

EFFECT OF PHAGOSTIMULANTS IN ARTIFICIAL DIETS ON HONEY BEE FEEDING BEHAVIOR

Anita Hanna and Justin O. Schmidt

Carl Hayden Bee Research Center, ARS, USDA, 2000 E. Allen Rd., Tucson, AZ 85719

ABSTRACT

The role of phagostimulants and diet formulation in dietary choices made by honey bees, *Apis mellifera* L., was studied in laboratory. Diets in which sucrose or pure glucose comprised the added carbohydrate sweetener rapidly lost water, hardened, and became difficult for the bees to eat. Reduction of the glucose level to 75% or less and replacement of sucrose with fructose yielded a soft, acceptable diet texture that lost little water and could be readily consumed by bees. A sugar combination of 50% fructose and 50% glucose appeared ideal for incorporation in bee diets. Feeding choice preference tests in 1-3 days old honey bees revealed that addition of phagostimulant increased diet consumption over untreated controls. Addition of as little as 1-5% pollen extract significantly enhanced dietary feeding behavior in all diets tested. Phagostimulant enhancement of seven commercial honey bee substitute diets or dietary ingredients was analyzed for nutritional potential in longevity tests. The diets differed in the time lengths of bee survival, suggesting potential differences among the nutritional values of the diets. A complicating factor was the presence of heavy infestations of varroa mites, *Varroa destructor* Anderson & Trueman, on the bees. Phagostimulants derived from pollen appear to play an important role in feeding behavior of honey bees and the potential for developing more successful artificial diets for bees.

INTRODUCTION

Honey bee foragers are known to collect a wide variety of nutritive and non-nutritive substances that are presumed to be food by the foragers (Dietz 1975). The general view had been that bees were not discriminatory in food selection and based decisions mainly on texture or particle size of the pollen or powdery substance collected. A role of taste was not recognized. The myth that bees were not selective in their food preference was challenged with experiments that showed foragers given choices of pollen from four different plant species exhibited distinct preferences among the pollen types (Schmidt 1982). Likewise, young nurse bees that actually consume the pollen brought to the hive exhibited strong preferences for pollen of some species over those of other species (Schmidt and Johnson 1984). Nurse bees given a choice of pollen from individual species, versus a mixture of pollen from those same species, preferred the mixed pollen (Schmidt 1984). These reports indicated that bees likely use taste, particularly the presence of pollen-produced phagostimulants, in their choice of food sources, a postulation confirmed with feeding trials involving extracts from pollen added to a laboratory artificial diet (Schmidt 1985).

Diets that substitute other proteins for pollen are an important aspect of modern honey bee management. Substitute diets are administered when bees are weakened due to periods

of pollen dearth, population loss after pesticide spraying, or from heavy varroa or tracheal, *Acarapis woodi* (Rennie), mite infestation (Haydak 1970, Standifer et al. 1978). Additionally, substitute diet is needed to manipulate colonies to have optimal populations at the times of nectar flows and for crop pollination (Shuel and Dixon 1986, Peng et al. 1984). Currently, all available substitute diets lack pollen phagostimulants, have ingredient or texture problems, and are generally poorly accepted by the bees.

An effective diet must provide most of the nutritive elements for growth and development of honey bees, have overall high nutritional value, have a proper texture and consistency, and not be toxic (Schmidt 1984). Crucial elements in insect diets, including those of honey bees, are the presence of phagostimulants and the absence of feeding deterrents (Schmidt 1985).

The effectiveness of nutritive diets can be evaluated both behaviorally and physiologically (Schmidt et al. 1987). A behavioral measure is relative quantities of diets consumed in choice tests. An estimate of the physiological value of a diet is the longevity of animals fed the diet. In order to improve the attractiveness of artificial diets to honey bees and to increase optimal feeding, we tested the role of phagostimulants in dietary acceptance and nutritional value in bees. The objectives of these studies were: 1) to determine the palatability of diets for honey bees (Texture and Moisture Test), 2) to determine the role of phagostimulant in diet choices of honey bees (Feeding Preferences), and 3) to determine the nutritional value of diets for honey bees (Survival Studies).

MATERIALS AND METHODS

Honey bee corbicular pollen from mixed floral sources was obtained from colonies located near Sierra Vista, Cochise County, Arizona, during the spring 2001. The pollen was stored at -20°C until extracted. Pollen was extracted for 8 h in a soxhlet extractor with ethyl acetate (Burdick and Jackson, Muskegon, MI), and the liquid fraction was evaporated to near dryness, dissolved in acetone, and stored at -20°C until used. Extract amount used in the tests is listed as a percentage. For example, 100 g of a diet containing 5% pollen extract would contain the extract of 5 g of pollen added to 100 g dry weight of diet.

Test diets were obtained from commercial sources and given the following abbreviated names: Bee Pol (International Ingredients, St. Louis, MO); BeePro (Mann Lake, Hackensack, MN); Beltsville bee diet, or BBD (Bio-Serv, Frenchtown, NJ); Casein (International Ingredients); Dadant (Brood BuilderTM, Dadant & Sons, Hamilton, IL); Kelley ("Pollen Substitute", Walter T. Kelley, Clarkson, KT); and Rice Protein (Product STD 50-MF1000, International Ingredients). Phagostimulant was added into diets by pipetting the appropriate amount of acetone solution into a beaker, evaporating solvent just to dryness, and mixing dry diet with the extract until uniformity was achieved. At this point, enough distilled water was added and mixed to form a moist cake. When not fed to bees, test diets were kept at 4°C for up to one week.

All tests were conducted in an environmental room designed to simulate the conditions inside the brood chamber of a normal honey bee hive. The room was illuminated by red fluorescent lights and maintained at 34°C and 60-70% relative humidity.

Texture and Moisture Test. Test diets in hives or in the environmental room often become hard, dry, and difficult for bees to consume. Preliminary tests revealed that diets formulated with sucrose quickly became hard and unsuitable. Since water loss within 24 hours from diets appeared to be good measure of potential hardness and unpalatability to bees, we determined water loss from diets formulated with different ratios of the sugars glucose and fructose. Five diet treatments consisting of 50% total sugar were formulated with the following ratios of fructose and glucose: 100:0; 75:25; 50:50; 25:75; 0:100. The test diets were placed in 50 mm Petri dishes in the environmental room and weighed daily for water gain or loss.

Feeding Preferences. Seven commercial diets or dietary ingredients were tested with five phagostimulant treatments: extract from pollen equivalent to 1%, 2.5%, 5%, 10% and 20% pollen added to the diet. All diets were made by adding equal amounts of 50:50 fructose:glucose to the dry diets and mixing with enough distilled water to make a soft kneadable texture.

For the tests, about 125 (15 g) 1- to 3-day old worker bees were placed in 9x6x15 cm acrylic plastic and screen cages as described by Schmidt (1984) and Schmidt et al. (1995). Each test consisted of three replications. The bees were offered a choice of test diet plus phagostimulant or test diet alone. They were also provided water, a piece of beeswax foundation upon which to rest, and a 50% sucrose solution. The test and reference diets in each cage were weighed after 48 hours, replaced with fresh diet in reversed cage positions, and weighed again after 48 hours. The bees were then released to a hive. A t-test was used to show the difference between test diets and control (SAS Institute 1994) with a significant value of $P < 0.05$.

Survival Studies. The effectiveness of the phagostimulant enhanced diets on the survival and longevity of bees was conducted on each of the seven diets. Tests followed the procedures of Schmidt et al. (1987, 1995) and consisted of three replicates each. Tests were conducted in the environmental room using 60 1-day old bees, heavily infested with *Varroa destructor* mites, placed in acrylic plastic and screen cages (9x6x15 cm). Each cage was provided water, a piece of beeswax foundation for the bees to rest upon, 50% sucrose solution, and a single test diet. Diet consumption was measured every 2 or 3 days until the last bee died. Mortality curves were generated with survival analysis by SAS Proc (SAS Institute 1994) followed by Wilcoxon and Log-Rank tests; diet consumption analysis was by analysis of variance.

Rates of Diet Consumption. The comparison of diet consumption of honey bees fed several artificial diets were conducted as described by Schmidt et al. (1995). The experimental conditions were described in the section on survival studies. The diets were replaced every second or third day for 30 days and data were analyzed by analysis of variance (SPSS 1990).

RESULTS AND DISCUSSION

Texture and Moisture Test. Initial treatments in which sucrose was used as the sugar tended to lose water rapidly, become very hard within 24 hours, and were difficult for the bees to consume. After dietary sugars were changed to 50% fructose and 50% glucose, evaporation rates decreased dramatically: Bee Pol from 25.8% to 4.8%; BeePro from 37.6% to 12.9%; Dadant from 29.9% to 11.6%; Kelley from 38.3% to 12.8% (Table 1).

Diets consisting of glucose as the only added sugar lost considerable weight over a 48 hour period and became hard. In contrast, diets containing at least 25% fructose either lost

TABLE 1. Mean Percentage Water Loss from Test Diets Formulated Using Sucrose or 50:50 Fructose:Glucose as the Sugar Component.

Diets	Sucrose (Mean $\pm$ SD)	50:50 Fructose:Glucose	Probability ^a (Mean $\pm$ SD)
Bee Pol	25.79 $\pm$ 1.66	4.80 $\pm$ 1.67	0.0001
BeePro	37.58 $\pm$ 2.94	12.86 $\pm$ 1.54	0.0001
Casein Protein	7.85 $\pm$ 0.94	6.02 $\pm$ 0.65	N.S
Dadant	29.90 $\pm$ 2.31	11.62 $\pm$ 2.34	0.0001
Kelley	38.33 $\pm$ 4.53	12.77 $\pm$ 1.99	0.0001
Rice Protein	36.22 $\pm$ 3.94	4.26 $\pm$ 0.76	0.0001

^aDifference in water loss between sucrose and 50:50 fructose:glucose (t-test)

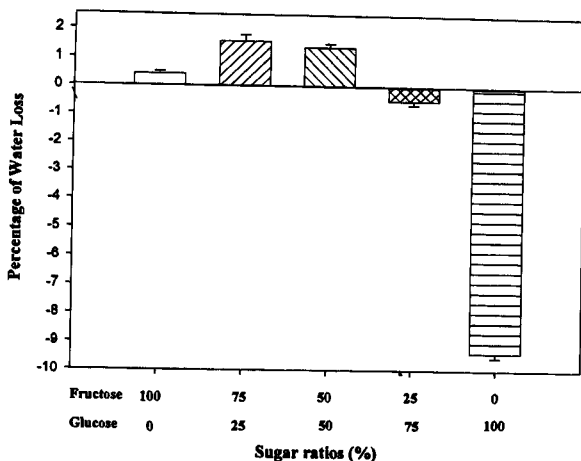


FIG. 1. Water Loss from Bee Diets Made with Fructose and Glucose.

little water or gained some atmospheric water to increase slightly in weight (Fig. 1). Those diets that gained weight were softer after 48 hours than at the beginning of the test. For the other tests, we chose the composition of 50% fructose and 50% glucose to be added to the diets, in part because this ratio maintained a very good diet texture and in part because honey, the normal sugar source in the hive, is composed of about equal portions of fructose and glucose (White 1992). Experiments using sucrose and high fructose corn syrup were not successful because these sugars tended to allow large water losses from the diets (data not shown).

Feeding Preferences. Honey bees readily discriminated between artificial diets with or without phagostimulant extract (Fig. 2). For many diets the difference was evident even with addition of as little as 1% (Beltsville Bee Diet, Casein protein, Rice Protein) or 2.5% pollen extract (Bee Pol, Dadant, Kelley). Only the BeePro diet required extract of 5% pollen to cause a difference. In general, increases in phagostimulant levels above those first eliciting preference produced little increase in preference. This effect suggests that honey bee feeding behavior might be strongly influenced by a threshold effect; that is, once a threshold phagostimulant concentration is reached to signal that the food is recognized and acceptable, little added stimulus enhancement occurs. In practical terms this indicates that very little pollen extract, typically less than 5%, is required to effect meaningful improvement of the acceptance of the substitute diet by the bees. This, of course, assumes all other factors in the diet are suitable for the bees.

Survival Studies. Cumulative survival curves of honey bees fed different diets are shown in Fig. 3. The average life span of the bees fed various diets ranged from 7 to 12.5 days, with a mean of 8.9 days (Fig. 4). Significant differences among groups of diets were observed ($P < 0.0001$, Wilcoxon and Log-Rank Tests). Three of the commercial diets, Kelley, Bee Pol and BeePro, prolonged the life span of honey bees relative to the average.

Rates of Diet Consumption. Diet consumption rates from seven diets are shown in Fig. 5. The bees generally consumed diet most rapidly during the first 5 days. Thereafter, consumption decreased sharply and by day 10 had almost ceased. Analysis of variance shows that the mean rate of consumption had a highly significant effect on the total of consumption of the diets ($P < 0.05$).

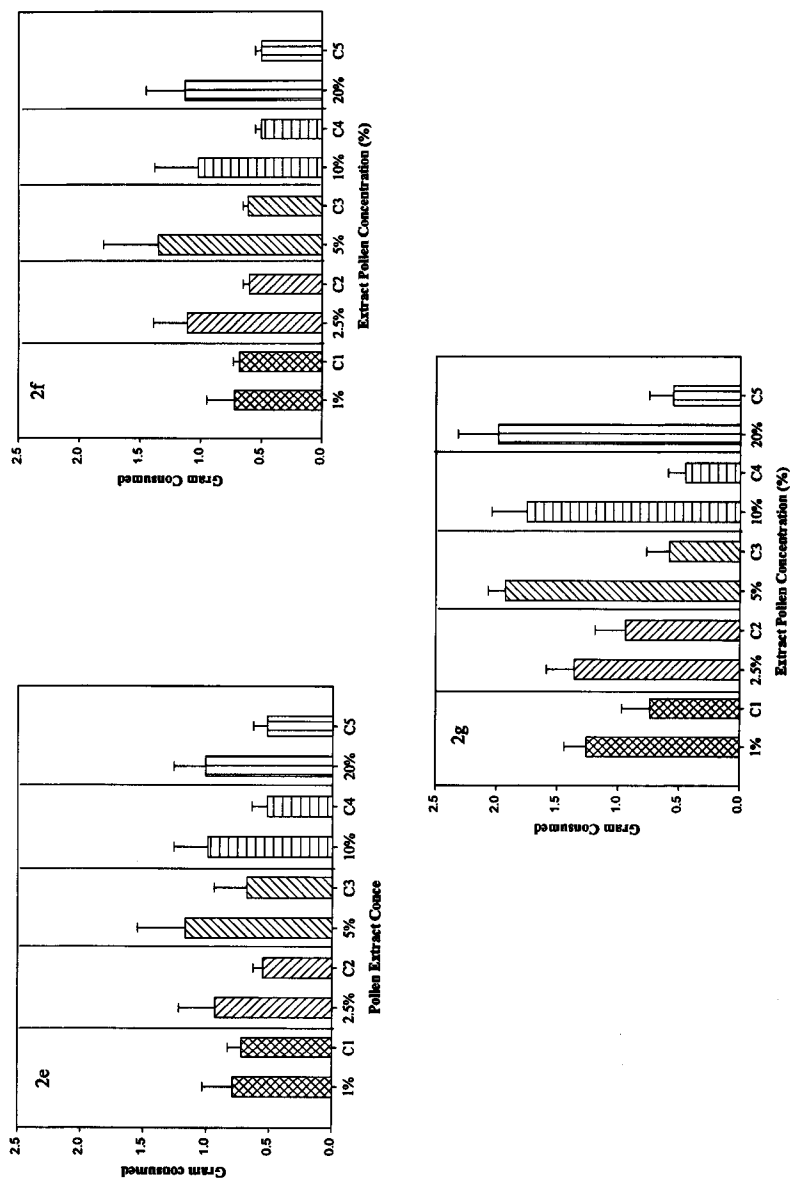


FIG. 2 (Cont.). Consumption by Honey Bees Given a Choice of Phagostimulant Enhanced and Control Diets. Diets With Pollen Extract Designated as %, Diets Without Extract as C: 2e, Dadant; 2f, Kelley; 2g, Rice Protein (Mean ± SD)

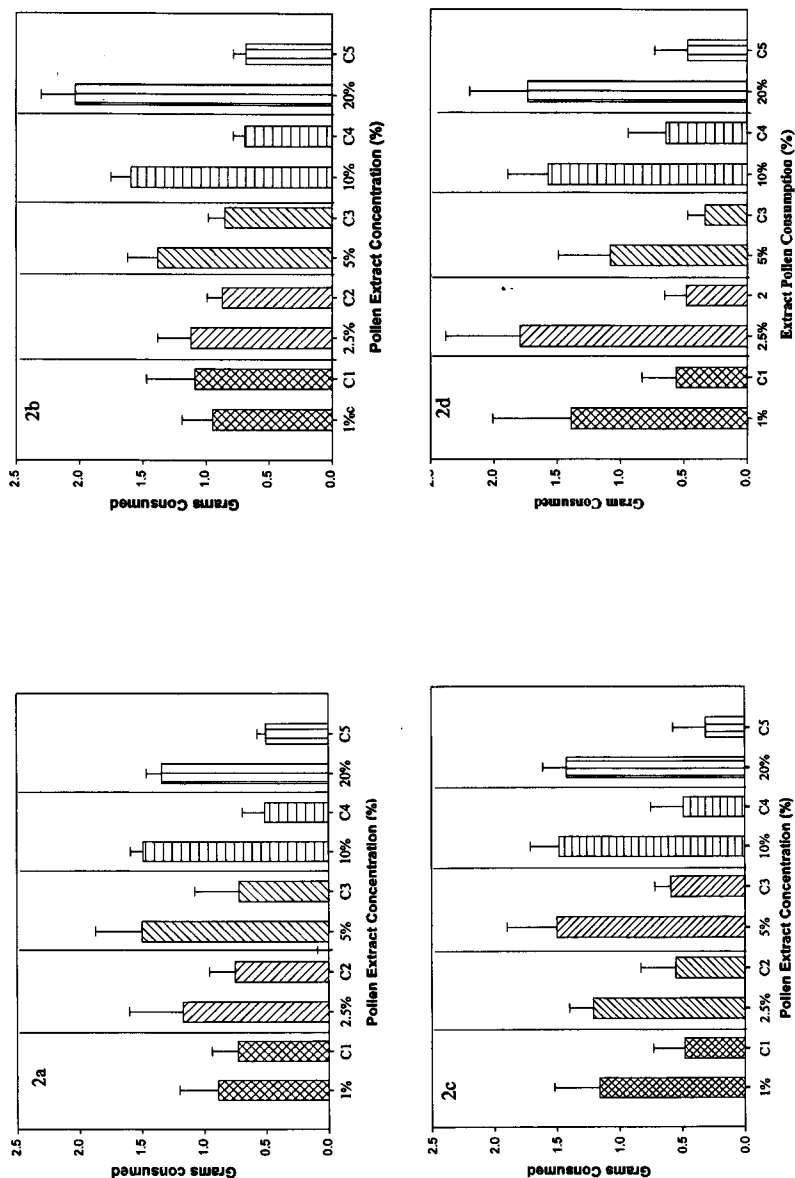


FIG. 2. Consumption by Honey Bees Given a Choice of Phagostimulant Enhanced and Control Diets. Diets With Pollen Extract Designated as %, Diets Without Extract as C: 2a, Bee Pol; 2b, BeePro; 2c, BBD; 2d, Casein Protein (Mean $\pm$ SD)

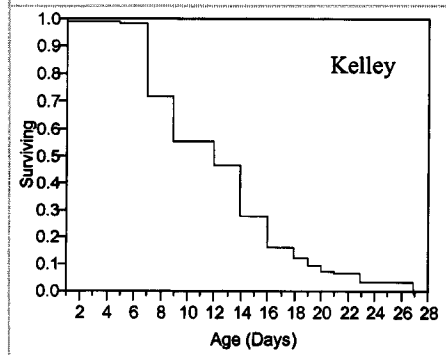
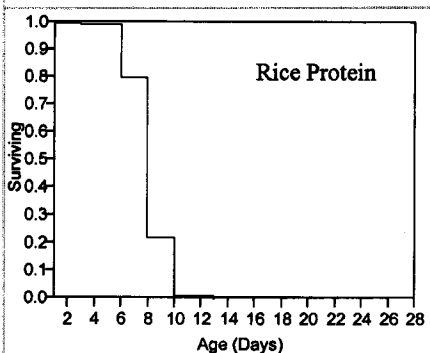
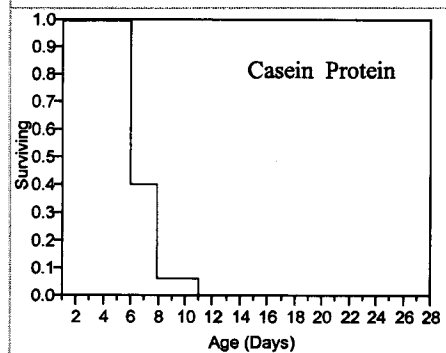
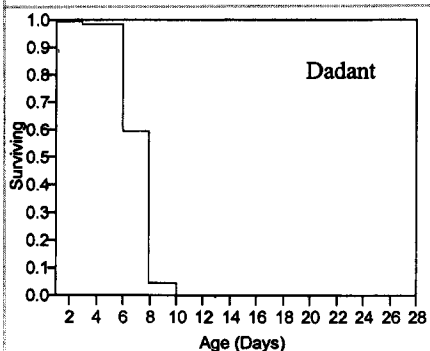
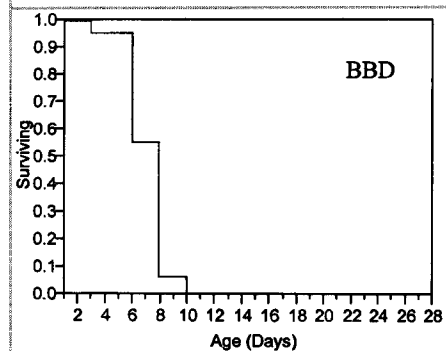
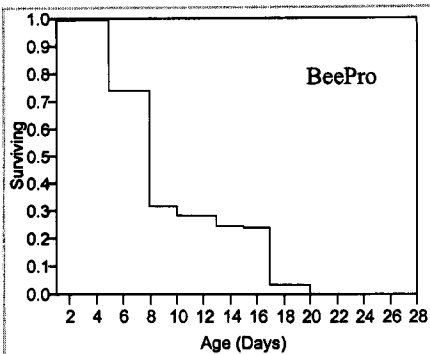
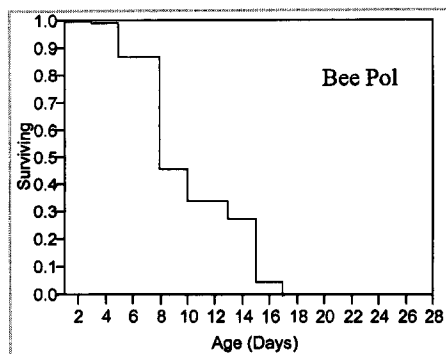


FIG 3. Survival Profiles of Honey Bees Fed Single Source Diets.

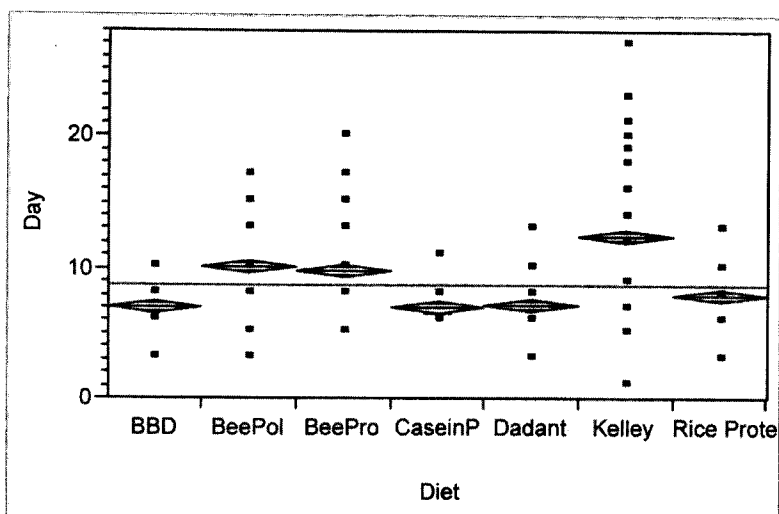


FIG. 4. Mean Longevities of Honey Bees Fed Single Source Diets. Diamond Shape is Group Mean of Each Diet.

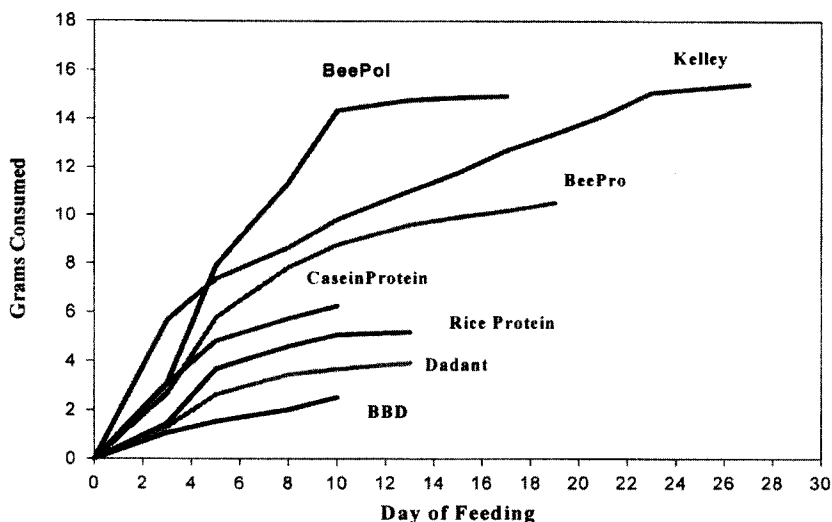


FIG. 5. Cumulative Food Consumption of Honey Bees Fed Single Source Diets.

Schmidt et al. (1987) reported a similar study using different pollens instead of substitute diets. In that study bees were fed 25 different pure pollens plus several blends of pollen as sole food sources (other than sugar). Survival times for these bees (30-35 days)

were better than in the present study (8.9 days). In another study, Schmidt et al. (1995) analyzed three types of pollen (sunflower, sesame and rape) and obtained average life spans of 31, 33, and 51 days, respectively. The difference between our studies and the earlier studies are both diet types and parasite infections of the bees. The first two studies fed bees with pollen, their natural food source; we fed pollen-free artificial diets enhanced with minor quantities of pollen flavor. Pollen provides all the nutrients needed by bees for growth and development, except for energy, and is readily digested (Schmidt and Buchmann 1985, 1992). Because the diets tested in the current experiments likely do not effectively replace the nutrients in pollen and because the bees used were heavily infested with varroa mites, results from this study cannot be directly compared to those of the earlier studies.

ACKNOWLEDGMENT

We are indebted to Leonard Hines for providing pollen and Steve Thoenes and Hayward Spangler for manuscript reviews.

LITERATURE CITED

- Dietz, A. 1975. Nutrition of the adult honey bee, pp. 125-156 *In* Dadant and Sons [eds.], *The Hive and The Honey Bee*. Dadant and Sons, Hamilton, IL.
- Haydak, M. H. 1970. Honey bee nutrition. *Ann. Rev. Entomol.* 15: 143-156.
- Peng, Y. S., Marston, J. M., and O. Kaftanoglu. 1984. Effect of supplemental feeding of honeybee (Hymenoptera: Apidae) populations and the economic value of supplemental feeding for production of package-bees. *J. Econ. Entomol.* 77: 632-636.
- SAS Institute 1994. *SAS/STAT User's Guide*. SAS Institute Inc., 4th edition.
- Schmidt, J. O. 1982. Pollen foraging preferences of honey bees. *Southwest. Entomol.* 7: 255-259.
- Schmidt, J. O. 1984. Feeding preferences of *Apis mellifera* L. (Hymenoptera: Apidae): individual versus mixed pollen species. *J. Kansas Entomol. Soc.* 57: 323-327.
- Schmidt, J. O. 1985. Phagostimulants in pollen. *J. Apic. Res.* 24: 107-114.
- Schmidt, J. O., and S. L. Buchmann. 1985. Pollen digestion and nitrogen utilization by *Apis mellifera* L. (Hymenoptera: Apidae). *Comp. Biochem. Physiol.* 82A: 499-503.
- Schmidt, J. O., and S. L. Buchmann. 1992. Other products of the hive, pp. 927-988. *In* J. Graham [ed.], *The Hive and The Honey Bee*. Dadant and Sons, Hamilton, IL.
- Schmidt, J. O., and B. E. Johnson. 1984. Pollen feeding preference of *Apis mellifera*, a polylectic bee. *Southwest. Entomol.* 9: 41-47.
- Schmidt, J. O., S. C. Thoenes, and M. D. Levin. 1987. Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. *Ann. Entomol. Soc. Amer.* 80: 176-183.
- Schmidt, L. S., J. O. Schmidt, H. Rao, W. Wang, and L. Xu. 1995. Feeding preference and survival of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. *J. Econ. Entomol.* 88: 1591-1595.
- Shuel, R. W., and S. E. Dixon. 1986. An artificial diet for laboratory rearing of honey bees. *J. Apic. Res.* 25: 35-43.
- SPSS Institute 1990. *SPSS Base System User's Guide*. Norusis/SPSS Inc. pp. 211-215.
- Standifer, L. N., F. E. Moeller, N. M. Kauffeld, E. W. Herbert, and H. Shimanuki. 1978. Supplemental feeding of honey bee colonies. *USDA Information Bulletin No. 413*. 8 pps.
- White, J. W. Jr. 1992. Honey, pp. 869-925. *In* J. Graham [Ed.], *The Hive and The Honey Bee*. Dadant and Sons, Hamilton, IL.