

ARTIFICIAL DIETS FOR HONEY BEES, *APIS MELLIFERA*¹

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The search for a satisfactory substitute for pollen in the nutrition of honey bee colonies has occupied the attention of apiculturists for many years. Whitcomb and Wilson (1927) reported that colonies of bees could rear brood when fed only sugar syrup. Haydak (1935) confirmed this earlier report, but found that when a colony was fed a pure carbohydrate diet, brood rearing continued only for a short period of time. He also reported that the adult bees used materials from their own body tissues to produce larval food. This conclusion was based on his finding that the nitrogen content of the bodies of the adult bees decreased by an amount equal to the nitrogen content of the bodies of the newly emerging bees.

Soudek (1929) reported that when newly emerged adult bees were fed fresh egg white or dried yeast, their pharyngeal glands developed fully. Ribbands (1953) cited Currie as reporting that dried milk and dried egg white were the most promising pollen substitutes of the materials tested. Both resulted in full gland development and some brood production. Haydak (1933) reported better brood production by bees fed dried yeast than with other substances tested. Later Haydak (1937) reported that brood rearing resulted when caged colonies were fed soy flour.

Investigations comparing the nutritive value of pollen diets with non-pollen diets containing proteins of animal or plant origin were reported by Standifer et al (1960). Using the degree of pharyngeal gland development and length of life of newly emerged worker bees as criteria for evaluation, these investigators found that egg albumin was equal to pollen in promoting gland development, and that skim milk powder, sesame seed meal, and soy flour were nearly as effective. However, they were not able to show a correlation between gland development and longevity among bees on a particular diet and concluded that longevity alone could not be used to evaluate food stuffs for growth or brood rearing.

Recently Haydak and Dietz (1965) reported that any appropriate protein source is satisfactory for the growth of emerging bees and development of their pharyngeal glands, but that additional vitamins are indispensable for brood rearing.

Most investigators have used the glandular development of the worker bee and egg laying by the queen as criteria for measuring the suitability of an artificial diet. While these factors are important, a diet cannot be considered as a substitute for pollen unless several generations of brood can complete their development and emerge as adult bees. The so-called pollen substitutes actually should be called pollen extenders, as there is no known replacement for pollen that will permit the maintenance, or build-up, of a confined colony of bees for extended periods of time.

Recently, Weaver (1964) described an artificial diet on which caged bees were maintained for long periods of time even though the diet was greatly inferior to pollen as measured by brood production. Attempts to

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maintain bees on this diet at Gainesville, Florida, were unsuccessful, as the bees would not feed on the dry portion of the diet.

METHODS

In March 1965, investigations were initiated to study the nutritional requirements of a diet that could be used to replace pollen in the diet of adult honeybees. Small colonies of bees consisting of one frame of brood, two empty combs, a laying queen, and three pounds of worker bees were confined in 6x6x6 ft. screen cages. Each colony was supplied with distilled water and 60% sucrose syrup *ad libitum*. The diets used in these tests were formulated either as liquid or solid preparations. Liquid diets were placed in pans outside the colonies. Solid diets were prepared by mixing various fractions of bee collected pollen with the ingredients of a basic artificial diet in the desired proportions. The mixture was moistened with a small amount of filtered honey and formed into small cakes. Colonies usually were examined every day and a visual estimate of the amount of food consumed was made. A fresh cake containing 20 g of the diet was placed on top of the frames after removing any of the old food that remained.

Separation of whole pollen into various fractions was accomplished by the following standard technique. Lipid material was extracted by refluxing for 30 minutes 250 g of pollen in 940 ml of a 3:1 mixture of chloroform and ethanol. The mixture was filtered and the procedure repeated a second and third time. The defatted residue was air dried and the filtrate was concentrated by evaporating the solvent at room temperature (25°C). The dried residue and the semi-solid lipid material were refrigerated until used.

Water soluble materials were removed by mixing the defatted residue with water in a Waring blender for 15 minutes at high speed. The mixture was centrifuged, and the liquid carefully decanted. The solid residue was dried and the liquid portion concentrated to 1/5 its original volume in a swiftly moving air stream at a temperature of 25°C. Both portions were refrigerated until used.

The final step in the fractionation of pollen was to reflux 100 g of the defatted residue in 250 ml of 4N HCl for eight hours at 100°C. The mixture was filtered, and after repeated washing to remove the excess acidity, the residue was air dried at 25°C. The filtrate was concentrated under reduced pressure, and both portions refrigerated until used.

The basic design of the experiments reported upon in this paper was a sequential screening program much like that commonly used in testing candidate insecticides. No attempt was made to compare various diet formulations with respect to degree of effectiveness.

During most of the experimentation nine colonies of bees were used simultaneously. Occasionally a sequence of inadequate diets would reduce the population so severely in a colony that the colony had to be replaced or at least a frame of brood ready to emerge added.

In Table 2 diets are listed in the sequence in which they were tested in a particular colony. No attempt was made to achieve a standardized condition in a colony before a new diet was introduced. New diets were usually introduced the next day after discontinuing the previous test diet. No colony was observed to store any of the solid portions of any diet;

therefore, any influence of a previous diet on the bees within a colony was reduced to the immediate physiological state of the bees. For a discussion of this effect, see the Discussion and Conclusions section.

RESULTS

Confining the colonies in the cages had no apparent effect on the behavior of the adult bees or on colony development, since normal brood development occurred in the check colonies feeding on honey and pollen.

The first artificial diet tested (Diet 1) was a liquid-dry formulation modified from the diet reported by Weaver (1964). It consisted of 400 ml water containing in solution 600 g sucrose, 204 μ g carnitine, 6000 μ g inositol, 6000 μ g choline, 600 μ g glutathione, 204 μ g p-amino benzoic acid, and 0.6 ml Deca-Vi-Sol vitamin mixture. The following quantities of vitamins are supplied by 0.6 ml Deca-Vi-Sol: Vitamin A, 3000 U.S.P. units; Vitamin D, 400 U.S.P. units; Vitamin C, 60 mg. B₁, 1 mg; B₂, 1.2 mg; B₆, 1 mg; B₁₂, 1 μ g.; niacinamide, 8 mg; panthenol, 3 mg; and biotin, 30 μ g.) The liquid portion of Diet 1 was fed *ad libitum*. The dry portion of this diet was a mixture containing 200 g vitamin-free casein, 10 g corn oil, 10 g RNA, 2.4 g tryptophan, 5.4 g histidine, 1.2 g cystine, 3.8 g glycine, 1.0 g cysteine, 10 g cholesterol, and 4 g Wesson's salt mixture.

Diet 1A contained the same components as Diet 1, but all vitamins were incorporated into the dry portion of the diet. In both Diets 1 and 1A, the dry portions were presented to the bees in small aluminum pans. The bees, however, failed to accept or feed on the dry food.

Diet 2 was a liquid formulation that contained the following nutrients dissolved in 400 ml water: 10 g gelatin, 10 g casein, 1 g corn oil, 1 g cholesterol, 0.1 g Wesson's salt mixture, 0.1 g RNA, 600 g sucrose, and 0.6 ml Deca-Vi-Sol mixture. A few drops of Triton X-100 emulsifier were added and these ingredients mixed in a Waring blender. The bees ate this preparation readily, but some difficulty was experienced with the gelatin and casein settling out on standing.

Diet 3 was the same as Diet 1 except that the dry ingredients were moistened with filtered honey and formed into small cakes that were placed inside the hive on top of the frames.

Diet 4 was an all solid formulation containing 10% gelatin, 10% casein, 5% corn oil, 5% Wesson's salt mixture, 1% RNA, 1% cholesterol, and 68% sucrose. Ten grams of Diet 4 were moistened with filtered honey and 0.6 ml of Deca-Vi-Sol, formed into a cake, and placed on top of the frames every other day.

It was clear that consumption of the artificial diets was poor when compared with consumption of control diets of honey and pollen. Various modifications of Diet 4 were tried in an attempt to improve the palatability to the bees. The addition of 1 drop of a mixture of the following essential oils resulted in slightly increased consumption but did not improve nutritive value: anise star wormseed, cedar wood, geraniol, pennyroyal, caraway, Bergamot, sassafras, and fennelseed. The addition of an ether extract from 5 g of fresh citrus pollen did not improve either acceptance or nutritive value of the diet. One modification, reducing the amount of cholesterol from 1% to 0.5%, and subsequently to 0.25% by weight of dry ingredients, seemed to significantly increase the acceptance of the food. The diet was further modified and designated as Diet 15 (Table 1). Al-

though colonies feeding on Diet 15 were unable to mature larvae beyond an age of 3-4 days, as shown in Fig. 1, it was selected for further testing and is referred to as basic artificial diet in the later tests described in this paper. Results obtained in tests with Diet 15 and subsequently modified diets are presented in Table 2.

TABLE 1. FORMULA OF BASIC ARTIFICIAL DIET (DIET 15)

Percent by Weight				
Gelatin	5.00			
Casein, Vitamin Free	5.00			
Zein	5.00			
Egg Albumin	5.00			
Wesson's Salt Mix	5.00			
Mazola® Corn Oil	5.00			
Cholesterol	0.25			
RNA	1.00			
Sucrose	45.00			
Cellulose	23.75			
Vitamin Mixture (Amount/20 gms. of above mixture)				
Riboflavin	1.2 mg	Inositol	12.0	mg
Pyridoxine	1.0 mg	Carnitine	0.46	mg
Niacinamide	8.0 mg	p-amino benzoic Acid	0.48	mg
Thiamine	1.0 mg	Glutathione	1.18	mg
Ascorbic Acid	60.0 mg	Biotin	0.0298	mg
Panthenol	3.0 mg	Vitamin B ₁₂	0.00064	mg
Choline	12.0 mg			

(Enough filtered honey added to allow the molding of 20 g cakes)

It was evident that the basic artificial diet was deficient in some way that was critical for development of older larval stages, and that this deficiency could be satisfied by the addition of small amounts of whole pollen. As shown by the data in Table 2, and in Fig. 2, addition of 10% by weight, or even as little as 7.5% by weight of whole pollen (Diets 28 and 40) to the basic artificial diet resulted in complete brood development by colonies 5 and 8, but when only 5% whole pollen was added (Diet 19) brood development was not possible in Colony 5.

The addition of 10% or 20% of pollen lipids (Diets 17 and 27) to the basic artificial diet did not promote brood development in colonies 3 and 1, but colony 7 reared all stages of brood throughout the entire test period while feeding only on the residue remaining after the lipids had been extracted from whole pollen (Diet 21) and the 60% sucrose syrup. Normal, complete brood development also occurred in Colonies 4 and 9 when they

Fig. 1 (above) — Inability of larvae in Colony 1 receiving Diet 15 to mature beyond the 3-4 day stage. Fig. 2 (below) — Normal brood in Colony 5 receiving Diet 28 plus 10% whole pollen.

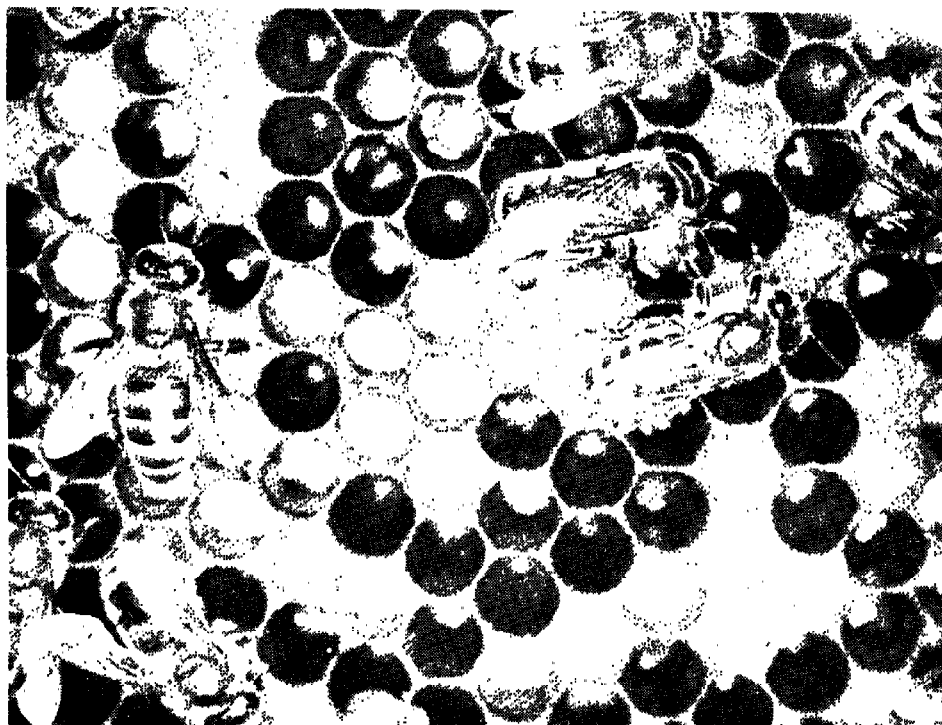
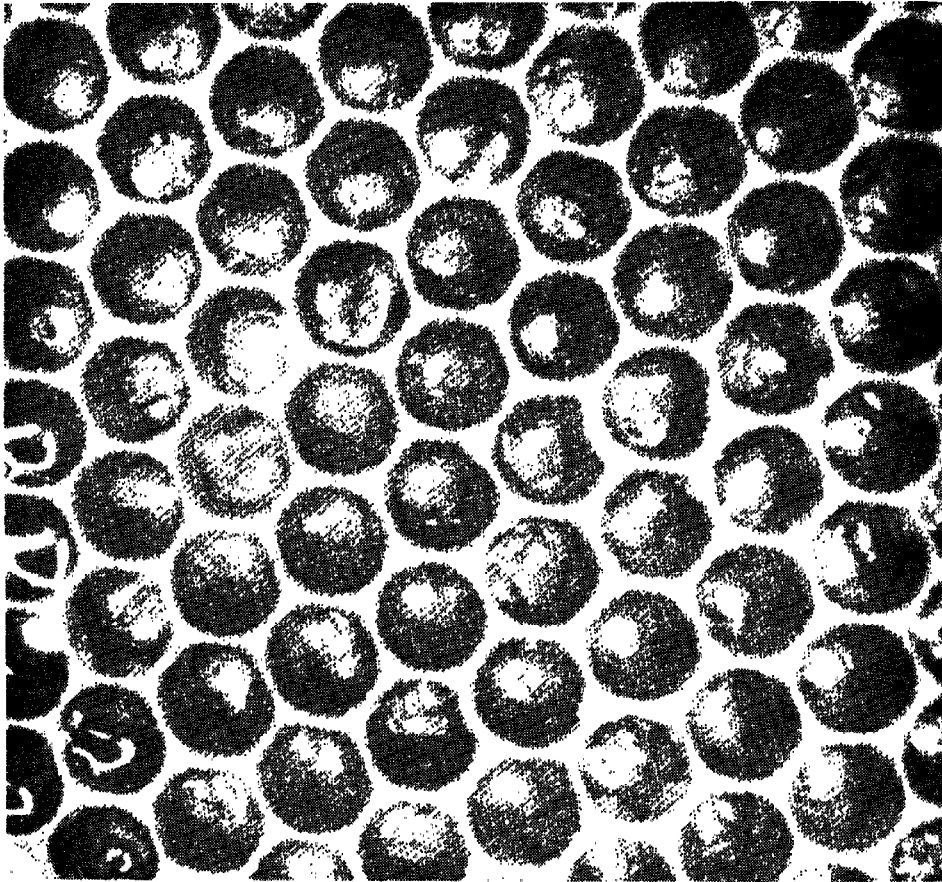


TABLE 2. FOOD CONSUMPTION AND BROOD REARING BY CAGED COLONIES OF HONEYBEES FEEDING ON VARIOUS DIETS.

Colony No.	Diet No. and Formula	Days Fed	Estimated Average Daily Consumption In Gms.	Oldest State of Brood Development Attained	Larval Food Supply*
1	15 Basic Artificial Diet (Table 1)	17	5	3-4 day larvae	Excellent
	27 Basic Diet plus 20% pollen lipids	8	15	2-3 day larvae	Excellent
2	22 Whole pollen	39	16	All stages including some emerging bees	Good
3	17 Basic Diet plus 10% pollen lipids	12	3	1 day larvae	Poor
	36 Basic Diet plus 20% condensed Hydrolysate obtained by hydrolyzing lipid free pollen	12	0	Eggs	
4	32 Basic Diet plus 33% residue after extraction of lipids and water soluble materials	20	9	All stages including some emerging bees	Good

Table 2 — Continued

5	19	Basic Diet plus 5% whole pollen	13	3	1-2 day larvae	Good
	28	Basic Diet plus 10% whole pollen	35	16	All stages including some emerging bees	Good
	37	Basic Diet plus 33% Residue remaining after hydrolyzing lipid free pollen	12	12	All stages including some emerging bees	Good
6	25	Wax Moth Diet** plus 20% pollen lipids	5	14	2-3 day larvae	Good
	29	Wax Moth Diet** plus 33% lipid-free pollen residue	34	14	All stages including some emerging bees	Good
7	21	Residue after removal of lipid material from pollen	53	11	All stages including some emerging bees	Good
8	40	Basic Diet plus 7.5% whole pollen	47	6	All stages including some emerging bees	Good
9	32	Formula given above	43	10	All stages including some emerging bees	Good

*Larval food supply rated according to amount. Good is equivalent to the amount found in the larval cells in a free flying colony.

**Developed by Haydak (1935).

were given the basic artificial diet fortified with 33% by weight of the residue remaining after the lipid and water soluble materials were removed from whole pollen (Diet 32).

The addition of 20% by weight of the acid hydrolysate of pollen to the basic diet (Diet 36) resulted in a mixture that the bees in Colony 3 would not eat. Although the queen continued to lay eggs, the adult bees did not feed the young larvae after the eggs hatched. Colony 5 was able to rear brood through all stages while feeding on the basic artificial diet plus 33% by weight of the residue remaining from the acid hydrolysis of lipid free pollen (Diet 37). A microscopic examination of the residue from the hydrolysis process revealed that some pollen grains were still intact and it is possible that the acid solution had not penetrated those grains. Further testing of this diet is needed to determine whether or not the essential principle of pollen can actually withstand such acid treatments, or if the satisfactory results obtained with Diet 37 were due to the addition of whole pollen in grains that were not broken.

As shown in Fig. 3, there was no significant difference between the rate of development of brood in an uncaged colony and that in caged colonies feeding on Diets 32, 21, and 28. In each case, brood was sealed on the fifth day after the eggs hatched, and there was no noticeable difference in the size or appearance of larvae of the same age.

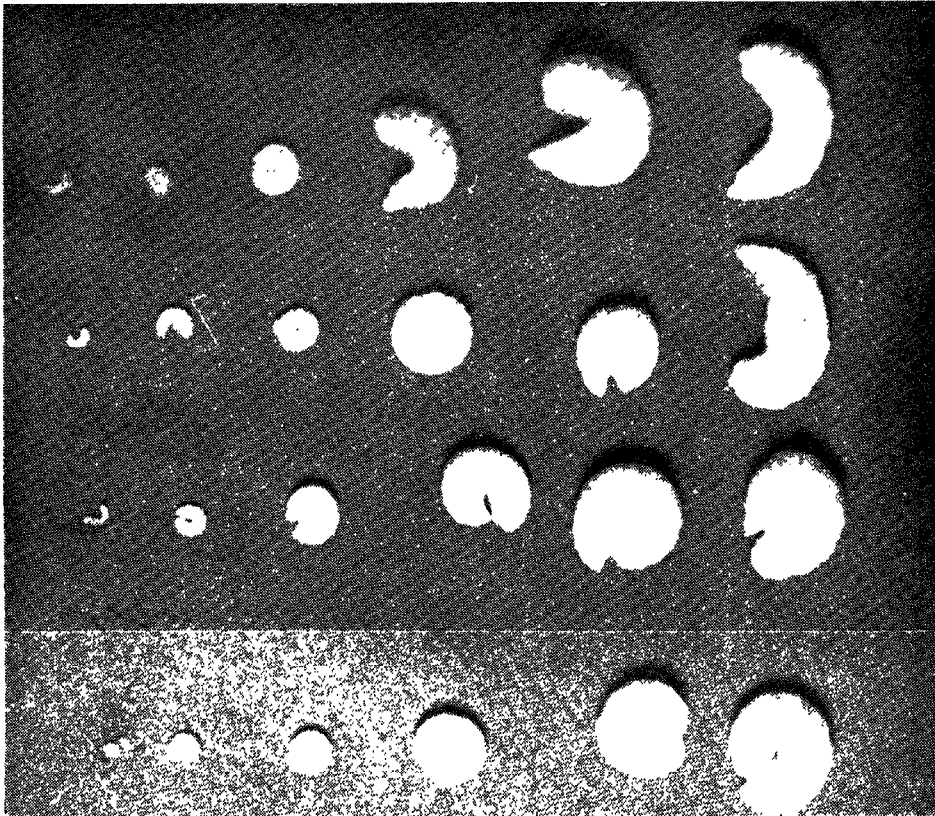


Fig. 3—Larval development at 24 hr. intervals in uncaged and caged colonies: (top to bottom) uncaged colony, Diet 32, Diet 21, Diet 28. See Table 2 for diet formulae.

DISCUSSION AND CONCLUSIONS

One possible source of error in diet studies conducted in a sequential manner as we have done is the ability of bees to draw upon their own body reserves to provide larval food for developing brood. Some estimate of the time such reserves might last is important. On 29 June colonies containing all stages of larvae, some sealed pupae, and a few cells of pollen were confined in cages and given cakes of artificial food. When checked on 6 July, only colony 6 (feeding on a wax moth diet, Haydak 1936) still had larvae of all ages. On 9 July no larvae over 4 days old were present in any of the colonies. On 12 July the diet of colony 6 was changed and it received Diet 25 for 5 days, but larval development still did not progress beyond the fourth day. On 17 July colony 6 was given Diet 34, and by 20 July, 5-6 day old larvae were present. Similar responses were observed in other colonies during these tests. These observations indicate that for 7 to 10 days confined bees may be able to supplement a deficient diet with reserves from their bodies. The response of depleted bees to a good diet is apparent in 1 to 3 days.

Although it is not possible to identify the deficiencies in the basic artificial diet, several conclusions can be drawn from these tests. First, it is evident that the particular lipids in pollen are not essential. The fact that Colony 7 was able to maintain apparently normal brood development for more than 50 days on a diet consisting of the residue after lipid extraction of whole pollen (Diet 21, Table 2) indicates that very little, if any, lipid material is needed in the diet. The amount of lipid material extracted from the mixed pollens used in our experiments amounted to 15-20% by weight of whole pollen. This is higher than some previously reported values (Vivino and Palmer 1944).

Since normal, complete brood development was obtained by fortifying the basic diet with relatively small amounts of pollen, we believe that the basic diet contained most of the necessary nutrients. It is reasonable to hypothesize that the essential missing material is in pollen in good quantities. Analyses show pollen to be rich in protein (Vivino and Palmer 1944), with some pollen containing 20% or more protein. Assuming that the pollen used in our tests contained 20% protein, the addition of 2 g of pollen to 18 g of artificial diet would supply 0.4 g of pollen protein/20 g of food consumed (Diet 28). The artificial diet in this case would supply 3.6 g of protein/20 g of food consumed. Hence, while it is possible that pollen proteins could be critical, it seems improbable to us that this is the case. Some pollens have been found to be unusually rich in the vitamin inositol; as much as 40 mg inositol/g of pollen may be present, (Augustin and Nixon 1957). At this level, 2 g of whole pollen would supply 80 mg inositol/20 g of food when mixed with 18 g of the basic artificial diet. The basic diet supplies only 12 mg of inositol/20 g of diet; thus adding whole pollen at the 10% level could possibly increase the inositol content more than six fold. Inositol is known to be an essential vitamin for some insects (Dadd 1963) and the possibility of the level of this vitamin being critically low in our artificial diet must be checked.

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