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ORIGINAL RESEARCH ARTICLE

Pollen substitutes increase honey bee haemolymph protein levels as much as or more than does pollen.

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Summary

Adequate substitutes for pollen are necessary for maintaining healthy bee colonies during periods of pollen dearth, but testing them objectively is both time consuming and expensive. We compared two commercial diets with bee collected pollen and acacia pod flour (used by beekeepers in some parts of Brazil) by measuring their effect on haemolymph protein contents of young bees exclusively fed on these diets, which is a fast and inexpensive assay. The commercial diets included a new, non-soy-based, pollen substitute diet (named Feed-Bee®) and a soy-based diet, named Bee-Pro®. The diets were each given in patty form to groups of 100 Africanized honey bees in hoarding cages, maintained and fed from emergence until six days of age. Sucrose, in the form of sugar syrup, was used as a protein free control. Feed-Bee®, Bee-Pro®, pollen and acacia pod flour diets increased protein titers in the haemolymph by factors of 2.65, 2.51, 1.76 and 1.69, respectively, over protein titers in bees fed only sucrose solution. The bees fed Feed-Bee® and Bee-Pro® had their haemolymph significantly enriched in protein compared to the controls and those fed acacia pod flour and to titers slightly higher than those fed pollen. All four proteinaceous diets were significantly superior to sucrose alone.

Los substitutos del polen incrementan los niveles de proteína en la hemolinfa de la abeja melífera igual o más que el polen.

Resumen

Los substitutos del polen son necesarios para mantener saludable a la colonia de abeja durante períodos de escasez del polen, pero en los estudios con estos substitutos suele invertirse mucho tiempo y son costosos. Comparamos dos dietas comerciales con polen y una harina de vainas de Acacia (utilizada por apicultores en algunas regiones de Brasil), midiendo su efecto sobre los contenidos proteínicos de la hemolinfa de abejas jóvenes alimentadas exclusivamente con estas dietas, una prueba rápida y barata. A las dietas comerciales, se le incluyó un nuevo substituto de polen, no basado en soja (conocido comercialmente como Feed-Bee®) y una dieta a base de soja, conocida como Bee-Pro®. Cada dieta se aplicó en forma de tarta a grupos de 100 abejas africanizadas en jaulas de observación, a partir de su emergencia hasta los seis días de edad. La sucrosa en jarabe de azúcar, fue utilizada como dieta control sin proteína. Las dietas con el Feed-Bee®, el Bee-Pro®, el polen y la harina de vainas de Acacia incrementaron los valores de proteína en la hemolinfa por factores de 2,65, 2,51, 1,76 y 1,69, respectivamente, con respecto a los valores de proteína en las abejas alimentadas solamente con jarabe de sucrosa. Las abejas que fueron alimentadas con Feed-Bee® y Bee-Pro® presentaron en su hemolinfa un significativo enriquecimiento de proteína comparada con las del grupo control y valores ligeramente más altos que aquellas alimentadas con polen.

Keywords: Pollen, substitute, diet, honey bee, protein, haemolymph, *Prosopis juliflora*, soybean, *Apis mellifera*

Introduction

Protein plays a major role in the life of honey bees (Amdam and Omholt, 2002) and other insects (House, 1961; Cohen, 2003). Honey bee longevity, brood rearing, and honey production is reduced when protein availability is inadequate (Crailsheim, 1990; Herbert, 2000). Colonies that have no access to pollen, the bees' natural source of protein, have a reduced capacity to rear brood, quickly decline in population, and may eventually die. Protein deficiency also affects the ability of honey bees to resist diseases (Matilla and Otis, 2006); consequently, it is suspected to be an important factor involved in "Colony Collapse Disorder" (Cox-Foster *et al.*, 2007). As pollen is not always available, an alternative protein source is sometimes necessary to ensure bee health and continued colony development, as well as to maintain colony strength for pollination, overwintering, and honey production (Standifer *et al.*, 1980; Goodwin *et al.*, 1994; Herbert, 2000). Various supplementary diets (i.e. those that contain pollen) are advocated and some are commercially available. Pollen is, however, expensive and can transmit disease organisms.

Testing artificial bee diets objectively is difficult. The collection of dietary material by the bees may not mean that it is adequate. During dearth periods, honey bees often collect substances that have consistencies similar to pollen, such as road dust and bird feed, even if they have no nutritional value for the bees. Measuring colony brood production, which is probably the most relevant characteristic, is time consuming, requires many colonies, and needs to be done under controlled conditions to avoid effects of pollen collection from natural sources (Herbert *et al.*, 1985). An alternative technique that allows rapid testing of diets consists of feeding recently emerged adult bees in small hoarding cages for six days and then measuring the protein contents in their haemolymph (Cremonez *et al.*, 1998). Both overall protein levels and concentrations of the key honey bee storage protein, vitellogenin, are increased by nutritionally superior protein diets. Bee bread (pollen stored in brood comb that has been fermented) gives the highest protein levels with this diet evaluation technique (Herbert and Shimanuki, 1978; Gilliam, 1997), especially when made from fresh pollen (Pernal and Currie, 2000; Gregory, 2006).

Various alternative bee diets have been found to be nutritionally poor or unpalatable and most are not well tested (Herbert, 2000). Commercially available pollen substitutes (i.e. those that do not contain any pollen) have been better studied, but beekeepers have experienced mixed results with their use. Those based on soy flour have been reported to be inferior (Pham-Delegue *et al.*, 2000; Manning *et al.*, 2007; Saffari, 2008); so a new non-soy-based diet, called Feed-Bee®, was developed (Saffari *et al.*, 2004) (the recipes for Feed-Bee® and Bee-Pro®, the non-soy-based pollen substitute we used, are proprietary).

Although consumption rates of pollen substitutes may prove their palatability, only through nutritional tests can their worth be evaluated. The method of investigating the efficiency of a protein source by detecting the level of protein in the haemolymph of worker bees fed on pollen and pollen substitute diets provides a means to determine the actual benefit that the bees obtain from pollen substitutes or supplements (Bitondi and Simões, 1996; Cremonez *et al.*, 1998; Szymas and Jedruszuk, 2003). Thus, the objective of our study was to determine the efficiency of Feed-Bee®, pollen, Bee-Pro®, and also acacia pod flour (*Prosopis juliflora*, which is commonly advocated by Brazilian beekeepers for feeding to honey bees, Perreira *et al.*, 2006) as protein supplements for honey bees by measuring total protein in the haemolymph of caged honey bees fed these diets.

Materials and methods

Groups of 100 newly emerged Africanized honey bees, collected from three different colonies in an apiary in Ribeirão Preto, SP, Brazil, were mixed and placed into hoarding cages (Kulincevic and Rothenbuhler, 1973; Mardan and Kevan, 2002). The cages with bees were maintained in an incubator, with no light, at 30ºC and about 70% relative humidity. The bees randomly received one of four proteinaceous diets: pollen; Feed-Bee®; Bee-Pro®; of acacia (*Prosopsis juliflora*) pod flour. All were administered as a paste and the bees were allowed to feed *ad libitum* (Cremonez *et al.*, 1998). The acacia pod flour was obtained from a local Brazilian market. Pollen was collected from pollen traps in our university apiary in Ribeirão Preto and was stored at about -4ºC in a refrigerator for up to a week, until it was incorporated into the diets. All diets had a similar final consistency and were prepared by mixing one part (by weight) of the proteinaceous powder with two parts of commercial grade finely granulated sucrose, which was ground with a spice grinder to powder consistency. Enough water was added to obtain a paste. Water was also supplied in glass tubes capped with cotton balls.

Approximately 20 g of each diet was placed in a hoarding cage, in a shallow plastic container and the diets were replaced every two days. A protein free control was provided by feeding a fifth group of bees with 50% (by weight) sucrose syrup. A trial consisted of five cages, one for each diet and three trials were made, sequentially. Consumption rates were not recorded.

After the newly emerged bees had been fed for six days, five to six bees were removed from each cage and haemolymph was collected from a small incision at the level of the 3rd dorsal tergite, into microcapillary tubes previously washed in a 0.1% (wt:vol) phenylthiourea solution in water. The protein concentration was determined spectrophotometrically for each bee (Cremonez *et al.*, 1998). The mean haemolymph protein concentrations of the bees fed the various diets were compared by ANOVA and comparisons between the diets were made by Tukey's test (Sigma Stat, 1995).

Table 1. Crude protein (%) of the protein sources and mean protein titers (µg/µl haemolymph) of the haemolymph of individual six day old *Apis mellifera* workers, fed on artificial diets or pollen from day 0 (when the newly-emerged bees were placed in the cages). The haemolymph was collected from five to six bees (analysed individually) from each of three sequential repetitions (cages of 100 bees). The protein contents of the diet materials (before mixing with sucrose to prepare the diet patties) were obtained by analyses made by Industrial Laboratories of Canada Inc. (Feedbee®), and by the manufacturer (Bee-Pro®), except for the pollen and acacia pod flour, which were analyzed in Brazil. *Protein titers followed by the same letter are not significantly different from each other (Tukey's test, α = 0.05).

Diet	Crude protein content	Protein titer* in the haemolymph	Standard deviation of the protein titers	N (number of bees tested)
Feed-Bee®	36.4	9.42[a]	4.09	18
Bee-Pro®	29.9	8.95[abc]	3.51	16
Pollen	20	6.26[bc]	2.19	17
Acacia pod flour	22	6.00[c]	2.67	15
Sucrose	0	3.56[d]	1.62	17

Results

The titers of protein in the haemolymph (µg/µl) of the caged honey bees varied significantly among the diet groups ($P < 0.01$, ANOVA, Table 1). Feed-Bee® gave the highest protein levels, but it was not significantly superior to the other commercial diet, Bee-Pro®. However, Feed-Bee® performed significantly better than pollen, acacia pod flour and sucrose, while Bee-Pro® was significantly superior to acacia pod flour and sucrose, but not to pollen. All of the proteinaceous diets were superior to sucrose syrup in their ability to elevate the protein content of bee haemolymph.

Discussion

Both Feed-Bee® and Bee-Pro® out performed pollen (Table 1), giving 2.65 and 2.51 times more protein in the bee haemolymph, respectively, than the sucrose controls, while the increase obtained with pollen was 1.76. This may seems a surprising result, but although pollen is the natural protein source for honey bees, they normally consume it after it has been fermented, in the form of "bee bread" (Herbert and Shimanuki, 1978). Bee bread is superior to bee collected pollen when haemolymph protein values of bees fed on these materials are compared (Cremonez *et al.*, 1998). Though pollen is rich in protein (Roulston and Cane, 2000), it is apparently not all fully available until it has been processed by the bacteria in bee bread (Herbert and Shimanuki, 1978; Gilliam, 1997). We also have no information on the plant origins of the pollen we used; this factor influences the nutritional value of pollen (Barbier, 1970; Baker and Baker, 1983; Pernal and Currie, 2000; Gregory, 2006). The acacia pod flour gave nearly the same protein titers as did the bee-collected pollen (1.69 times the protein found in sucrose fed bees), indicating that this local pollen substitute has some value for honey bees. All four protein sources originally contained at least 20 % crude protein (Table 1), before being mixed with sucrose and water. The acacia pod flour we tested contained about 20 % protein, similar to the levels found in pollen, but can vary from 8 % to 40 % crude protein, depending on the fraction of seeds included when it is produced (Perriera *et al.*, 2006). Gregory (2006) reported similarly high levels of haemolymph proteins (*ca.* 30 µg/ml) in both Africanized and European honey bees at Weslaco, Texas, USA fed pollen and Feed-Bee®, but lower levels in those fed Bee Pro®. Amdam and Omholt (2002) reported much higher amounts of vitellogenin in haemolymph than did Gregory (2006) and what we found in the present study. Their results may reflect the temperate adaptations of the race of honey bees they studied, the time of year of their studies, and the requirements for storage proteins in northern temperate zone honey bees as they prepare physiologically for winter. Our study was of tropically adapted Africanized honey bees. Queenless groups of worker honey bees in hoarding cages might moreover be expected to divert protein ingested into ovarian development.

Overall, both Feed-Bee® and Bee-Pro® were superior, the former statistically so, to fresh bee collected pollen and therefore are confirmed to be adequate alternatives for feeding bees. The laboratory trials measuring haemolymph protein in small groups of caged bees successfully paralleled experience with colonies in the field and also demonstrated that modern pollen substitutes can be superior to, or as good as, bee collected pollen, with the added advantages of lower cost and no risk of spreading bee diseases. This protein substitute evaluation technique (Cremonez *et al.*, 1998) also provides a means of determining whether locally available protein materials such as acacia pod flour are good candidates for the development of efficient and inexpensive bee diets.

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