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Short communication

A defined artificial diet for the larvae of *Manduca sexta*

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The tobacco hornworm, *Manduca sexta* (Johannson) (Lepidoptera: Sphingidae), has become an important and widely used laboratory animal. It was first cultured on the foliage of its host plants (Waldbauer *et al.*, 1964; Hoffman *et al.*, 1966), but is now typically cultured on undefined artificial diets that contain such complex substances as wheat germ and yeast (Yamamoto, 1969; Bell & Joachim, 1976; Baumhover, 1985). A diet that includes such ingredients, each containing the gamut of nutrients, is useful for maintaining cultures but is not appropriate for most nutritional studies, including studies of dietary self-selection. We here describe a defined diet (containing no ingredients that are chemically less well characterized than linseed oil) that is suitable for studies of self-selection and other aspects of nutrition. To the best of our knowledge, no defined diet for *M. sexta* has been previously published.

The defined diet (Table 1), a modification of Vanderzandt's (1968) diet, is prepared as follows: The group A ingredients, water and agar, are poured into a flask that is capped with aluminum foil, autoclaved for 20 min, cooled for 10 min, poured into a blender with the group B ingredients, and then thoroughly mixed. When the mixture cools below 60 °C, the group C ingredients are added, the diet is blended for 5 min and then poured into plastic molds. After the diet has cooled to room temperature, each mold is wrapped with aluminum foil and stored at 4 °C until used.

Table 1. Composition of defined diet for rearing *Manduca sexta* larvae

Group A:	
Agar	30 g
Water (distilled)	1000 ml
Group B:	
Vitamin-free casein	67.0 g
Ovalbumin	22.0 g
Sucrose	97.0 g
Wesson's salt mixture	15 g
Cobalt, molybdenum and zinc salts*	47 mg
Cholesterol	4 g
Alphacel	50 g
4M KOH	6.0 ml
Linseed oil	3.7 ml
Group C:	
Ascorbic acid	6.7 g
Choline chloride	0.98 g
Cysteine HCl	0.98 g
Beta carotene	0.24 g
Calcium pantothenate	98 mg
Niacinamide	98 mg
Riboflavin	4.9 mg
Pyridoxine HCl	2.4 mg
Thiamine HCl	2.4 mg
Biotin	0.16 mg
Folic acid	4.15 mg
Vitamin B12	0.016 mg
Inositol	0.24 g
Streptomycin sulphate	0.12 g
Kanamycin sulphate	72 mg
Sorbic acid	2 g
Methyl-p-hydroxybenzoate	2 g
10% formalin	32 ml
Alpha-tocopherol	0.08 ml

* A mixture of 2 parts zinc acetate, 1 part sodium molybdate, 1 part cobalt chloride.

Table 2. Survival, growth and reproduction parameters of *Manduca sexta* reared through the larval stage on a defined diet or on a diet with wheat germ and yeast. Each treatment began with 25 newly hatched larvae. Values are $\bar{x} \pm$ S.E. Number of insects is given in parentheses. Not all surviving adults were used to determine longevity and egg production

		Defined diet	Wheat germ diet
% Survival to adult		80.0 (20[a])	96.0 (24[a])
Days to pupa		23.4 ± 0.4[b] (20)	22.1 ± 0.1[b] (24)
Fresh weight of pupa (g)	Female	5.41 ± 0.13[b] (8)	6.57 ± 0.13[b] (10)
	Male	4.98 ± 0.11[b] (12)	6.24 ± 0.13[b] (14)
Adult longevity (days)	Female	12.8 ± 1.6[c] (7)	15.6 ± 0.4[c] (5)
	Male	10.1 ± 0.8[b] (7)	14.4 ± 0.8[b] (5)
Total eggs per female		643.5[d] (7)	1412.2[d] (5)
% egg hatch		96.3[a]	84.0[a]

[a] Means significantly different, χ^2-test of independence between samples, continuity corrected, $p < 0.05$; [b] means within a row significantly different, $p < 0.05$, or [c] not significantly different, $p > 0.05$, paired t-test; [d] sums of eggs laid each day divided by number of females alive on that day; no statistical comparison made.

The culture is kept in a room at 26 °C and 60-65% r.h. A light : dark cycle of 16 : 8 prevents the induction of diapause. The insects are maintained as described by Waldbauer *et al.* (1964), with the following exceptions: Each newly hatched larva is individually confined with diet in a capped, 30 ml, plastic cup. Fifth instar larvae are transferred to larger containers, one larva per container, and given more diet. Larvae that are ready to pupate are placed in holes bored in a wooden block (Bell & Joachim, 1976). The holes are closed with corks, and the blocks are placed so that the larvae lie horizontally. Seven days later the pupae are removed from the blocks and transferred to small cages where the adults emerge. Adults are then moved to a cage covered with cheesecloth (120 × 78 × 99 cm). They are hand-fed (Waldbauer *et al.*, 1964) a 20% sucrose solution daily, and a potted tobacco plant is placed in the cage as an oviposition site. Eggs are removed from the plant, surface-sterilized for 15 min in a 0.25% solution of sodium hypochlorite, rinsed for 1 min in luke-warm running water, and then dried on filter paper.

We successfully reared several groups of *M. sexta* larvae on the defined diet, including two consecutive generations from egg to egg. However, as Table 2 shows, insects on the defined diet were inferior in several developmental parameters to *M. sexta* reared simultaneously and under the same conditions on a modification of Bell and Joachim's (1976) diet containing wheat germ and yeast. Nevertheless, the defined diet supports growth and reproduction at levels that are often superior to those reported for larvae fed solanaceous foliage. For example, although *M. sexta* reared on the defined diet attained mean pupal weights of 4.98 g (males) and 5.41 g (females) (Table 2), Stewart and Baker (1970) reported a mean weight of only 3.66 g for pupae reared on tobacco, and Waldbauer (1962) reported a mean weight of only 3.92 g for pupae reared on tomato. While females reared on the defined diet laid a mean of 643 eggs, females reared on tomato leaves laid a mean of about 1,000 eggs (Waldbauer, 1962), but females reared on tobacco laid only from 200 to 300 eggs (Madden & Chamberlin, 1945; Hoffman *et al.*, 1966). These parameters, especially fecundity, are not determined by larval nutrition alone but may also be affected by extrinsic factors such as lighting, temperature, humidity, and the quality of the oviposition substrate.

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