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EFFECTS OF THIAMINE- OR RIBOFLAVIN-DEFICIENT DIET FED TO NEW EMERGED HONEY BEES, *APIS MELLIFERA L.*

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SUMMARY

Newly emerged honey bees, Apis mellifera L., were fed pollen, vitamin-free casein fortified with ten B-vitamins, or vitamin-free casein fortified with B-vitamins but lacking thiamine or riboflavin. The longevity, hypopharyngeal gland development and total body nitrogen was determined for each treatment. The hypopharyngeal gland development of bees fed the complete vitamin mixture nearly equalled that of pollen-fed bees. The glands of bees fed the diets deficient in thiamine and riboflavin failed to develop beyond the stage found in newly emerged bees. The total nitrogen content of bees fed the vitamin-deficient diets was substantially less than the nitrogen content of bees fed either pollen or casein fortified with the complete vitamin mixture. Longevity studies showed that 95 % of bees fed the diets deficient in either thiamine or riboflavin died by 20 days after emergence. A 35 % mortality rate was recorded for those bees fed the complete vitamin diet. The lowest percentage of mortality after 20 days was recorded in those bees fed the pollen diet. This study clearly demonstrated the requirements of newly emerged bees for riboflavin and thiamine.

INTRODUCTION

It has not been demonstrated that fat-soluble vitamins A, D, E, and K are required in honey bee diets. The vitamin requirements of insects thus appear to be of the water-soluble type, particularly the B-complex. Nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamine are probably essential in all immature insects, and biotin, choline, and folic acid may also be necessary.

The natural foods of honey bees are honey, a source of carbohydrates, and pollen, a source of proteins, fats minerals, and vitamins. Pollen is the food basic for rearing brood, for feeding the laying queen, for developing the brood food glands, and for

maintaining a normal protein balance in older bees. The pollen supply is therefore a limiting factor in the life of a honey bee colony.

Pollen is eaten chiefly by the young nurse bees, and from it they elaborate brood food, the so-called " royal jelly ", a rich, nitrogenous, creamy substance produced by the hypopharyngeal glands in the head of worker bees. This is fed to the queen, and also to the young larvae in the early days of their development. Feeding pollen to confined newly emerged bees not only activates the hypopharyngeal glands, but also builds up the fat bodies, develops the wax glands and ovaries, and prolongs life. Likewise, young bees reared on a pollen-free diet exhibit a regression in the size and vacuolation of the hypopharyngeal glands. According to MAURIZIO (1954) the development of the hypopharyngeal glands in worker bees, as a consequence of their diets, offers an opportunity to study diets.

HAYDAK (1934) reported that bees gained weight and showed an increase in the nitrogen content of their bodies when the supply of pollen was plentiful. Later, HAYDAK (1937) tested the effect of a protein-free diet on adult worker bees and observed that a substantial decrease both in weight and the nitrogen content was observed in all parts of the body. Pollen was found to be rich in vitamins, especially B-vitamins (HAYDAK and PALMER, 1938; KOCK and SCHWARZ, 1956). Of the B-vitamins present, pantothenic acid, nicotinic acid, thiamine, riboflavin, and ascorbic acid were present in the greatest amounts.

This investigation was conducted to determine the influence of diets deficient in thiamine or riboflavin on the hypopharyngeal gland development of worker bees, Kjeldahl nitrogen content of body regions, and daily mortality.

MATERIALS AND METHODS

Newly emerged honey bees were obtained for the diet studies by placing combs of sealed brood in an incubator at 35 °C. Bees were brushed from the combs every 4 hours. Newly emerged bees were used for the tests because their hypopharyngeal glands were undeveloped and because they had not fed on pollen or honey as adults. Each test diet was fed to four cages, each containing 25 bees (Fig. 1). Diet and water were fed *ad libitum*. The following diets were tested : pollen in powdered sugar (control), vitamin-free casein fortified with a complete water-soluble vitamin complex, vitamin-free casein fortified with the vitamin complex less thiamine, and vitamin-free casein fortified with the vitamin complex less riboflavin.

The control diet consisted of 10 g of pollen, mixed in 90 g of powdered sugar. The mixture was moistened with 5 ml of tap water and molded into a cake. The complete vitamin diet was a modification of a diet developed by VANDERZANT (1959) for rearing the boll weevil. The mixture consisted of the following vitamins (g/500 ml H_2O) : choline chloride (50.000); niacin (18.000); calcium pantothenate (2.000); thiamine chloride (0.900); riboflavin (1.800); pyridoxine (0.500); folic acid (0.250); biotin (0.025); inositol (18.000); B_{12} (0.002).

One ml of this Vitamin B mixture was added to 100 g of dry diet. The dry portion of the diet consisted of vitamin-free casein (81.25 % protein, 36 g), powdered sugar (containing 3 % starch, 56 g), peanut oil (2 g), and alphacel (6.00 g).

FIG. 1. — *Test cages each containing 25 newly emerged honey bees fed various test diets.*

Additional diets were prepared from vitamin mixtures deficient in either thiamine or riboflavin. Each diet was evaluated for the degree to which it developed hypopharyngeal glands, for the nitrogen content of body regions, and for longevity.

Three bees were removed from each cage daily. The head of each bee was removed for hypopharyngeal gland development, and the thoraces and abdomens to be used for nitrogen determinations (digestive tracts removed) were placed in a dry-air oven for 24 hours at 100 °C. Hypopharyngeal glands were removed from the front region of the head with forceps and a water mount prepared. The degree of development of both right and left glands was evaluated on a numerical system of 1-4 after MAURIZIO (1954) as follows :

Stage 1 : Main and side canals readily visible, gland lobi underdeveloped, irregular, transparent.

Stage 2 : Main and side canals visible, gland lobi irregularly rounded with clearly visible spaces between, transparent.

Stage 3 : Main canal visible, side canals mostly concealed by tightly filled gland lobi. Gland lobi more or less transparent with small spaces between.

Stage 4 : Main and side canals completely concealed, no spaces visible between the gland lobi.

Total nitrogen content of the various body regions was determined by the " Association of Official Agricultural Chemists " Micro-Kjeldahl Method (1960). Each 30 ml Kjeldahl flask, containing 100 mg dry sample, was digested for 1 hour, and the distillation was made as soon as the digested solution cooled. The alimentary tracts of the bees were removed before the nitrogen content was determined.

Dead bees were removed from each cage daily, and the percentage of mortality was recorded.

RESULTS

Hypopharyngeal gland development (Table 1). Eighteen days after the bees emerged the hypopharyngeal glands of bees fed the test diets had shown no improvement. The gland lobi were still small and flaccid, and the common ducts were clearly visible. Bees fed the 10 % pollen diet began to show gland development on the 20th day. After 26 days the glands had reached a 3.5 classification (Fig. 2) and though not completely developed, they were developed enough to be functional. The hypopharyngeal glands of bees fed the complete vitamin complex (Fig. 3) did not develop as much as those of bees fed the 10 % pollen diet, but they too were still developed enough to be functional. The glands of bees fed the diets deficient in thiamine and riboflavin (Fig. 4 and 5 respectively) were not developed, the lobes of the glands were shrunken,

TABLE 1. — *Hypopharyngeal gland development (*) of newly emerged bees fed various test diets.*

Diet	Rating of gland development at indicated day												
	2	4	6	8	10	12	14	16	18	20	22	24	26
10 % Pollen	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	2.5	3.0	3.5
Complete Vitamin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5	2.0	2.0	3.0
Thiamine-Deficient	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Riboflavin-Deficient	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

(*) Development classified on a scale of 1 (no development) to 4 (maximum development) after MAURIZIO (1954).

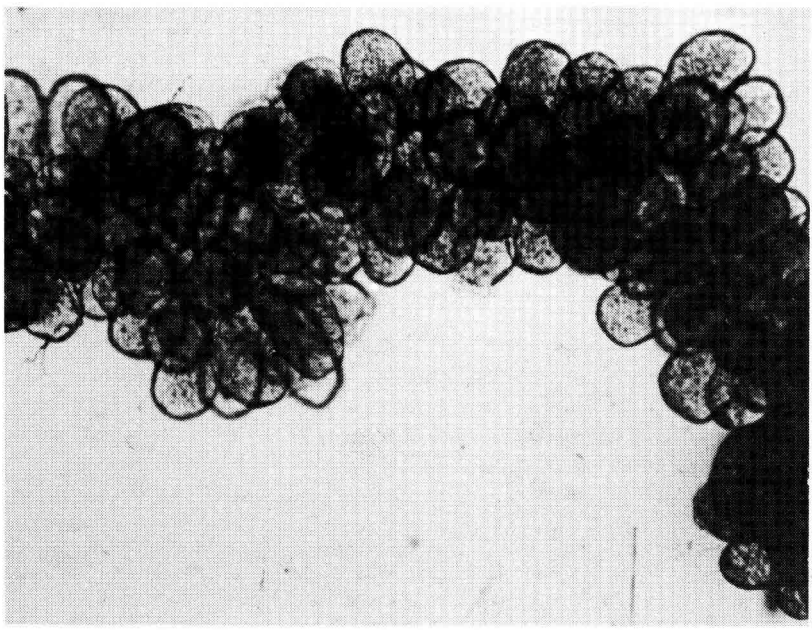

FIG. 2. — *Hypopharyngeal gland development of worker bees fed a 10 % pollen diet (× 40).*

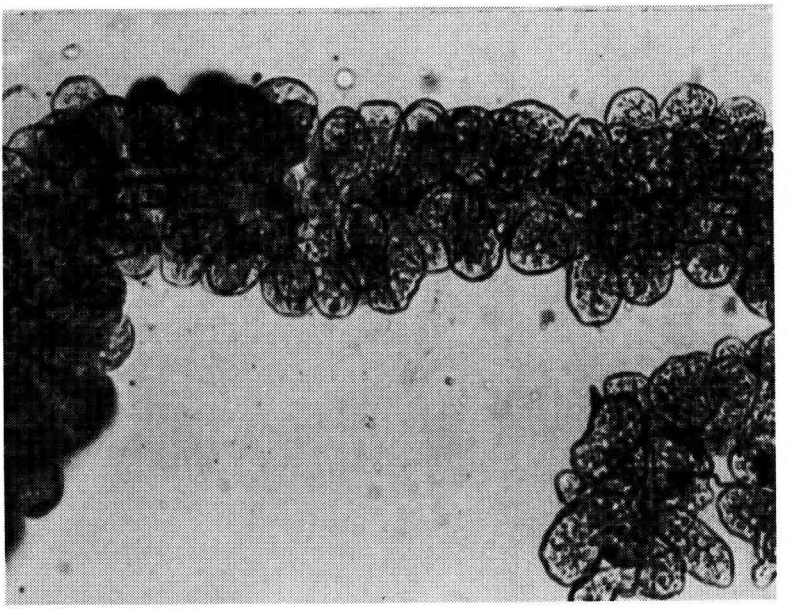

FIG. 3. — *Hypopharyngeal gland development of worker bees fed a complete water soluble vitamin complex (× 40).*

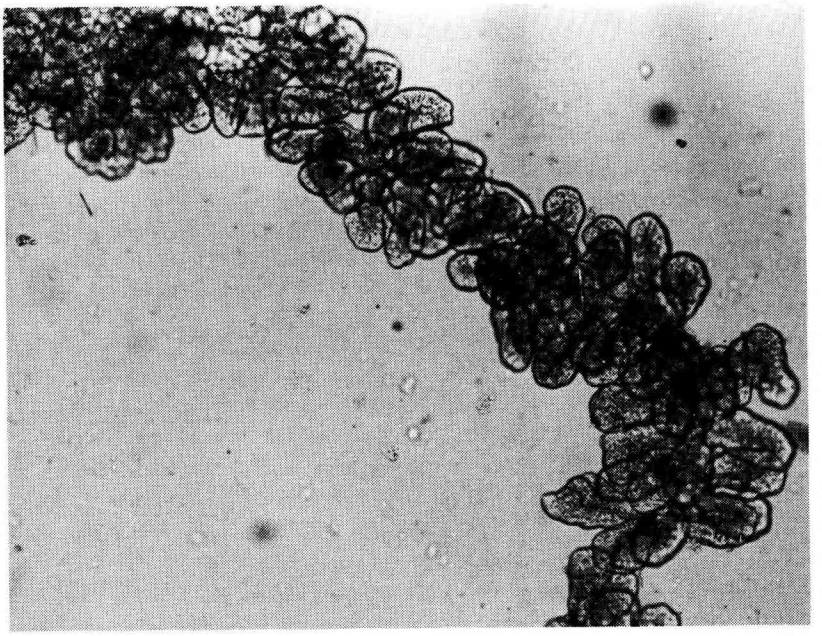

FIG. 4. — *Hypopharyngeal gland development of worker bees fed a thiamine deficient diet (× 40).*

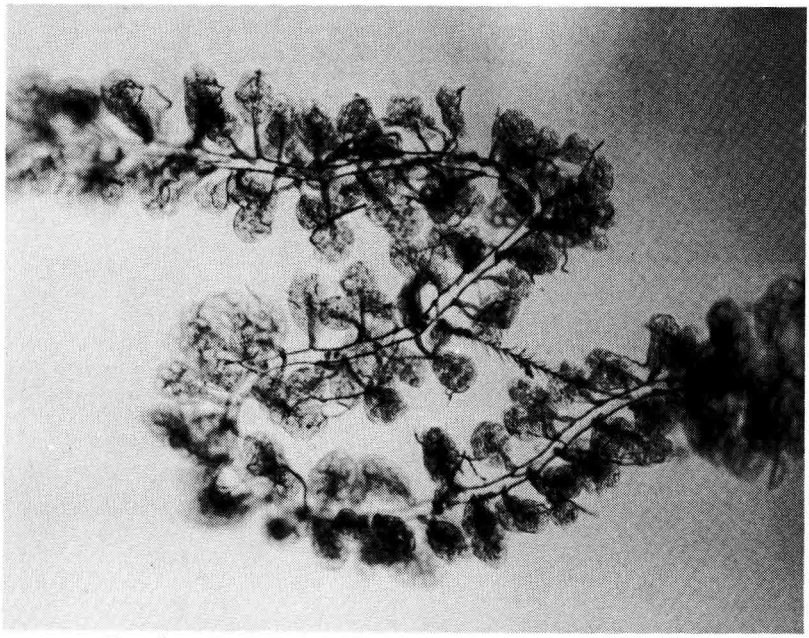

FIG. 3. — *Hypopharyngeal gland development of worker bees fed a riboflavin deficient diet.*

and the common ducts were clearly visible. The glands had not developed beyond the stage observed in newly emerged bees.

Kjeldahl nitrogen (Table 2). The total nitrogen content of the thoraces and abdomens varied greatly. The nitrogen content of bees fed the 10 % pollen diet increased 3 days after emergence but decreased thereafter. After 15 days the total N_2 increased and continued to do until the 21st day. The highest total nitrogen was recorded in those bees fed the 10 % pollen diet. The total nitrogen of bees fed the complete vitamin diet approached that of the pollen diet. The bees deficient in thiamine and riboflavin were lowest in total nitrogen per bee.

TABLE 2. — *Kjeldahl nitrogen contents of thoraces and abdomens of honey bees fed various test diets.*

Diet	Nitrogen content at indicated days after emergence *(mg per bee)*						
	0 (*)	3	6	13	15	18	21
10 % Pollen Diet	2.20 mg	4.18	3.64	3.65	4.38	4.42	4.66
Complete Vitamin Diet	2.20 mg	3.90	3.87	4.00	4.03	4.14	4.60
Thiamine-Deficient	2.20 mg	4.00	3.82	3.50	3.20	3.57	3.83
Riboflavin-Deficient	2.20 mg	3.54	4.17	4.07	4.03	4.01	3.66

(*) Newly emerged honey bees less than 2 hours of age.

Longevity (Fig. 6). Ninety-five percent of bees fed the diets deficient in thiamine and riboflavin died by 20 days after emergence; a 35 % mortality rate was recorded for those bees fed the complete vitamin diet. At the termination of the study the lowest percentage of mortality (20 %) was recorded in those bees fed the pollen diet.

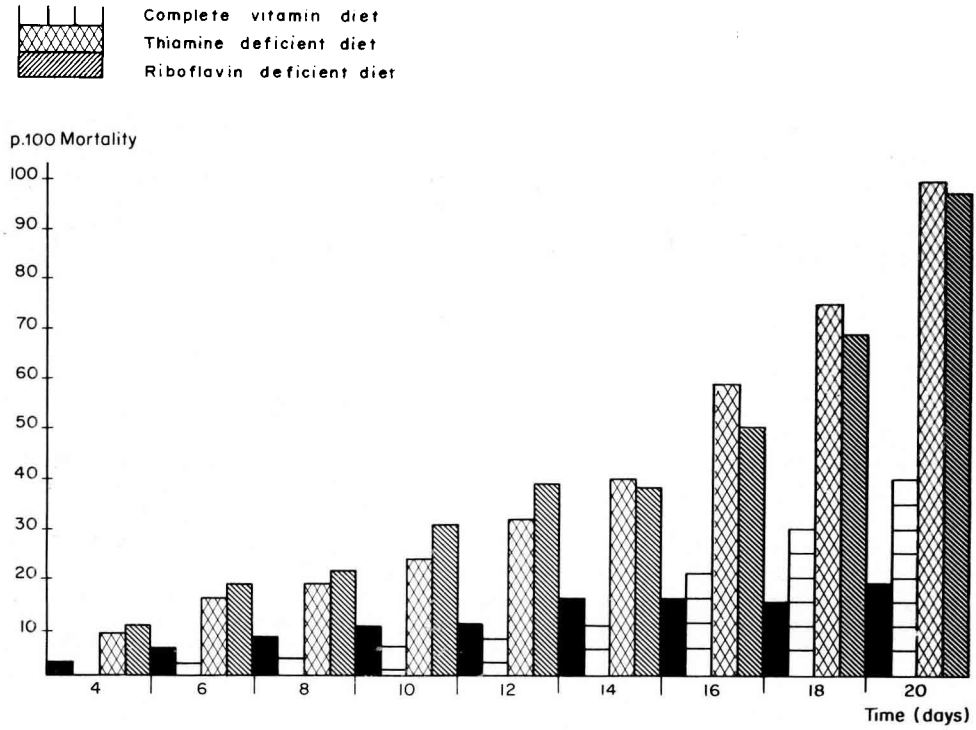

FIG. 6. — *Longevity of newly emerged honey bees fed complete and vitamin-deficient diets.*

Additional studies were undertaken to determine the brood-rearing capability of newly emerged bees fed the test diets. Three hundred grams of newly emerged bees (approximately 2 500 bees) were placed in small nuclei containing three frames. A fertile queen was added to each nucleus. Test diets and water were fed *ad libitum.*

Newly hatched larvae were observed in the colonies fed the pollen diets 2 weeks after the test diet was initially fed. Eggs were observed in the cages of bees fed the complete vitamin diet, but no larvae were present. No eggs were observed in those cages fed the vitamin-deficient diets.

In bees fed the complete vitamin diets, hypopharyngeal gland development, body nitrogen, and longevity were greater than in newly emerged bees fed diets deficient in either thiamine or riboflavin. However, in all cases, the pollen diet was most effective.

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ZUSAMMENFASSUNG

EFFEKTE EINER THIAMIN- ODER RIBOFLAVIN-MANGELDIÄT AUF FRISCHGESCHLÜPFTE HONIGBIENEN, *APIS MELLIFERA* L.

Frischgeschlüpfte Honigbienen *(Apis mellifica)* wurden entweder mit Pollen gefüttert, oder mit vitaminfreiem Casein, das mit 10 B-Vitaminen angereichert war, oder mit Casein mit Vitamin B-Zusatz, dem aber entweder Thiamin oder Riboflavin fehlte. Für jeden Ansatz wurden Lebensdauer, Entwicklung der Hypopharyngealdrüsen und der Gesamtstickstoffgehalt bestimmt. Der Entwicklungsgrad der Hypopharyngealdrüsen von Bienen, die mit der kompletten Vitaminmischung gefüttert waren, kam beinahe mit der pollengefütterten Biene gleich. Die Drüsen der Bienen mit einer Thiamin- oder Riboflavin-Mangeldiät entwickelten sich nicht über das Stadium der frisch geschlüpften Bienen hinaus. Der Gesamtstickstoffgehalt der Bienen mit der Vitamin-Mangeldiät war beträchtlich niedriger als der Stickstoffgehalt der Bienen mit der Pollendiät oder mit der Casein-Vitamin-Volldiät. Lebensdauerversuche zeigten, dass von den Bienen mit thiamin- oder riboflavinfreier Ernährung 95 % bis zum 20. Lebenstag gestorben war. Bei Bienen mit kompletter Vitamindiät betrug die Mortalität 35 %. Die niedrigste Sterberate wurde bei den Bienen mit der Pollendiät beobachtet. Diese Untersuchung beweist also den Bedarf frischgeschlüpfter Bienen an Riboflavin und Thiamin.

RÉSUMÉ

ACTION D'UN RÉGIME DÉFICIENT EN THIAMINE OU EN RIBOFLAVINE SUR DES ABEILLES, *APIS MELLIFICA*, RÉCEMMENT ÉCLOSES

On a administré à des abeilles récemment écloses du pollen, de la caséine dévitaminée et renforcée avec 10 vitamines B, ou de la caséine dévitaminée et renforcée avec le complexe vitaminique moins la thiamine ou la riboflavine. Pour chaque traitement la longévité, le développement des glandes hypopharyngiennes et l'azote total ont été déterminés. Le développement des glandes hypopharyngiennes des abeilles nourries avec le régime comportant le complexe vitaminique entier était sensiblement équivalent à celui des abeilles nourries au pollen. Les glandes des abeilles qui avaient reçu les régimes déficients en thiamine et en riboflavine n'ont pu se développer au-delà du stade trouvé chez les abeilles récemment écloses. La teneur en azote total des abeilles nourries avec les régimes déficients en vitamines était nettement plus faible que celui des abeilles nourries au pollen ou avec la caséine plus le complexe vitaminique entier. Les études de longévité ont montré que 95 % des abeilles qui avaient reçu les régimes déficients soit en thiamine, soit en riboflavine sont mortes dans les 20 jours suivant l'émergence. Un taux de mortalité de 35 % a été enregistré chez les abeilles qui avaient reçu le complexe vitaminique entier. Le plus faible pourcentage de mortalité après 20 jours a été enregistré chez les abeilles nourries au pollen. Ces expériences montrent clairement les besoins en riboflavine et en thiamine des abeilles nouvellement écloses.

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