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EFFECT OF SOME FOOD ADDITIVES ON CAGED BEES UNDER LABORATORY CONDITIONS

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ABSTRACT: This work was carried out during spring and early summer of 2015 in Apiculture and Sericulture Research Laboratory, Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt for evaluating biological and physiological effects of 18 different diets as compared with control fed on sucrose syrup (1:1) on caged worker bees. The longest leathal time $_{50}$ (the time at which 50% of bees were dead) (LT_{50}) and leathal time $_{90}$ (the time at which 90% of bees were dead) (LT_{90}) and the maximal activity surface area of hypopharyngeal gland (HP) acini were recorded for caged bees fed on diet 16; composed of 4.5 pollen: 4.5 banana: 1 coriander: 90 sucrose (35.8, 54.1 days and 0.64 mm^2, respectively), followed descindingly by diet 18; 4.5 pollen: 4.5 date paste: 1 coriander: 90 sucrose (35.0, 50.2 days and 0.56 mm^2, respectively) and diet 12; 4.5 lentil flour: 4.5 date paste: 1 coriander: 90 sucrose (28.9, 38.5 days and 0.54 mm^2, respectively). The heaviest mean body weight was detected in case of the caged bees fed on diet 14; 4.5 pollen: 4.5 guava: 1 santonica: 90 sucrose (95.33 mg), followed by diet 16; 4.5 pollen: 4.5 banana: 1 coriander: 90 sucrose (88.92 mg), and diet 18; 4.5 pollen : 4.5 date paste: 1 coriander : 90 sucrose (86.38 mg) and diet 12; 4.5 lentil flour: 4.5 date paste: 1 coriander : 90 sucrose (81.75 mg). Therefore, survival and development of hypopharyngeal (HP) of caged bees could be depend upon for evaluating the efficiency of the test feed.

Key words: Food additives, caged bees, laboratory conditions.

INTRODUCTION

Pollen substitutes are necessary for honeybee colonies, especially when natural resources of nectar and pollen are unavailable or reduced. These are essential for development of young bees, rearing brood, reproduction and colony maintenance. These are ideal materials that provide required nutrients to bees **(Saffari *et al*., 2004; Zahra and Talal, 2008)**. The pollen substitute diet and pollen are equally accepted by the bees. The pollen substitute diet is thus, as highly palatable as natural pollen and easily provided as patties to colonies in standard hives **(Saffari *et al*., 2004)**.

Beekeepers feed colonies to stimulate brood rearing in the late winter or early spring. Colonies with limited nutritional reserves suffer from reduced brood rearing and a shorter adult workers lifespan. When the amounts of pollen and nectar were low or unavailable, protein supplements may help the colony survival and subsequently more honey production **(Yousif-Khalil, 1983; Nabors, 2000; Mattila and Otis, 2006)**.

The quality of food collected by honeybees has an important relationship to the overall hive development, and special attention must be given to the role that food plays on the development of the hypopharyngeal glands (HPG) **(Wcislo and Cane, 1996)**. HPG of workers of *Apis mellifera* L. (Hymenoptera: Apidae) have been morphologically and physiologically studied due to their importance on the production of royal jelly **(Gatehouse *et al*., 2004; Pinto *et al*., 2012)**.

Thus, such vital role of these glands provides motivation for improving the current knowledge on the development of adequate diets that stimulate the development of the glands, with the aim of empowering the production of this

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apicultural product of great nutritional properties. The size and secretion of hypopharyngeal glands (HPG) vary with the age of nurse bees, as well as the seasonal and regional conditions that surround them **(Deseyn and Billen, 2005; Costa-Leonardo *et al*., 2008)**.

The objective of this study was to evaluate the physiological and biological effects of different diets on caged bees under laboratory conditions.

MATERIALS AND METHODS

The present investigations were carried out in Apiculture and Sericulture Research Laboratory, Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt, during spring and early summer of 2015.

Test Workers

Newly emerged F_2 hybrid Carniolan bees (0-24 hr.), before consuming any pollen or honey, needed for the present study were obtained by screening sealed brood combs of colonies (nearly with the same strength and headed by sister queens of the same age) 24 hrs prior to emergence. The collected workers were divided into groups of 50 emerged worker bees each.

Cages Used

Wooden frame cages, each measuring 22 × 22 × 25 cm., with a side opening covered with plastic screen containing 14 mesh/inch were used.

Test Diets

The test diets were offered to caged bees in form of cakes (patties) using 90% sucrose for each cake. Basic tested materials were soybean flour, lentil flour, clover pollen, banana, guava, date paste (agwa), santonica and coriander. Plant seeds were ground until flour. Pollen were collected by pollen trap attached to the hive entrance during clover flow and kept in air tight plastic bags in freeze until use.

Feeding Bees

Groups of newly emerged bees (each of 50 individuals) were introduced in each cage. Such bees were offered about 40 g of tested diets in form of cakes at 6 day-intervals. Three replicates were made for each tested diet and control.

Judging Test Diets

The efficiency of the diets was judged basing upon three parameters (measurements); the longevity, the development of body weight and hypopharyngeal glands of caged bees.

Longevity of Caged Bees

Fifty newly emerged worker bees were confined all together and offered the tested diet in the aforementioned cages and all cages of each treatment were inspected daily to count and eliminate the dead bees. This experiment continued until all the bees died. For percentages of mortality, the LT_{50} and LT_{90} values (days) as well as the percentage of increase or decrease as compared to the control were calculated and also recorded.

Body Weight of Caged Bees

Samples of 5 bees of each cage were taken at 3-day intervals and weighed immediately until the end of their lives. The procedures were repeated three times.

Development of the Hypopharyngeal Glands

The development of the hypopharyngeal glands of caged bees was measured at 6, 9 and 12 days of workers age that fed on the aforementioned diets. The glands were dissected out, then the maximum length and width of 20 acini of the glands were measured for each worker in each experiment. Stereoscope microscope provided with ocular micrometer lens was used for this purpose. Fifteen workers were used to represent each treatment. Thereafter, the values of acinal surface area were modified into mm^2 according to the power of ocular and objective lenses used. Acinal surface was calculated according to Maurizio's formula **(Maurizio, 1954)**:

$$\text{Acinal surface area} = T_T \frac{a \times b}{2}$$

Where:

a= maximum length

b= maximum width and T_T= 3.14

Data obtained were statistical analyzed (analysis of variance) according to **Snedecor and Cochran (1967)** method.

Table 1. The test food mixtures composition

Diet No.	Food mixture (diet)						
1	4.5 soybean flour	:	4.5 banana	:	1 santonica	:	90 sucrose
2	4.5 soybean flour	:	4.5 guava	:	1 santonica	:	90 sucrose
3	4.5 soybean flour	:	4.5 date paste	:	1 santonica	:	90 sucrose
4	4.5 soybean flour	:	4.5 banana	:	1 coriander	:	90 sucrose
5	4.5 soybean flour	:	4.5 guava	:	1 coriander	:	90 sucrose
6	4.5 soybean flour	:	4.5 date paste	:	1 coriander	:	90 sucrose
7	4.5 lentil flour	:	4.5 banana	:	1 santonica	:	90 sucrose
8	4.5 lentil flour	:	4.5 guava	:	1 santonica	:	90 sucrose
9	4.5 lentil flour	:	4.5 date paste	:	1 santonica	:	90 sucrose
10	4.5 lentil flour	:	4.5 banana	:	1 coriander	:	90 sucrose
11	4.5 lentil flour	:	4.5 guava	:	1 coriander	:	90 sucrose
12	4.5 lentil flour	:	4.5 date paste	:	1 coriander	:	90 sucrose
13	4.5 pollen	:	4.5 banana	:	1 santonica	:	90 sucrose
14	4.5 pollen	:	4.5 guava	:	1 santonica	:	90 sucrose
15	4.5 pollen	:	4.5 date paste	:	1 santonica	:	90 sucrose
16	4.5 pollen	:	4.5 banana	:	1 coriander	:	90 sucrose
17	4.5 pollen	:	4.5 guava	:	1 coriander	:	90 sucrose
18	4.5 pollen	:	4.5 date paste	:	1 coriander	:	90 sucrose
Control	bees were offered sucrose solution (1:1) alone						

RESULTS AND DISCUSSION

The influences of the aforementioned test diets on the longevity, body weight and the development of the hypopharyngeal glands of the caged bees were studied and the results can be summarized as follows:

Effect of Feed Additives on Longevity of Caged Emerged Bees

The effect of different diets on the longevity of caged emerged bees is presented in Table 2.

The longest LT_{50} (The time at which 50% of bees were dead) that reached 35.83 days was recorded in case of the caged bees fed on diet 16, followed by 34.98 days which recorded for diet 18 and 28.9 days with diet 12. The longevity of caged bees fed on diet 14 and diet 13 was nearly similar (10.27 and 10.18 days, respectively) with control (10.3 days). Meanwhile, caged bees fed on most other diets lived shorter than the control.

The longest LT_{90} (54.08 days), representing 292.4% of control and 50.17 days representing 271.4% of control was recorded in case of the caged bees fed on diets 16 and 18, respectively, followed by 38.53 days representing 208.1% of control, with diet 12. In case of the diets contained soybean flour plus coriander, the length of life of caged bees was longer than that recorded in the control, while the opposite was noticed for diets contained soybean flour + santonica.

A highly significant differences ($P < 0.01$) were observed between the mean LT_{50} and LT_{90} after feeding with different diets.

Table 2. The mean longevity of caged bees as affected by feeding on the test food mixtures, expressed as LT_{50} and LT_{90}

Diet No.*	LT_{50} (day)	Control (%)	LT_{90} (day)	Control (%)
1	5.23	50.5	8.83	47.6
2	4.27	41.7	7.30	39.5
3	3.90	37.9	6.33	34.1
4	16.00	155.3	28.60	154.6
5	9.00	87.4	21.50	116.2
6	9.20	89.3	21.70	117.3
7	7.32	70.9	14.42	77.8
8	5.33	51.5	9.58	51.9
9	8.25	80.1	14.05	76.2
10	16.27	158.3	31.83	171.9
11	15.52	150.5	29.43	158.9
12	28.90	280.6	38.53	208.1
13	10.18	99.0	18.73	101.1
14	10.27	100.0	20.93	113.0
15	11.58	112.6	19.48	105.4
16	35.83	347.6	54.08	292.4
17	17.10	166.0	28.65	155.1
18	34.98	339.8	50.17	271.4
Control	10.30	100.0	18.53	100.0
LSD 0.05	3.80		16 .16	
LSD 0.01	5.09		7.14	

(*) The composition of diets mentioned in Table 1.

It could be concluded that the longevity of caged bees seems to be related to diet constituents, as the longest longevity was observed when pollen plus coriander was the basic constituent of the diet. On the contrary, soybean flour + santonica and lentil flour + santonica as basic constituents in the diet had the adverse effect on the longevity of caged bees. Also, the results showed that the effect of feed protein materials on the longevity of caged bees was variable because it dependent on the supply of the required nutrients and lack of poisonous materials. The pollen which is a natural food for honeybees provides most of the protein, vitamins and minerals for honeybees resulting longer longevity of bees, but raw soybean flour led to the reduction of longevity probably due to its poisonous materials such as trypsin inhibitor **(Saffari *et al.*, 2010)**. In connection, **Irandoust and Ebadi (2013)** stated that feeding bees on soybean flour caused highest mortality of caged bees compared to the control bees fed on sucrose solution. The comparison of mean mortality according to the type of protein ingredients showed that pollen was the best protein source, compared to soybean meal that had more effect on the longevity of honeybees in the incubator.

Effect of Food Additives on Body Weight of Caged Bees

Results presented in Table 3 indicate that the heaviest mean body weight of caged bees at one-day old was 82 mg in caged bees fed on diets 7, 8 and 9 followed by diet 3 (81.33 mg) and diet 14 (79.33 mg), while the lightest weight was recorded in caged bees fed on diet 17 (73.33 mg). Diets 15, 16, 18 possessed nearly similar workers weight of control (74.67 mg). Statistical analysis showed insignificant difference between the mean weights of one day old bees, because these bees did not feed on the test diets.

At 3 days old caged bees, it was found the highest significant mean body weight was 90.67 mg, followed by 89.33 mg and 86.33 mg in case of caged bees fed on diets 14, 16 and 18, respectively. On the other hand, the other diets showed lighter weights nearly similar to that of the control.

Results presented in Table 3 indicate that caged bees of 6 days old fed on diet 14 showed the heaviest body weight (100.67 mg), followed by caged bees fed on diet 16 (87.33 mg) and diet 18 (83.33 mg). On the other hand, feeding caged bees on diets 11, 10, 5 and 9 appeared the lowest mean body weights recording 63.33, 68.67, 72.67 and 73.33 mg, respectively, and nearly similar to that of the control (74.67 mg).

At 9 days old bees, the results indicated that the highest significant mean body weight was (100.67 mg) in caged bees fed on diet 14, followed by diet 16 (90.67 mg) and diet 18 (90.00 mg), while the lowest one was recorded in caged bees fed on diet 11 (72.67 mg), being lighter than the control (78.00 mg).

At 12 days old bees, it was found the highest significant body weight was 99.33 mg, followed by 88.00 mg and 87.33 mg in case of caged bees fed on diets 14, 16 and 18, respectively. On the other hand, the lowest one was noticed with diet 9 (64.00 mg) and less than the control (68.00 mg).

At 15 days old bees, results presented in Table 3 indicate that caged bees fed on diet 14 showed the highest significant mean body weight (98.00 mg), followed by bees fed on diet 16 (94.67 mg) and diet 18 (94.67 mg). Meanwhile, control caged bees possessed the lightest one (67.33 mg). The other diets showed intermediate means.

At 18 days old bees, results in Table 3 indicate that caged bees fed on diet 14 showed the highest significant mean body weight (98.67 mg), followed by caged bees fed on diet 16 (96.67 mg) and diet 18 (86.67 mg). On the contrary, control caged bees showed the least one (70.00 mg).

At 21 days old bees, the results indicated that the maximum significant body weight was (90.00 mg) in caged bees fed on diet 16, followed by diet 18 (88.00 mg) and diet 12 (80.67 mg), while the lowest one (73.00 mg) was recorded in caged bees fed on diet 5.

In general, it could be mentioned that feeding caged bees on diet 14 (4.5 pollen + 4.5 guava + 1 santonica + 90 sucrose) gave the best results followed by diets 16 (4.5 pollen + 4.5 banana + 1 coriander + 90 sucrose), 18 (4.5 pollen + 4.5 date paste + 1 coriander + 90 sucrose) and 12 (4.5 lentil flour + 4.5 date paste + 1 coriander + 90 sucrose), regardless of age of caged bees. This may be due to pollens are considered the natural source of protein for the bees.

Effect of Food Additives on the Development of Hypopharyngeal Glands of Caged Bees

Results presented in Table 4 show that the acinal surface area of the glands in 6, 9 and 12 days old workers after confinement and feeding.

At 6 days old bees, results in Table 4 indicate that the maximum significant acinal surface area of HP was 0.59 mm^2 in caged bees fed on diet 16, followed by diet 18 (0.52 mm^2) and diet 12 (0.50 mm^2), while the lowest one was recorded in caged bees fed on diet 3 (0.18 mm^2), and less than the control (0.28 mm^2).

At 9 days old bees, it was found that the highest significant surface area was 0.84 mm^2, followed by 0.78 mm^2 and 0.76 mm^2 in case of caged bees fed on diets 16, 18 and 12, respectively. On the other hand, the lowest surface area was 0.25 mm^2 in offered diet 3 and it was less than the control (0.44 mm^2). The maximum activity of hypopharyngeal glands was observed during this age (9 days old).

Table 3. The mean body weight of caged emerged bees (mg) fed on the different diets

Diet No.*	1 day	3 days	6 days	9 days	12 days	15 days	18 days	21 days	Mean
1	78.00	77.33	76.00	79.33	75.33	——	——	——	77.20
2	77.33	78.00	79.33	80.67	——	——	——	——	78.83
3	81.33	78.67	80.00	80.67	——	——	——	——	80.17
4	76.67	80.67	82.00	81.33	79.33	80.67	83.3	73.33	79.67
5	76.00	76.00	72.67	78.00	62.67	72.67	72.67	73.00	74.63
6	76.00	80.67	80.00	81.33	78.67	80.00	80.67	75.33	79.08
7	82.00	77.33	74.67	78.00	74.67	78.67	71.33	76.00	76.58
8	82.00	77.33	77.33	80.00	72.00	——	——	——	77.73
9	82.00	74.00	73.33	75.33	64.00	76.67	74.00	——	74.19
10	76.00	73.33	68.67	74.67	76.00	78.67	75.67	78.00	75.13
11	78.67	68.67	63.33	72.67	77.33	78.67	80.67	78.67	74.84
12	78.00	82.67	82.67	84.00	79.33	83.33	83.33	80.67	81.75
13	75.33	77.33	78.00	78.00	74.00	77.33	74.67	79.33	76.75
14	79.33	90.67	100.67	100.67	99.33	98.00	98.67	——	95.33
15	74.67	85.33	82.67	85.33	81.33	84.00	84.67	——	80.57
16	74.67	89.33	87.33	90.67	88.00	94.67	96.67	90.00	88.92
17	73.33	80.00	80.00	81.33	76.67	78.67	76.67	79.33	78.25
18	74.67	86.33	83.33	90.00	87.33	94.67	86.67	88.00	86.38
Control	74.67	76.67	74.67	78.00	68.00	67.33	70.00	——	72.76
LSD 0.05	——	9.27	6.58	8.10	8.16	9.16	22.69	8.46	
LSD 0.01	——	12.41	8.82	10.85	10.93	12.27	30.39	11.33	

(*) The composition of diets mentioned in Table 1.

Table 4. The effect of different diets on the acinal surface area (mm^2) of hypopharyngeal glands of caged bees at different ages

Diet No.*	Workers age			
	6 days	9 days	12 days	Mean
1	0.27	0.43	0.16	0.29
2	0.24	0.52	——	0.38
3	0.18	0.25	——	0.22
4	0.32	0.53	0.27	0.37
5	0.36	0.53	0.24	0.38
6	0.28	0.51	0.14	0.31
7	0.48	0.68	0.34	0.50
8	0.32	0.61	0.33	0.42
9	0.34	0.58	0.21	0.38
10	0.27	0.46	0.17	0.30
11	0.29	0.52	0.17	0.33
12	0.50	0.76	0.36	0.54
13	0.27	0.50	0.19	0.32
14	0.31	0.43	0.20	0.31
15	0.39	0.63	0.23	0.42
16	0.59	0.84	0.48	0.64
17	0.41	0.65	0.34	0.47
18	0.52	0.78	0.37	0.56
Control	0.28	0.44	0.16	0.29
LSD 0.05	0.20	0.26	0.17	
LSD 0.01	0.26	0.35	0.23	

(*) The composition of diets mentioned in Table 1.

Results presented in Table 4 indicate that caged bees of 12 days old fed on diet 16 showed the largest significant acinal surface area (0.48 mm^2), followed by caged bees fed on diet 18 (0.37 mm^2) and diet 12 (0.36 mm^2). On the other hand, feeding caged bees on diet 1 manifested the lowest surface area (0.16 mm^2) being similar to that of the control. The other diets showed to give surface area more than the control. The activity of hypopharyngeal glands was less than the previous age.

Generally, it could be mentioned that the activity of hypopharyngeal gland (HP) increased sharply until reached its maximum at 9 days old caged bees then decreased until reached its minimum at 12 days old caged bees.

However, the development or activation of this structure is linked to some factors such as protein availability and quantitative and qualitative variations of this source **(Al-Ghamdi *et al.*, 2011)**. Obtained results from this study demonstrated that the type of diet does affect the development of caged bee glands. The highest activity of HP was observed in caged bees fed on diet 16, followed descindingly by diet 18 and diet 12. On the other hand, the least activity of this structure was detected in caged bees offered diet 3.

Honeybee workers prefer natural pollen grains over pollen substitutes which were less beneficial to bees than pollen as a source for protein. Therefore, the diets contained pollen plus coriander gave the best results. On the other hand, diets contained soybean flour + santonica gave the least results. These results are in agreement with those of **Al-Ghamdi *et al.* (2011)** who stated that food palatability and absorption are factors that should be taken into account when providing honeybees with supplemental diets. They also, found that supplements with greater protein content may not always be the most efficient. Foods based on soybean might be rejected or only consumed in small amounts for not presenting attractive organo leptic characteristics. Also, **Abdilla (2005)** stated that addition of pollen to honeybee diet activated the hypopharyngeal glands development. **Alqarni (2006)** indicated that the normal source of protein for honeybee workers as bee bread or date palm pollen was the best source for hypopharyngeal gland development. **Pinto *et al.* (2012)** stated that bees that were fed on sucrose solution or soybean extract presented the smallest acini areas of hypopharyngeal gland as compared to the other treatments.

In conclusion, the longest LT_{50} and LT_{90} and the maximum activity of HP were observed in case of the caged bees fed on diets 16, 18 and 12. Meanwhile, the heaviest mean body weight was detected in case of the caged bees fed on diets 14, 16, 18 and 12. Therefore, survival and development of HP of caged bees seem to be useful criteria for evaluating the effect of tested food.

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تأثيـــر بعـــض الإضـــافـــات الغـــذائيـــة علـــى نحـــل الأقفـــاص تحـــت ظـــروف المعمـــل

سلوى أحمد محمد رمضان – سعد إبراهيم يوسف خليل – سعد محمد على الشكعة – ولاء مجاهد محمد هلالي

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

أجرى هذا العمل أثناء الربيع وبداية الصيف عام ٢٠١٥م بمعمل بحوث النحل والحرير، قسم وقاية النبات، كلية الزراعة، جامعة الزقازيق، لتقييم التأثيرات البيولوجية والفسيولوجية لـ ١٨ وجبة غذائية على شغالات نحل العسل المرباة في أقفاص مقارنة بالكنترول الذي تغذت شغالاته على محلول سكري تركيز (١:١ سكروز: ماء)، سجل نحل الأقفاص عمراً أطول (LT_{50} ، LT_{90}) وقياسات أعلى لمساحة سطح فصوص الغدة فوق البلعومية عند تناوله للوجبات الغذائية رقم ١٦ والتي تتكون من ٤,٥ حبوب لقاح: ٤,٥ موز: ١ كسبرة: ٩٠ سكروز مسجلة ٣٥,٨ ، ٥٤,١ يوم، ٠,٦٤ مم^2، على التوالي، جاءت الوجبة ١٨ المكونة من ٤,٥ حبوب لقاح + ٤,٥ عجوة + ١ كسبرة: ٩٠ سكروز فى المرتبة الثانية مسجلة ٣٥,٠، ٥٠,٢ يوم، ٠,٥٦ مم^2، على الترتيب، ثم الوجبة ١٢ المكونة من ٤,٥ دقيق عدس: ٤,٥ عجوة: ١ كسبرة: ٩٠ سكروز والتى سجلت ٢٨,٩ ، ٣٨,٥ يوم، ٠,٥٤ مم^2، على التوالى، لوحظ أن أثقل وزن لجسم نحل الأقفاص (٩٥,٣٣ مجم/شغالة)، كان عند التغذية على الوجبة رقم ١٤ المكونة من ٤,٥ حبوب لقاح: ٤,٥ جوافة: ١ شيح: ٩٠ سكروز، تلاه الوجبة رقم ١٦ والتى سجلت ٨٨,٩٢ مجم/شغالة، ثم الوجبة ١٨ والتي سجلت ٨٦,٣٨ مجم/شغالة ثم الوجبة رقم ١٢ (٨١,٧٥ مجم/شغالة)، لذلك فإن مدة بقاء ونمو الغدة فوق البلعومية لنحل الأقفاص يبدو أنهما مقياسان مهمان لتقييم الغذاء المختبر.

المحكمـــون:

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٢- **أ.د. علي مرسي سليمان حجاب** أستاذ الحشرات الاقتصادية المتفرغ – كلية الزراعة – جامعة الزقازيق.