The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.)

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ABSTRACT

Elucidating the mechanisms by which honey bees process pollen vs. protein supplements are important in the generation of artificial diets needed to sustain managed honeybees. We measured the effects of diet on protein concentration, hypopharyngeal gland development and virus titers in worker honey bees fed either pollen, a protein supplement (MegaBee), or a protein-free diet of sugar syrup. Workers consumed more pollen than protein supplement, but protein amounts and size of hypopharyngeal gland acini did not differ between the two feeding treatments. Bees fed sugar syrup alone had lower protein concentrations and smaller hypopharyngeal glands compared with the other feeding treatments especially as the bees aged. Deformed wing virus was detected in workers at the start of a trial. The virus concentrations increased as bees aged and were highest in those fed sugar syrup and lowest in bees fed pollen. Overall results suggest a connection between diet, protein levels and immune response and indicate that colony losses might be reduced by alleviating protein stress through supplemental feeding.

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1. Introduction

Honey bee colony losses are greater now than any time in recent history (vanEngelsdorp et al., 2007, 2009). The causes for colony deaths have been attributed to parasitic mites (Amdam et al., 2004) and disease, but reasons for other losses such as those dying from colony collapse disorder (CCD) have been elusive (vanEngelsdorp et al., 2009). Perhaps an underlying factor contributing to colony losses is inadequate nutrition. A steady supply of pollen insures the growth of colonies because it provides protein to adult bees and stimulates brood rearing. Colonies with low nutritional reserves have reduced brood rearing (Keller et al., 2005; Mattila and Otis, 2007; DeGrandi-Hoffman et al., 2008) and workers that transition from nest activities to foraging earlier in their adult life (Schultz et al., 1998; Toth et al., 2005; Toth and Robinson, 2005). Worker longevity is affected by the age of first foraging (Guzman-Novoa et al., 1994; Rueppell et al., 2007). Workers that become foragers earlier in life die sooner than their nestmates that perform tasks in the hive. Thus, colonies with limited protein intake decline from the combination of reduced brood rearing and a shorter lifespan for adult workers. If parasitic mites and pathogens are present, the population decline can be even more severe so that the colony perishes.

Honey bees rely on pollen as their source of protein, lipids, sterols, vitamins, minerals and certain carbohydrates (Todd and Betherick, 1942). Nectar is primarily a carbohydrate source, but can contain some amino acids and lipids (Percival, 1961; Baker and Baker, 1975). Some digestion of pollen occurs in the midgut, but the primary means by which the nutrients from pollen are made available to the colony is its conversion to worker jelly. The conversion occurs in the paired food glands called hypopharyngeal glands (HPG) located in the frontal area of the worker's head (Hrassnigg and Crailsheim, 1998). The glands are comprised of acini that produce the protein-rich worker jelly that is fed to larvae of all castes and to the queen. Nurse bees distribute the jelly to nestmates through trophallactic interactions (Crailsheim, 1991). Via this system, the nutrients from the pollen are circulated throughout the colony.

In addition to the need for pollen in brood rearing and to optimize worker longevity, nutrition (particularly protein availability) is a key factor in resistance to pathogens (Ford et al., 2001; Kaminogawa and Nanno, 2004; Ritz and Gardner, 2006; Rowley and Powell, 2007). Encapsulation, phenyloxidase, and lysozyme activity are enzyme-based immune responses to foreign invaders such as virus and to wounding and are affected by protein deficiencies (Siva-Jothy et al., 2005; Lee et al., 2006). In honey bee colonies, protein deficiencies that affect the immune response could accelerate the spread of disease among nestmates and cause pathogen levels to increase so that adult longevity and survival are reduced. Thus, what began as a nutritional deficiency could develop into colony loss from disease.

Pollen is not always available to colonies, so in managed hives, protein supplements are fed to bees to stimulate brood rearing and prevent colony populations from declining (e.g. Nabors, 2000; Mattila and Otis, 2006a). The supplements might not contain pollen, but instead have protein derived from sources such as soy or whey. Ideally, feeding protein supplements causes a flow of nutrients through a colony that is similar to pollen because the supplement stimulates the HPG of young bees to produce worker jelly. In some instances, feeding colonies protein supplements can cause colony populations to grow at rates that are similar to those fed pollen (DeGrandi-Hoffman et al., 2008; Mattila and Otis, 2006a).

Whether protein supplements are metabolized in a similar manner to pollen is not known. Here we compare protein levels and HPG acini size of young worker bees fed either bee-collected pollen or a protein supplement. The effect of nutrition on immune response was indirectly inferred by comparing virus concentrations over time in workers fed different diets. Specifically, we examined the effects of diet on titers of deformed wing virus (DWV). This positive stranded RNA virus causes morphological deformities (i.e., stick wings) and early death in newly emerged adult bees (Bowen-Walker and Gunn, 1998). DWV is one of the most widespread and prevalent viral infections of *A. mellifera* worldwide (Allen and Ball, 1996; Ellis and Munn, 2005). There is a significant correlation between DWV titers and overwintering colony losses (Highfield et al., 2009), and along with other picorna-like viruses it is associated with colony losses from CCD (Johnson et al., 2009).

We compared the effects of a protein supplement with pollen and a protein-free feeding regime on newly emerged worker bees placed in cages. There are strengths and limitations with using caged bees rather than colonies in studies such as ours. A limitation is that it does not account for colony-level interactions that might affect worker metabolism and immune response. However, a strength of this method is that the cages eliminate variability from differences that occur among colonies in terms of incoming nectar and pollen, size of brood areas, queen egg laying rates, and colony population size and age structure. We interpret the results with respect to the limitations of cages, but also discuss how our results might be extended to colony-level effects of supplemental protein feeding on colony health and population growth.

2. Materials and methods

The study was conducted at the Carl Hayden Bee Research Center, Tucson Arizona, U.S.A. from June through July of 2008. All bees used in the study were from European honey bee (*Apis mellifera ligustica*) colonies headed by commercially produced and mated European queens (Kona Queens, Captain Cook, HI). We conducted three trials over a 6-week period. A trial lasted for 11 days. In each trial we established 5 cages per diet treatment for a total of 15 cages per trial. A different source colony for the bees was used in each trial. Protein concentrations and HPG measurements were made using three bees collected randomly from each cage during each sampling interval.

2.1. Preparation of diets

Bees were fed ad lib either pollen patty or a protein supplement that lacks pollen (MegaBee® patty). MegaBee was chosen as a protein supplement because in full sized queen right colonies it is consumed at rates that are similar to pollen patties (DeGrandi-Hoffman et al., 2008). Pollen patties contained a mixture of pollens collected by bees in the Sonoran desert in Arizona, USA. The pollen was collected less than 12 months before the start of the study, and kept frozen until using it for making the patties. After the patties

were made, they were kept frozen until fed to the bees. Previous studies examining the degradation of stored pollen over time indicate that the nutritional value does not decline in the first year of storage so that the lifespan of workers in cages (deGroot, 1953) or brood production in colonies is reduced (Hagedorn and Moeller, 1968; Haydak, 1970).

Pollen patties were made by combining pollen with equal parts (by weight) of granulated sucrose, Drivert sugars (a mixture of equal parts of sucrose and dry fructose) and tap water (DeGrandi-Hoffman et al., 2008).

2.2. Feeding diets

At the start of each trial, frames with sealed worker brood were put in a temperature controlled room (32–34 °C). When the adults emerged, they were pooled and transferred into cages. Twenty workers were sampled from the pool of newly emerged bees (five each for estimates of protein and HPG development and 10 for detection and quantification of virus) prior to the treatment to establish a baseline for protein concentration, HPG development and virus titer (day 0 samples) for the trial. Then, an average of 124 ± 2 newly emerged bees were transferred into each of the plexiglas cages (15 11.5 cm × 7.5 cm × 16.5 cm) used per trial. All bees in the cages were <24 h old at the start of the trial. The bees were fed pollen patty, or a protein supplement. Vials with distilled water and high fructose corn syrup also were provided to treatment and control cages. Control cages received no pollen patty or protein supplement. We calculated the amount of diet consumed during the 11-day period of each trial by weighing the pollen and protein supplement patties at the beginning and end of the trial. Bees were sampled from all cages after feeding for 4, 7 and 11 days. Sampled workers were kept frozen until protein and HPG analysis.

2.3. Protein analysis

Protein concentrations were estimated by removing the heads from three worker bees sampled per cage, and homogenizing them individually in 2 ml Eppendorf microcentrifuge tubes (Eppendorf North America, Westbury, NY) containing 75 mM phosphate buffer solution (pH 7.4). The sample was centrifuged and analyzed for protein content using the 500-0202 Quick Start Bradford Protein Assay Kit 2 (Bio-Rad Laboratories, Hercules, CA) (Sagili et al., 2005; Sagili and Pankiw, 2007). The protein concentrations in the pollen cake and the protein supplement were estimated using the same protein assay kit. Standard curves to estimate protein concentration in the samples were prepared using bovine serum albumin (BSA). Protein absorbance was measured at 595 nm using a Biotek Synergy HT spectrophotometer.

2.4. Hypopharyngeal gland measurements

HPG were removed from 3 bees per cage. The glands were placed in a Petri dish with wax depressions containing water. A photo of the glands was taken using a Leica MX12 microscope with a Leica DC-300 camera and Leica IM-50 image manager software program (v 1.2). For calibration, a photograph of a 1 mm line was taken at the same magnification used for observing the HPG. The 2-dimensional areas of five acini were measured for each HPG sample using the Photoshop (Adobe) pixel counting routine. The area of each acini was estimated by the equation: $N_a \times (N_{mm})^{-2}$ where N_a = the number of pixels for each acini and N_{mm} = the number of pixels in a 1 mm line that is 1 pixel wide. N_{mm} was squared to estimate the number of pixels in a 1 mm × 1 mm area and multiplied by 0.001 to convert it to microns. The size of the five acini was averaged and used as acini measurement for the bee.