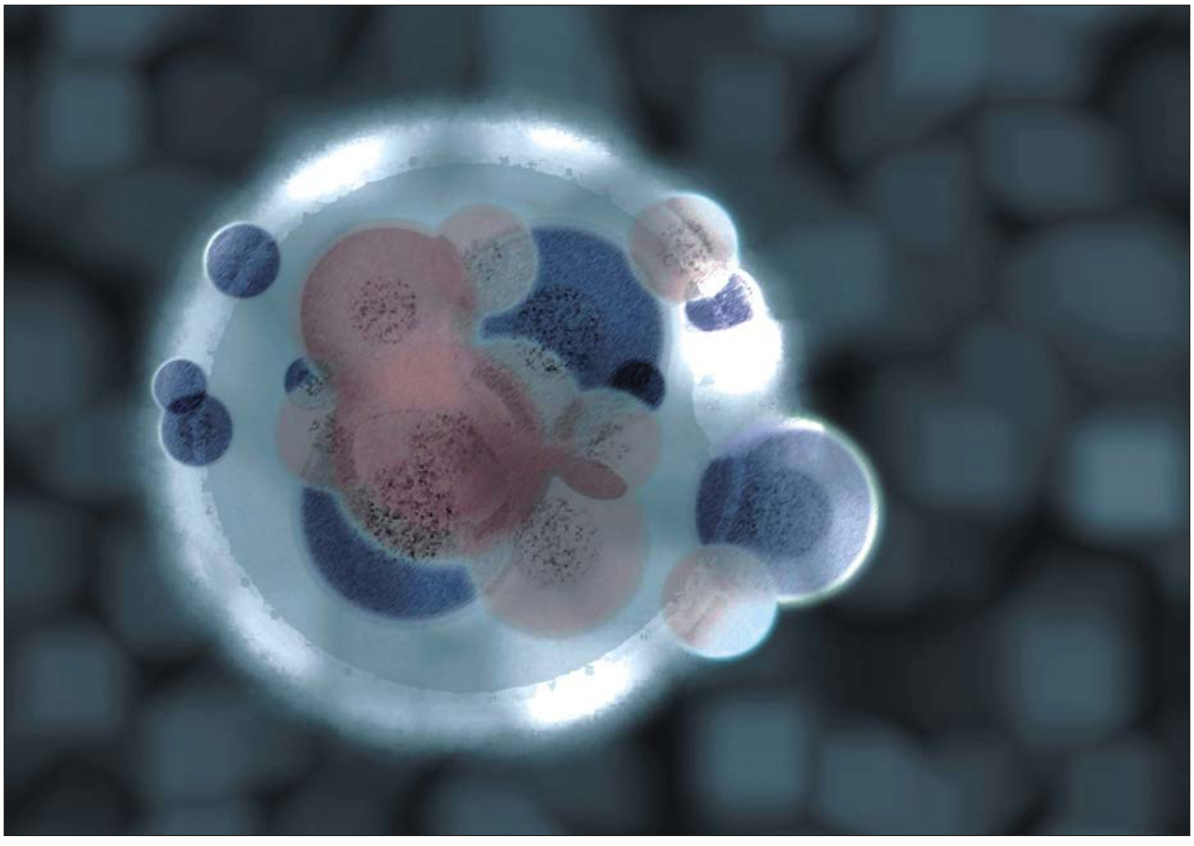

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Influence of Some Protein Diets on the Longevity and Some Physiological Conditions of Honeybee *Apis mellifera* L. Workers

Abdulaziz S. Alqarni
Department of Plant Protection, College of Food Science and Agriculture,
King Saud University, P.O. Box 2460 Riyadh 11451, Saudi Arabia

Abstract: The experiments were carried out under laboratory conditions in two groups to evaluate the suitability of some protein diets for supplementary feeding of honeybee. In the first group, food consumption and cumulative mortality percentage of newly emerged bees fed on some protein diets were determined. The highest food consumption rate was recorded in bees fed on diet II (Improved traditional substitute, ITS), followed by diet III (mixed date palm pollen + soybean flour, DP+Sb) whereas the lowest one was for diet I (Traditional Substitute, TS). Feeding bees on beebread or diets containing date palm pollen gave the longest LT_{50}. In the second group, development of hypopharngeal glands and rectal content was evaluated. Results indicated that the normal source of protein for honeybee workers as beebread or date palm pollen was the best source for hypopharngeal gland development. The rectal content weight reflected the suitability of diets which contain pollen grains for feeding honeybee colonies.

Key words: *Apis mellifera* L., honeybee nutrition, supplementary feeding, protein diets, longevity, hypopharyngeal glands, rectal contents

INTRODUCTION

Honeybee workers required different nutrients soon after emergence to build up their final body composition. The basic food for honeybee is represented by honey as energetic source and pollen grains that encompass proteins, vitamins, enzymes, minerals and lipids that are necessary for the growth and development of honeybees (Haydak, 1970).

Beekeepers used to feed bees with pollen supplements or substitutes to promote colony development at periods of pollen shortage. The useful pollen substitute should stimulate colony growth and support aspects of worker quality, such as high brood survival and the length of adult stage (Winston *et al*., 1983). Meanwhile, the pollen substitute should be acceptable and has the necessary stimulation for bees to consume it (Doull, 1973).

Mostafa (2000) used single and mixed protein material under laboratory conditions for feeding honeybee workers. Abdalla (2001) and Abdilla (2005) tested some pollen substitutes for feeding honeybee worker. They considered the pollen substitute consumption in the period after worker emergence and the development of hypopharyngeal gland and rectal content, important criteria for estimating the suitability of proteins diets.

Bee colonies in Saudi Arabia experience long periods of pollen shortage across the country. Short spring is followed by a long hot summer in most regions where temperature exceeds 40°C and bees become in need to nectar and water in addition to pollen. Alqarni (1995) and Alghamdi (2002) found that pollen area was less than 10 sq. inch/colony during summer season in central Saudi Arabia and supplementary feeding was very essential.

This experiment aimed to study the suitability of some protein diets for honeybee workers feeding. Such information will be useful for improving honeybee colonies development in Saudi Arabia especially during the period of pollen shortage.

MATERIALS AND METHODS

Experimental research was carried out at the Honeybee Research Unit, King Saud University, Riyadh during fall 2005 in laboratory conditions on Carniolan honeybee *Apis mellifera* L. workers. Three food materials were used to make four diets to feed the bees under investigation.

Bee pollen was collected at the time of experiment from honeybee colonies to be used as control 1. In another control treatment (control 2) bees were fed only on sugar syrup to evaluate the effect of protein absence on the death percentage.

Date palm pollen (*Phoenix dactylifera* L.) collected directly by hand from male date palm trees and kept under freezing condition until used.

Traditional substitute used by beekeeper as pollen substitute consists of defatted soybean meal (*Glycine max* L.), brewer's dried yeast and skimmed milk powder. All diets were tested against control treatment as explained in Table 1. Frames of only sealed brood were

placed in screen cages and kept in an incubator at 32±1°C, 70 R.H to obtain newly emerged bees (0-24 h). Fifty workers were transferred to experimental wooden cages of 15×15×5 cm dimensions with two sides of glass and black muslin. Every cage was provided with a vial of tap water, a vial of sugar solution 1:1 (w/v) and a piece of wax comb attached to the top of cage. Four cages were assigned to each tested diet that was prepared as paste-like material and introduced to each cage into a small plastic feeder. Diets were weighed before introduction, reweighed and changed every 3 days. Another control treatment was made in which bees fed only on sugar syrup to evaluate the effect of protein absence on the death percentage. All cages were held in the dark in an incubator at 32±1°C and 70% RH. This method was described by Mostafa (2000), Abdalla (2001) and Abdilla (2005).

Experiment was carried out on two groups, each of which consists of twenty cages. Daily food consumption and honeybee workers longevity were measured in the first group, whereas development of hypopharyngeal glands and rectal contents were measured in the second group.

In the first group, food consumption for every cage was calculated every 3 days and represented as mg/cage/3 days until 12 days old. Dead bees in each cage were counted and removed every 3 days until half of the initial number of honeybees were dead (LT_{50}). Values in days of bees fed with different diets were determined by a computerized probit analysis program.

In the second group, the development of hypopharyngeal glands was carried out on honeybee workers of 3, 6, 9 and 12 days-old. Twenty bees were used to assimilate each age from each treatment. The degree of development of hypopharyngeal glands was determined according to Maurizio (1954). An arbitrary scale (I to IV) was used to determine the degree of development.

The same twenty bees were also used to determine the weight of rectal content by extracting the rectum with a fine forceps, put on a cover glass, previously weighed and then reweighed on an analytical balance. F-test was used in order to calculate and test the significance of the administered diets. The multiple scale of Duncan (1955) was used in order to compare the means.

RESULTS AND DISCUSSION

First group: Data presented in Table 2 showed the rates of food consumption (mg/cage/3 days) of newly emerged bees fed on three protein diets tested against those fed on beebread. General trend showed that honeybee workers food consumption increased after 1-3 days after emergence (a.e.) and reached its highest peak at 4-6 days a.e., then decreased significantly during the 3rd and 4th periods (7-12 days a.e.). Hagedorn and Moeller (1967) reported that worker bees begin protein mass consumption at 42 to 52 h a.e. and reach the highest consumption rate at five days a.e. During this early adult stage, all nitrogen requirements of a worker bee come from pollen protein that is highly essential for the development of body tissues, muscles and glands (Herbert, 1999). Results indicated that consumption of diets decreased significantly at the third period and beyond. At this stage, protein requirements decreased as the nursing tasks are completed and the need for carbohydrates increased as field tasks are carried out (Jaycox, 1981; Herbert, 1999). Total food consumed by workers during the twelve days after emergence was calculated for each tested diet. The highest food consumption was recorded for diet II (149 mg/cage/12 days), followed by diet III (110.8 mg/cage/12 days), then bee bread (86.75 mg/cage/12 days) whereas the lowest consumption was on diet I. All mentioned amounts were significantly different (Table 2).

Table 1: Description of some protein diets administered to honeybee *Apis mellifera* L. workers (%)

Diets (%)	Bee bread	Soybean meal	Dried skimmed milk	Brewers yeast	Date palm pollen	Mixed pollen grain (%)	Sugar
Bee bread (Bb) (Cont. 1)	100	-	-	-	-	-	-
Traditional Substitute (TS) Diet I	-	30	10	10	-	-	50
Improved Traditional Substitute (ITS) Diet II	-	30	10	10	-	5	45
Mixed date palm pollen + soybean (DP+ SB) Diet III	-	25	-	-	25	-	50
Control (2) Sugar syrup only (without any protein diet)							

Table 2: Rates of food consumption by honeybee *Apis mellifera* L. workers fed on 4 protein diets during 4 periods for 12 days long

	Means of consumption (mg/cage/3 day)				
Tested diets (mg)	1-3 days	4-6 days	7-9 days	10-12 days	Total mg/cage/12days
Bee bread (Bb)	29.0B	40.75Ef	13.50H	3.50L	86.75(c)
Diet I Traditional Substitute (TS)	14.75H	14.75H	14.00H	9.75H	53.25(d)
Diet II Improved Traditional Substitute (ITS)	38.00F	86.00C	13.50H	11.50H	149.00(a)
Diet III mixed date palm pollen + soybean flour (DP + SB)	38.00F	46.00E	14.75H	12.00H	110.80(b)
Mean	29.94B	46.88A	13.94C	9.19D	

Means followed by the same letter do not differ significantly at 0.05

Among the compared diets, diet II (Improved traditional substitute) showed the highest consumption by worker bees. The type of basic material used in pollen substitute greatly affected the consumption of the foods. Addition of pollen grains to pollen substitute (Diet II) significantly increased the diet consumption (Kleinschmidt and Kondos, 1977), (Herbert and Shimanuki 1980). Chiang *et al*. (1992), Mostafa (2000) and Abdilla (2005) indicated that inclusion of pollen in a pollen supplement increases its palatability to honeybees.

The cumulative mortality percentages as well as the longevity of workers at each treatment were recorded. The half-life was estimated as LT_{50} (number of days required for 50% of the bees to die). Data showed that feeding caged bees on sugar syrup only significantly shortened the longevity of honeybee workers (LT_{50} 17.99 days) in comparison to all treatments (Table 3). Diet I (lack bee pollen) showed the lowest LT_{50} (23.15 day), whereas the highest LT_{50} was for diet II (beebread) and diet III (includes date palm pollen) (25.56 and 27.71 day, respectively).

The results indicated that protein source played an important role influencing the longevity of honeybee workers. The mortality rates of newly emerged workers fed on different protein sources were highly related to the type of materials and their contents of protein. It appears from the results obtained that the commonly accepted protein source used as pollen substitute for bee is soybean meal. It can be used as protein source for feeding honeybee colonies mixed with other ingredients such as yeast, skimmed milk and pollen. Pollen collected by hand such as date palm pollen or pollen grains could be added to soybean meal to act as an attractant or to increase its nutritive value, while yeast could be added as a source of protein and B vitamin (Erickson and Herbert, 1980).

Second group: The Results indicated that the age of bees in which the development of hypopharyngeal glands could be influenced occurred during the first 12 days after emergence. The considered increment in the gland has been obtained in this period (Table 4).

The highest degree of the hypopharyngeal gland development was recorded in workers fed on beebread (control), followed by those fed on diet III (mixed date palm pollen+soybean). Glands of workers fed on diet I and diet II showed significantly lower development than those of workers fed on beebread and diet III. The poorest result obtained from workers fed on sugar syrup only.

The present result are in agreement with those obtained by Standifer *et al*. (1960) who indicated that the development of the glands is related to/or promoted by high protein content of the diet. Many authors described that soybean flour, dried yeast and dried milk were the most suitable substances used for supplementary feeding

Table 3: Cumulative mortality percentages of honeybee *Apis mellifera* L. workers fed on some protein diets (TS: Traditional substitute, ITS: Improved TS, DP: Date palm pollen, SB: Soybean flour)

	Protein diets				
Age of bees (days)	Bee bread (Bb)	Diet I (TS)	Diet II (ITS)	Diet III DP + SB	Control (sugar syrup)
3	0.0	4.0	2.0	0.0	2.5
6	2.0	7.5	5.0	3.0	9.0
9	4.0	12.5	7.5	6.0	15.0
12	7.0	22.0	11.0	8.0	26.0
15	11.0	27.5	19.0	10.5	33.5
18	22.0	34.5	33.0	13.5	47.5
21	33.0	43.5	35.5	21.5	64.5
24	40.0	53.0	45.5	37.5	76.5
27	47.0	61.5	52.6	48.5	86.5
30	57.5	73.5	63.5	54.5	95.0
33	64.0	84.0	74.5	74.0	100.0
LT_{50} days	27.57a	23.15b	25.56a	27.71a	17.99c

Means followed by the same letter do not differ significantly at 0.05

Table 4: Development of hypopharyngeal glands in honeybee *Apis mellifera* L. workers fed on some protein diets

	Degree of hypopharyngeal gland development				
Tested Diets	3 days	6 days	9 days	12 days	Means
Bee bread (Bb)	3.56B	3.98A	3.50b	3.46B	3.60(A)
Diet I Traditional Substitute (TS)	2.74F	3.00De	1.85ij	1.54K	2.28(D)
Diet II Improved Traditional Substitute (ITS)	2.98De	3.29C	2.19g	1.79J	2.56(C)
Diet III Mixed date palm pollen + soybean flour (DP+Sb)	3.53B	3.84A	3.13d	2.08Gh	3.14(B)
Control sugar syrup only	1.99Hi	2.95E	1.89ij	1.76J	2.15(E)
Mean	2.96B	3.39A	2.51C	2.13D	

Means followed by the same letter do not differ significantly at 0.05

Table 5: Weight of rectal contents of honeybee *Apis mellifera* L. workers fed on some protein diets

Tested diets	Weight of rectal contents (mg/bee) 3 days	6 days	9 days	12 days	General mean
Bee bread (Bb)	11.53F	26.13A	27.24a	13.95e	19.71AB
Diet I Traditional Substitute (TS)	10.84F	11.70F	22.73b	19.05d	16.08D
Diet II Improved Traditional Substitute (ITS)	13.02E	19.15D	20.47c	18.06d	17.67C
Diet III Mixed date palm pollen + soybean flour (DP + Sb)	10.59Fg	22.56B	22.36b	18.88d	19.41B
Control Sugar syrup only	9.40G	22.75B	26.66d	22.03b	20.20A
General mean	11.73D	20.46B	23.89A	18.39C	

Means followed by the same letter do not differ significantly at 0.05

of honeybee colonies after pollen of bee bread (Spencer-Booth, 1960; Standifer *et al.*, 1973). Pollen consumption is positively correlated with gland development (Hrassnigg and Crailsheim, 1998; Mostafa, 2000).

The rectal contents started with low weight on the first inspected age then grew up to reach the highest weight at 9-days old. The means of rectal contents weight regardless of the age of honeybee workers show that values of those bees fed on a diet containing date palm pollen was similar to those fed on beebread (control). The next descendent mean belongs to the bees fed on diet II (improved traditional substitute) with a significant difference from the mean of honeybee workers fed on bee bread (Table 5). The lowest mean of rectal weight was reported for the bees fed on diet I (traditional substitute, TS) that differs significantly than any compared diet. This result is correlated with the decrease of the amount of diet consumed by honeybee workers. These results reflect the suitability of diets containing pollen for feeding honeybee colonies during nursing period of honeybee workers.

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