكندرية – قسم الحشرات الأقتصادية**

أجرى البحث لدراسة تأثير التغذية ببدائل حبوب اللقاح على الغذاء الملكى والهيموليف فى نحل العسل . وقد تمت مقارنة بروتينات الهيموليف و نوعية الغذاء الملكي المنتج في نحل العسل بواسطة خلايا غذيت فقط على بدائل حبوب اللقاح مع نظائرهما المنتجة من الخلايا التي تتغذى طبيعياً على حبوب اللقاح التي يجمعها النحل من الأزهار. هذه البدائل هي الحمص مخلوط باللبن البودرة و كذلك فول الصويا المنزوع الدهن. وقد أظهرت النتائج أن السكر الكلي ، البروتين الخام و 10-HDA في الغذاء الملكي كانوا مختلفين إختلافا طفيفا فيما بينهم و بين نفس المكونات في الغذاء المنتج من خلايا طبيعية ، و لم توجد فروق محسوسة بالـ electrophortic densitogram لكل من بروتينات الغذاء الملكي المنتج من خلايا تم تغذيتها على بدائل حبوب اللقاح و الأخرى الطبيعية. و حدث هذا أيضا بالنسبة للهيموليمف المأخوذ من خلايا بدائل حبوب اللقاح و كذلك الطبيعية. ومن جهه اخرى لم توجد فروق كبيرة بين صفات الملكات المرباة على الغذاء الملكي في كل من الخلايا المغذاة على حبوب لقاح و الأخرى المغذاة على بدائل حبوب اللقاح.