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Protein levels and colony development of Africanized and European honey bees fed natural and artificial diets

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ABSTRACT. Pollen substitute diets are a valuable resource for maintaining strong and health honey bee colonies. Specific diets may be useful in one region or country and inadequate or economically unviable in others. We compared two artificial protein diets that had been formulated from locally-available ingredients in Brazil with bee bread and a non-protein sucrose diet. Groups of 100 newly-emerged, adult workers of

Africanized honey bees in Brazil and European honey bees in the USA were confined in small cages and fed on one of four diets for seven days. The artificial diets included a high protein diet made of soy milk powder and albumin, and a lower protein level diet consisting of soy milk powder, brewer's yeast and rice bran. The initial protein levels in newly emerged bees were approximately 18-21 µg/µL hemolymph. After feeding on the diets for seven days, the protein levels in the hemolymph were similar among the protein diet groups (~37-49 µg/µL after seven days), although Africanized bees acquired higher protein levels, increasing 145 and 100% on diets D1 and D2, respectively, versus 83 and 60% in the European bees. All the protein diets resulted in significantly higher levels of protein than sucrose solution alone. In the field, the two pollen substitute diets were tested during periods of low pollen availability in the field in two regions of Brazil. Food consumption, population development, colony weight, and honey production were evaluated to determine the impact of the diets on colony strength parameters. The colonies fed artificial diets had a significant improvement in all parameters, while control colonies dwindled during the dearth period. We conclude that these two artificial protein diets have good potential as pollen substitutes during dearth periods and that Africanized bees more efficiently utilize artificial protein diets than do European honey bees.

Key words: *Apis mellifera*; Nutrition; Pollen substitute; Hemolymph; Protein diets

INTRODUCTION

A major concern for beekeepers is the necessity to maintain honey bee colonies in good condition during times when pollen is in short supply or not available. Pollen, one of the floral resources collected by bees, is the natural source of protein for these insects (Pernal and Currie, 2001). During times of pollen dearth, the pollen reserves in the combs and protein reserves in bees are rapidly expended. Consequently, supplementary pollen or pollen substitutes are required to maintain the colony's strength for pollination services or honey production (Herbert, 1992). If there is no pollen, or a good pollen substitute is not available to the colonies, brood production can diminish or even stop completely (Haydak, 1963). Pollen collected from honey bee colonies could be used in times of food shortage; however, this incurs the risk of spreading pathogens (De Jong et al., 2009; Brodschneider and Crailsheim, 2010). Provision of artificial diets is a safe way to feed bees protein. Also, bee-collected pollen tends to be expensive and of limited supply. Consequently, beekeepers often provide pollen substitute diets to colonies, although these are often formulated without considering the costs of the diet components versus the benefits of providing such diets (Herbert et al., 1977; Li et al., 2012; Morais et al., 2013).

Bees usually consume pollen after it is fermented, in the form of bee bread (Gilliam, 1997; Brodschneider and Crailsheim, 2010). Proteins are responsible for 66-74% of the dry matter of adult workers (Hrassing and Crailsheim, 2005). This protein content increases during the first days due to protein anabolism and decreases as the workers age (Crailsheim, 1986).

During periods of famine or food shortage, honey bee colonies can maintain brood rearing for a short period of time, initially using stored bee bread in the combs and later using adult bee body reserves (Haydak, 1935). Supplementation with artificial alternative diets or pollen substitutes can help to improve colony performance (Herbert, 1992). Moreover, adequate nutrition promotes the development of healthy colonies. This is because the health of a honey bee colony is not defined solely as an absence of disease but also depends on having well-nourished individuals that can withstand stressors such as parasites, infections, pesticides, high temperatures, and lack of water and food shortages (Naug, 2009; Brodschneider and Crailsheim, 2010).

In *Apis mellifera*, accumulation of the storage proteins vitellogenin and hexamerins in the hemolymph of adult workers is significantly influenced by nutrition (Bitondi and Simões, 1996; Cunha et al., 2005; Bitondi et al., 2006, Martins et al., 2008). Adequate nutrition ensures proper maintenance of honey bees. Studies have shown a connection between nutrition and the transition of tasks from inside the nest to the outside tasks performed by workers. Foragers preferentially consume nectar (Crailsheim et al., 1992) and a low protein diet (Slansky and Scriber, 1985). Studies made with Africanized and European honey bees have shown that workers that had the gene encoding vitellogenin silenced by RNAi made foraging flights earlier (Nelson et al., 2007; Marco Antonio et al., 2008).

Given the fact that Brazil is a country of continental proportions, we encounter large extremes in nutritional realities in different regions of the country. Although warm weather potentially allows for flowering throughout the year, many beekeepers lose large numbers of colonies due to absconding during periods of drought and food scarcity (Lima, 1995). As part of an effort to address this problem, we examined the efficacy of artificial diets that had been developed using low-cost materials to which beekeepers have easy access, through laboratory and field tests. We also compared the efficacy of these diets in European and Africanized honey bees.

MATERIAL AND METHODS

Combs containing emerging worker brood were removed from colonies and placed in an incubator at 34°C and 80% humidity. Africanized bees (Ribeirão Preto, SP, Brazil) and European honeybees (Gainesville, FL, USA) that emerged within a period of 15-20 h were collected. Groups of 100 of these workers were maintained in plastic confinement cages (8 x 11 x 13 cm). Each group was fed 5 g portions of one of the diets for seven days (freshly-prepared diet paddies were provided every two days). Seven cages of bees were prepared for each diet and for each kind of bee. Diet 1 (D1; 20.2% crude protein, given as a % of dry matter) consisted of 30 g soy milk powder, 10 g albumin, 20 g sucrose, and sufficient 50% sucrose syrup to make a paste. Diet 2 (D2; 14.4% crude protein) consisted of 20 g brewer's yeast, 20 g soy milk powder, 10 g rice bran, 20 g sucrose, and sufficient 50% sucrose syrup to make a paste. The natural diet used for comparison consisted of bee bread freshly collected from combs of field colonies. A fourth group was given no protein supplements. The cages were maintained in the dark in an incubator, at 34°C and 80% humidity. Sucrose syrup (50% w/v) was given *ad libitum* to all groups.

Extraction and quantification of hemolymph protein

Hemolymph was collected from 10 newly emerged workers (15-20 h, designated day 0). After three and seven days of confinement and feeding with the different diets, hemo-

lymph was collected from 10 workers taken from each cage with the aid of a pipette from a droplet resulting from a small incision made at the base of the bee's wing. The protein concentrations in the hemolymph were determined by spectrophotometry (Ultrospec 2100 pro, Pharmacia, Brazil) by the Bradford (1976) method, using bovine serum albumin to produce a standard curve (Cremonez et al., 1998).

Electrophoretic patterns of hemolymph protein

Hemolymph soluble proteins were separated by SDS-PAGE according to the method of Laemmli (1970), using a 7.5% polyacrylamide gel; 0.5 µL samples of the hemolymph obtained from pools of hemolymph collected from 10 workers were added to sample buffer and subjected to a constant current of 15 mA; the gels were run at 7°-10°C. After electrophoresis, the gels were stained with 1% Coomassie brilliant blue dissolved in a solution of glacial acetic acid, ethanol and water (1:5:5 v/v), which was also used for gel discoloration.

Evaluation of the colonies in the field and mapping of the brood and food areas

After evaluation of the efficacy of the diets in the laboratory, field experiments were conducted using five standard frame Langstroth hives kept on digital scales. The apiaries were located in two different regions of Brazil (FMRP, USP, Ribeirão Preto, SP, 21°12'S-47°48'W and UFERSA, Mossoró, RN, 5°11'S-37°20'W). We ran these experiments in Ribeirão Preto, in winter (June - July, 2009); during this season, there is considerably reduced availability of floral resources in the field.

Two colonies in each location were fed diet D1, two colonies were fed diet D2 and two colonies were given no protein supplements. The protein diets were administered in paste form and had a similar pasty consistency. Each hive was supplied with 100 g of diet weekly, wrapped in waxed paper and placed on the top bars of the brood frames. During the course of the experiment, 800 g of diet was given to each colony. When there was leftover food at the end of the week, the diet patties were collected and weighed to estimate consumption.

The experiments in Mossoro (semi-arid Northeast region of Brazil; a region known for a severe lack of food resources during the dry season, mainly due to lack of rain and high temperatures) were conducted from February to March 2010, a period when the region was going through a four-month drought. The same experimental design was repeated as in Ribeirão Preto, SP.

The daily weight of each colony was recorded to evaluate the impact of the diets on colony growth. Each of the colonies was fed on one of the diets for 45 days. The development of the colonies was evaluated by mapping the brood and food areas, according to methodology adapted from Al-Tikrity et al. (1971). The maps were made immediately before providing the diets and after 45 days.

Statistical analysis

Data obtained from the protein quantification in the hemolymph were compared using ANOVA on ranks and pairwise comparisons were made using the Student-Newman-Keuls test. The average weight gain and the comb map parameters of all colonies in the field were compared using ANOVA, and comparisons between treatments were made using the Student *t*-test. Statistical analyses were carried out using SigmaStat 3.5.

RESULTS

The mean protein titers in the hemolymph of workers fed protein diets were significantly higher than those of workers fed only sucrose (Table 1). Total protein content decreased in workers fed only sucrose. Diets D1 and bee bread tested on Africanized bees in Brazil and European bees in the USA resulted in a significant increase in the hemolymph protein levels after three days. There were no significant differences in protein levels in the hemolymph in bees fed D1, D2 or bee bread, in both Africanized and European, after seven days. All of the protein diets were significantly superior to the sucrose diet.

Table 1. Mean and standard deviation of the concentration of total protein (µg/µL) in the hemolymph of honey bees confined in hoarding cages at emergence and after feeding on sucrose syrup, bee bread, or one of the artificial protein diets (D1, D2) for 0-7 days.

Bee type	Age/days	Diets				
		None (initial)	Sucrose	Bee bread	D1	D2
EHB	0	21.6 ± 3.7				
	3		18.4 ± 5.4^{a}*	24.2 ± 7.4^{b}*	14.4 ± 3.5^{a}*	18.2 ± 4.4^{a}*
	7		14.2 ± 5.0^{a}*	48.0 ± 18.2^{b}**	39.4 ± 13.0^{b}**	34.4 ± 16.5^{b}**
AHB	0	18.5 ± 4.6				
	3		23.7 ± 5.4^{a}*	27.3 ± 8.2^{a}*	21.8 ± 9.8^{a}*	24.6 ± 8.7^{a}*
	7		16.4 ± 6.7^{a}*	48.7 ± 10.9^{b}**	45.2 ± 17.5^{b}**	36.8 ± 14.5^{b}*

In comparing data between diets, different superscript letters indicate significant differences. Within the columns, different numbers of asterisks indicate significant differences. EHB = European bees collected in Gainesville, Florida. AHB = Africanized bees collected in Ribeirão Preto, São Paulo. D1 = Diet 1 20.2% crude protein, 30 g soy milk powder, 10 g albumin, 20 g sucrose, and sufficient 50% sucrose syrup to make a paste. D2 = Diet 2 14.4% crude protein) consisted of 20 g brewer's yeast, 20 g soy milk powder, 10 g rice bran, 20 g sucrose, and sufficient 50% sucrose syrup to make a paste.

The protein profile of hemolymph collected from European workers (Figure 1) treated with both artificial diets was similar to that of the profile of workers fed beebread, although diet D1 produced bands of greater intensity than those of bees fed diet D2; the bands of the former were similar to those obtained from bees fed bee bread. Hemolymph from bees fed only sucrose and no protein produced relatively weak bands.

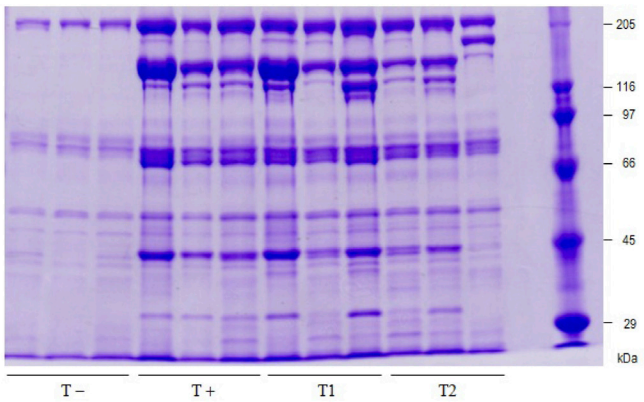

Figure 1. Coomassie brilliant blue-stained SDS-polyacrylamide gel showing hemolymph protein patterns of European honey bee workers fed on the different diets. T- = sucrose; T+ = bee bread; T1 = protein diet D1; T2 = protein diet D2 (see Table 1 for details of diets). Each sample consisted of 0.5 µL hemolymph obtained from a pool of hemolymph collected from 10 workers fed on each diet. Molecular mass markers (kDa) are indicated at the right.

Tests in field colonies during dearth periods in Ribeirão Preto, SP, and Mossoro, RN

In Ribeirao Preto, SP, the most consumed diet was D1 (mean consumption of 74%); for diet D2 mean consumption was only 34%. One of the colonies consumed very little (26%) of the D2 diet. On the 35th day, this colony absconded. In Mossoro, RN, mean consumption levels of the two diets were similar (66% for D1 and 58% for D2).

Comparisons of weight and comb mapping parameters in the field experiments

The overall average gain of weight and comb map parameters in all hives was significantly greater in the colonies that received the supplementary protein diets (Table 2). Colony weight increased approximately 3 kg, compared to a loss of nearly 4 kg in the colonies that did not receive the protein supplement. There was mean gain of about 14% (% of total comb area) in brood area in the colonies fed the protein diets, compared to a mean loss of 9% in colonies in the controls. The percentage of comb filled with honey increased a mean of 10-12% in colonies fed the protein supplements, compared to a loss of about 14% in the controls.

Table 2. Mean gain of the colony performance parameters fed different protein diets (N = 4 colonies for each diet).

Diet	D1	D2	Control
Colony weight (kg)	2.95 ± 0.8^{a}	2.8 ± 1.4^{a}	-3.7 ± 1.3^{b}
Sealed brood (%)	7.8 ± 1.3^{a}	7.8 ± 2.0^{a}	-4.8 ± 4.8^{b}
Open brood (%)	5.6 ± 4.1^{a}	6.6 ± 0.1^{a}	-4.1 ± 0.7^{b}
Sealed honey (%)	1.7 ± 7.1^{a}	-10.2 ± 4.5^{a}	-17.9 ± 10.5^{b}
Unsealed honey (%)	10.2 ± 6.4^{a}	-0.4 ± 3.2^{b}	4.5 ± 9.6^{b}

Different superscript letters in the same row indicate significant differences. See Table 2 for details of diets. Control colonies were not given protein supplements.

DISCUSSION

We found the two protein diets (D1 and D2) to be adequate substitutes for natural bee bread, since bees fed in the laboratory with these diets developed hemolymph protein concentrations and protein profiles similar to those of bees fed bee bread.

Qualitatively and quantitatively, hemolymph proteins were quite similar in bees fed bee bread versus diet D1, based on the SDS-polyacrylamide gel bands. This suggests that this artificial diet was able to provide the amino acids needed for protein synthesis characteristic of a worker fed a natural diet (bee bread).

Bee bread and the artificial protein diets D1 and D2 were well accepted by the bees. Cremonez et al. (1998) also found the highest protein values in bees fed bee bread. We observed an increase in the protein titers of workers during the seven days that the bees were confined, whenever they had access to a high protein diet (Table 1). Results similar to ours were reported by De Jong et al. (2009), who found that workers fed only a carbohydrate developed low levels of protein in the hemolymph, below the values found in newly emerged workers.

In our experiments, we registered high levels of protein in the hemolymph of both Africanized and European bees; although the final protein titers in European bees were lower (Table 1). Protein titers in the European bees fed only sucrose were reduced 34% after seven days, compared to 11% in the Africanized bees. Protein levels increased 83% in European

bees fed diet D1, compared to 145% in Africanized bees fed the same diet. Similar tendencies were found for bee bread and diet D2. Cappelari et al. (2009) also reported that Africanized bees developed higher protein levels in the hemolymph than European bees fed the same diet, suggesting more efficient metabolism of ingested nutrients.

In the field experiments, the high-protein diets also increased colony growth parameters during periods of scarcity of pollen resources, as also reported by Mattila and Otis (2006) and Somerville and Nicol (2006). Garcia et al. (1986) supplied various protein foods with 20, 30 and 40% crude protein; they found that crude protein negatively influences food collection. Contrarily, we found a higher consumption of the D1 diet, which had a larger amount of crude protein in its composition (20.2%) than did the D2 diet (14.4%). In addition to the amount of protein in the diet, other factors could influence food consumption, including the number of bees in the colony (Bitioli and Chaud Netto, 1992) and essential nutrients (Stace and White, 1994).

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