Causes of Deficiency of Soybean Flour as a Pollen Substitute for Honeybees[1]

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Soybean flour is used as an ingredient for preparing pollen substitutes or pollen supplements for feeding colonies of bees when the latter lack adequate amounts of pollen—the main source of protein, lipids, minerals and vitamins in the nutrition of the bees.

Although soybean flour when fed alone is adequate for rearing brood (Haydak, 1940, Haydak & Tanquary 1942) the amount of brood reared is considerably below that reared when dried skim milk or dried brewers' yeast is added to the soybean flour. It has been pointed out that the heat-treated soybean flour is an excellent source of protein of a high biological value and that its mineral and fat contents are high enough to justify the assumption that those constituents are supplied in amounts sufficient for the proper development of bees. The same cannot be said of the vitamins present in soybean flour, which is rather low in riboflavin. Its niacin (nicotinic acid) content is also considerably below that of either pollen or the dried brewers' yeast (Haydak 1945).

It is known (Fraenkel 1943) that both riboflavin and niacin are indispensable for the normal growth and development of insects. In order to ascertain whether the insufficiency of these two vitamins plays an important role in the inadequacy of soybean flour alone as food for bees or whether a lack of some other factors is contributing to this deficiency, experiments were undertaken in the summers of 1947 (preliminary) and 1948.

Experimental colonies were formed using bees which had never eaten pollen (not over 10 hours old). They were hived on pollen free combs placed in three-frame nuclei located in isolated cages. A paste made of 70 gm. soybean flour, 400 gm. honey, 20 cc. ethyl alcohol and 20 cc. water was placed in cells of the combs given to the control colonies. For the positive controls 70 gm. soybean flour-dried brewers' yeast mixture (4:1) was used. To ascertain the value of vitamin supplementation pure soybean flour was fortified with 40 mg. riboflavin, 100 mg. niacin, or with a mixture of 40 mg. riboflavin and 100 mg. niacin and thus fortified foods were given to the respective experimental colonies.

The vitamins were dissolved in the alcohol-water mixture and the solution was incorporated into the food. The resulting paste was distributed to the cells of the combs by means of a spatula. Two colonies were used for testing each food.

The bees were weighed before being added to the nuclei. On an average, there were 8.61 bees per gram. A good laying queen was introduced in each of the colonies. On the fifth day water was given and the pollen substitute candy was placed directly over the frames of the nucleus.

The candy was made by mixing 70 gm. of dry food, 170 gm. honey, 10 cc. of ethyl alcohol and 15 cc. of water. The vitamins were dissolved in the alcohol-water mixture which was incorporated into the candy. Two control colonies received pure soybean flour candy. The other two were offered soybean flour-dried brewers' yeast mixture (4:1). Each other group of two experimental colonies was given the pure soybean flour candy with an addition of 20 milligrams of riboflavin, 50 mg. of niacin, or a mixture of

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20 mg. riboflavin and 50 mg. niacin, respectively.

From the seventh day on sugar solution was supplied to all colonies *ad libitum*. On the seventh day the dead bees were counted in each colony, and discarded. This was done because the mortality of young bees during the first few days after hiving may be due to various causes irrespective of food (Haydak 1933). However, the weight of the dead bees was calculated and approximately equal weight of new emerged bees was added to make the colonies of about equal strength at the beginning of the actual test. After that the dead bees were removed and counted more often. For computation of mortality only those that died during the first 10 days of the test were taken into consideration.

For ascertaining the brood rearing activity of the colonies, the latter were examined daily. After the first sealed brood was noted, the bees were examined only to ascertain the presence of the queen. Ten days later both sealed and unsealed brood was counted. Such counts, for each colony, were repeated in three more 10-day intervals. The weight of the food consumed by the bees was recorded throughout the experiment. The results of the preliminary experiments are presented in table 1, those of 1948 experiments in table 2.

From table 2 it is evident that mortality rate in all cases did not differ appreciably. All colonies reared brood. However, the character of the brood rearing was different.

On examining the amount of sealed brood at the end of the first 10-day period, it is seen that the lowest count was in the colonies supplied either riboflavin or niacin alone, the highest in those in which soybean flour was supplemented with both of the vitamins or with dried brewers' yeast. However, the character of the open brood at the end of the first period was distinctly different in the colonies fed soybean flour alone and those offered the fortified foods. While the colonies fed soybean flour alone had the larvae mostly 3.5 days old and there were very few older larvae present, the colonies supplied with vitamins or yeast had all brood stages well represented, although in the colonies receiving single vitamins the brood was somewhat scattered.

In the second and following periods the age differences of the open brood were still more obvious. As time progressed, the numbers of the sealed brood cells in the colonies fed soybean flour alone or fortified with riboflavin were markedly diminishing while in those supplemented with niacin this decrease was more gradual. The slowest decrease was in the colonies fed either both vitamins or dried brewers' yeast in addition to soybean flour. These differences in the character of the brood rearing are still more obvious when one compares the number of sealed cells at the end of each period with the number of unsealed larvae at the end of the preceding period (Table 3).

It is obvious that the rate of normal brood rearing in the colonies fed soybean flour alone or supplemented with riboflavin in the third period abruptly decreased while in the colonies fed soybean flour supplemented with niacin, both riboflavin and niacin or the dried brewers' yeast the rate of the normal brood rearing started to diminish gradually and by the end of the fourth period still a considerable percentage of larvae reached maturity and were sealed. This means that while a relatively normal brood rearing continued uninterruptedly throughout the whole four periods in the colonies in which soybean flour was supplemented with niacin, riboflavin and niacin, or with dried brewers' yeast, the brood rearing in the colonies fed soybean flour alone or supplemented with riboflavin was impaired since only a small percentage of larvae reared during the third and fourth periods reached maturity and was sealed.

This behavior could be explained by a supposition that the young bees fed deficient diets at the beginning used the dispensable part of the vitamins stored in their bodies to supplement the larval food. It is known that nurse bees are able to use materials stored in their bodies for rearing brood (Haydak 1935). Haydak & Vivino (1943) found that the concentration of the B vitamins in the whole bees is higher than the values reported for other insects. As long as there was a physiological possibility of using those stored vitamins they were presumably used; when they were no more available, the brood rearing dropped off abruptly. This is reflected in the total number of sealed cells produced in those colonies (Table 4).

Table 1.—General results. Feeding experiments of 1947.

Food	Strength of Colonies	Brood Rearing Activity in Periods: I Sealed cells	I Un-sealed larvae	II Sealed cells	II Un-sealed larvae	III Sealed cells	III Un-sealed larvae	Total Sealed Cells	Indices of the Food Efficiency Periods: I	II	III	Total	Candy Consumed for Rearing One Bee
	gm.	No.	No.	No.	No.	No.	No.	No.					gm.
Soybean flour (SBF)	822	1855	453	197	580	3	327						
	873	1939	507	328	621	0	476						
Ave.	848	1897	480	263	601	2	402	2161	1.2	1.0	1.0	3.2	.22
SBF+ riboflavin	812	2007	476	281	895	10	444						
	867	1011	1099	288	1427	112	588						
Ave.	840	1509	738	285	1161	61	516	1855	1.0	1.1	30.5	32.6	.25
SBF+ riboflavin +niacin	816	1744	448	481	541	298	405						
	866	1821	737	504	407	210	227						
Ave.	841	1783	543	493	474	254	316	2529	1.2	1.9	127.0	130.1	.16
SBF+ dried yeast	839	2010	573	439	794	497	463	2946	1.3	1.7	249.0	252.0	.16

The number of sealed cells is the most important because it represents the number of larvae reaching maturity and resulting in emerged bees.

In order to find out what influence, if any, the number of adult bees present in the colonies at the beginning of each period had on the amount of young bees produced in this period, the strength of each colony at the beginning of each 10-day period was established by subtracting the number of dead bees from the original number of bees used in establishing the nucleus and adding to it the number of the young bees produced by the colony during the previous period (number of sealed cells).

From table 2 it is evident that the differences were not so marked as to influence the outcome. Since young bees normally perform nursing duties in outside colonies, the number of young bees added through the brood rearing in the experimental colonies could have been, to a great extent, instrumental in influencing the difference in the type of brood rearing, especially because the number of the young bees reared by all colonies during the first two periods differed (Table 4). However, when we compare the character of the brood rearing (Table 3) in the riboflavin or niacin fortified colonies, which produced about the same number of sealed cells (2025 and 2073 respectively) with the type of brood rearing in the niacin and niacin plus riboflavin supplemented colonies, where this difference was considerable (2073 and 2457 respectively), then we can infer that such influence was probably quite small.

It has been demonstrated (Lotmar 1930) that *Nosema* infection interferes with the development of the pharyngeal glands. However, periodical examinations of dead bees in all colonies did not show even traces of infection throughout the duration of the experiment. Therefore, those differences in brood rearing activity cannot be explained by the influence of that disease.

Probably the difference in the vitamin content of the foods supplied to the colonies played the most important role in the results obtained (Table 5). The amount of vitamins added to the diet was so calculated that the content would not be smaller than that present in pollen. According to Vivino & Palmer (1944), pollen collected by the bees at the University apiary had about 18 micrograms of riboflavin and 200 micrograms of niacin per gram of fresh matter. The amount of riboflavin present in the experimental foods was considerably greater, namely 76 micrograms per gram. This was done on purpose, in order to compensate for a possible loss of this vitamin during preparation of the food and while feeding the cakes to the bees (the experimental cages were located in a lighted laboratory and although the food was covered with a black waterproof paper there was a possibility of loss). The amount of niacin was equal to 198 micrograms per gram of fresh food—about equal to that found in pollen. However, the food that produced

Table 2.—General results. Feeding experiments of 1948.

Food	Grams per Colony	Dead Bees (Grams)	Mortality per Cent	Brood Rearing Activity in Periods: I Sealed Cells	I Unsealed Larvae	II Sealed Cells	II Unsealed Larvae	III Sealed Cells	III Unsealed Larvae	IV Sealed Cells	IV Unsealed Larvae
Soybean flour	842 854	33 86		1848 1466	1016 276	730 182	546 860	32 129	711 459	1 3	447 333
Ave.	848	60	7.1	1657	646	456	703	80	585	2	390
Soybean flour+ riboflavin	856 846	49 90		1946 1045	957 586	485 572	854 998	14 145	810 410	0 0	446 246
Ave.	851	70	8.2	1496	772	529	926	80	610	0	346
Soybean flour+ niacin	861 854	30 112		2032 865	730 537	529 718	758 586	368 345	888 191	325 28	240 163
Ave.	858	71	8.3	1449	634	624	672	357	540	177	202
Soybean flour+ riboflavin+ niacin	845 848	37 83		1932 1877	967 839	581 523	591 1074	388 574	598 548	262 210	337 428
Ave.	847	60	7.1	1905	903	552	833	481	573	236	383
Soybean flour+ dried yeast	847 852	44 63		2288 2091	783 478	605 760	986 517	594 531	932 541	270 345	379 401
Ave.	850	53	6.2	2190	631	683	752	563	737	308	390

Food	Strength of the Colonies at the Beginning of Periods in Grams: II	III	IV	Indices of Food Efficiency Periods: I	II	III	IV	Total	Candy Consumed From Rearing One Bee
Soybean flour	1024 938	1012 796	747 657						
Ave.	981	904	702	1.1	1.0	1.0	1.0	4.1	.31
Soybean flour+ riboflavin	1033 878	993 756	750 554						
Ave.	956	875	652	1.0	1.1	1.0	0	3.1	.32
Soybean flour+ niacin	1067 843	1031 729	777 588						
Ave.	955	880	896	1.0	1.3	4.5	88.0	94.8	.27
Soybean flour+ riboflavin+ niacin	1033 983	952 840	736 700						
Ave.	1008	896	718	1.3	1.2	6.0	118.0	126.5	.25
Soybean flour+ dried yeast	1069 1032	906 932	800 774						
Ave.	1050	919	787	1.5	1.5	8.0	154.0	164.0	.25

Table 3.—Character of brood rearing. (Sealed brood cells percentages of the number of unsealed larvae in each of the previous periods.)

Food	The End of Periods II	III	IV
	per cent	per cent	per cent
Soy bean flour			
1947	55.0	.3	—
1948	70.5	11.3	.3
Soy bean flour+ Riboflavin			
1947	38.0	5.2	—
1948	68.5	8.6	0
Soy bean flour+ Niacin			
1948	98.4	53.1	32.8
Soy bean flour+ riboflavin+ Niacin			
1947	90.8	53.6	—
1948	61.1	57.7	41.5
Soy bean flour+ Dried yeast			
1947	76.6	62.6	—
1948	108.2	74.9	41.8

the best results was the one containing dried brewers' yeast; this mixture had concentrations of only 5 micrograms of riboflavin and 40 micrograms niacin per gram.

It is apparent that niacin is the main limiting factor in soybean flour. However, the riboflavin content of the latter is very low and an addition of this vitamin to the diet improved the quality of the food still further. On the other hand, an addition of dried brewers' yeast produced still better results. This is true both for the preliminary and the subsequent experiments (Tables 1 and 2). Apparently there is some other factor or factors in which soybean flour is deficient and which are supplied by dried brewers' yeast.

The indices of food efficiency were obtained by assigning one point to the food giving the lowest average brood production in each category (sealed cells) and dividing by this lowest average the averages of the brood counts in the colonies supplied with other foods. The sum of the points for all periods gave the total index of food efficiency.

Table 4.—Total average brood rearing activity of the colonies used in 1948 experiments.

Food	Sealed Cells	Unsealed Cells	Total	Index of Food Efficiency	Total Sealed at the Beginning of III Period
	No.	No.	No.		No.
Soybean flour	2195	2324	4519	1.0	2113
Soybean flour+ riboflavin	2105	2654	4759	1.0	2025
Soybean flour+ niacin	2607	2048	4665	1.2	2073
Soybean flour + riboflavin+niacin	3174	2692	5866	1.5	2457
Soybean flour+ dried yeast	3744	2510	6254	1.8	2373

Table 5.—Riboflavin and niacin content of candy (micrograms per gram of fresh matter).

Candy	Riboflavin	Niacin
Soy bean flour	.8	9
Soy bean flour+riboflavin	76.0	9
Soy bean flour+niacin	.8	198
Soy bean flour+riboflavin +niacin	76.0	198
Soy bean flour+dried yeast	5.0	40

Since the record of the food consumption was kept there is a possibility of computing roughly an approximate amount of nitrogen used for the rearing of one bee (Table 6). The protein content of the soybean flour used in the experiment was equal to 50 per cent, that of dried brewers' yeast was also 50 per cent. Taking this as a basis the total amount of protein consumed by each group was computed. The nitrogen content of bees reared on soybean flour was found to be equal to 1.96 mg. per bee (Haydak, 1937). When the quantity of N contained in reared bees is substracted from the amount consumed by each group, then the amount of N used for the development and metabolism of original bees and of those that were produced by the experimental colonies (equal to the number of sealed cells, except those counted at the end of the fourth period) could be obtained. Haydak (1934) found that the average N content of fully developed (6 to 20 days old) bees, with the digestive tracts removed is equal to 2.50 mg. The average N content of emerging bees is equal to 1.98 mg. of which 0.24 mg. is for the digestive tract. The latter value could be accepted as the N content of digesitve tracts free of pollen for all ages of bees, because the digestive tracts of bees 31 days old, which are also almost free of pollen contain 0.25 mg. N. The N content of the whole bees, 6 to 20 days old, is then equal to about 2.74 mg. Therefore the increase in the N content of fully developed over emerging bees is equal to 0.76 mg. By subtracting this value from the total amount of N used by the adult bee one can obtain the amount of N used by each adult bee in catabolism and the synthesis of the larval food. Thus the amount of N used by the adult bees in catabolism and synthesis of the larval food for rearing one bee can easily be calculated. Adding to this number the amount of N content of an emerged bee reared in experimental colonies, the total approximate amount of N used in the rearing of one bee can be ascertained. It is interesting to note that the numbers for the colonies having about normal brood rearing are almost equal to the numbers given by Alfonsus (1933), who fed colonies made of adult developed bees, a weighed quantity of pollen and ascertained that for rearing one bee the nurse bees used 144.9 mg. of pollen, which is equal to 4.67 mg. N. The number given by Haydak (1935) is smaller (3.21 mg.). However, the latter value was calculated from the results obtained from a colony fed only sugar solution, the bees of which used their stored protein reserves for the production of the larval food. There was no waste in the digestive processes. From tables 1, 2, and 6 it is also obvious that more protein was used for the rearing of one bee in those colonies that were fed deficient foods. It appears that the amount of N used in rearing one bee is inversely correlated with the index of food efficiency (Tables 2 and 6).

SUMMARY.—Colonies consisting of newly emerged bees which have never eaten pollen were kept in isolated cages and fed candy made of soybean flour alone and that fortified with riboflavin, niacin, both riboflavin and niacin, and dried brewers' yeast. The mortality of bees was noted. The brood rearing activity was followed for four consecutive 10-day periods.

All colonies reared brood. However, in the colonies fed soybean flour alone or soybean flour fortified with only riboflavin there was an abrupt impairment in the brood rearing activity beginning with the third period. The decrease in the brood rearing activity in the colonies fed soybean flour supplemented with niacin was more gradual. The brood rearing activity in the colonies offered soybean flour fortified with both niacin and riboflavin or dried brewers yeast, although gradually decreasing, was the closest to the normal brood rearing. The best results were obtained in the colonies, the food of which was fortified with 20 per cent of dry brewers' yeast.

The results indicated that niacin probably is the main limiting factor in soybean flour. The amount of riboflavin in the latter is also inadequate since an addition of riboflavin to the niacin-fortified diet increased the nutritive value of the food still further. There seem to be some other factors in which soybean flour is deficient and which are supplied by dried brewers' yeast.

The calculated amount of nitrogen used

Table 6.—Quantity of Protein N used by the experimental colonies.

			Nitrogen						
					Used for Development & Metabolism of Adults		Spent in Catabolism & Synthesis of Larval Food		
Food	Number of Bees	Protein	Nitrogen	Contained in Reared Bees	Total	Per Bee	Per Adult Bee	Per Reared Bee	Used in Rearing One Bee
		gm	mg	mg	mg	mg	mg	mg	mg
Soy bean flour	9488	124.76	19962	4202	15760	1.66	.90	3.4	5.36
Soy bean flour+ Riboflavin	9329	124.23	19877	4126	15751	1.70	.94	4.2	6.16
Soy bean flour+ Niacin	9809	126.74	20278	5110	15168	1.55	.79	3.0	4.96
Soy bean flour+ Riboflavin+ Niacin	10222	140.73	22517	6221	16296	1.60	.84	2.7	4.66
Soy bean flour+ Dried yeast	10746	156.57	25061	7338	17723	1.60	.84	2.4	4.36

in rearing one bee was equal to 4.96, 4.66, and 4.36 mg. for the colonies fortified with niacin, both niacin and riboflavin and dried brewers' yeast respectively. That amount in the colonies fed soybean flour alone or that supplemented with riboflavin was greater, being 5.36 and 6.16 mg. respectively. It appears that the amount of N used in rearing one bee is inversely correlated with the index of food efficiency.

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