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【Research report】

飼養小菜蛾之改良飼料及其對脂肪酸之需求【研究報告】

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Abstract

摘要

以半合成飼料研究小菜蛾之人工飼育法，本飼料經以加熱之麥胚及用少量乙醚溶解膽固醇與亞麻子油來改進以往所用飼料之配製方法，提高飼育效果。小菜蛾幼蟲經用此改良飼料飼育後，其存活率及成蟲羽化率均達約84%。用甘藍與芥蘭菜所製成的菜葉粉供配製飼料，可得最好之飼育效果。Linoleic acid 和Linolenic acid，兩種脂肪酸均無法供小菜蛾單一脂肪酸來源，加入維他命E亦未能有助於此兩種脂肪酸，供小菜蛾發育之營養需求。

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AN IMPROVED DIET FOR REARING THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA*, AND ITS REQUIREMENTS FOR FATTY ACIDS

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ABSTRACT

Rearing of the diamondback moth, *Plutella xylostella*, was studied by feeding on a semi-synthetic diet. The diet was improved by autoclaving wheat germ and by dissolving cholesterol and linseed oil in ether before adding to other components, compared with the previous preparation. Larvae fed on the improved diet had ca. 84% survival and adult emergence. Addition of leaf powder of common cabbage or kale to the diet was superior to the powders of other cruciferous vegetables for diamondback moths rearing. Linoleic acid or linolenic acid cannot be a sole fatty-acid source for this species. Addition of α tocopherol with both fatty acids to the diet did not enhance insect development.

INTRODUCTION

Rearing of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) on semi-synthetic diets has been accomplished by some groups of workers, however, results were variable, possibly due to differences in dietary composition and preparation and to geographic variations of the insect (cf. Hou, 1985). Hsiao and Hou (1978) could obtain only 60% of pupation by feeding the larvae on a semi-synthetic diet, therefore this diet was considered suboptimal for the insect development. It was reported that heat treatment of soybean meal and wheat germ before adding to other components could improve the diet for certain insects (Lipke et al 1954, Adkisson et al 1960). Obviously procedures of dietary preparation are important while making artificial diets.

Since addition of plant materials to artificial diets can provide unknown nutrients or chemical feeding stimulants (Vanderzant, 1974), leaf powder is always incorporated into the diets for rearing insects attacking vegetables. The question arises as to whether leaf powders of different vegetables can be equally efficient for the diamondback moth rearing. Van Etten et al (1976) found significant differences in chemical components in varieties of cabbage; therefore, dietary effect of various vegetables on rearing of diamondback moths needs to be studied.

Our previous report indicated that linolenic acid could be a sole fatty acid for diamondback moth development when feeding on solvent-washed diets (Hou and Hsiao 1978). In this study, we used the improved diet and better fat-free ingredients for testing fatty acid requirements.

MATERIALS AND METHODS

Insects

The colonies of *P. xylostella* were collected from vicinity of Taichung city. The larvae of third generation of the laboratory colonies were used for various experiments.

Composition and preparation of diet

The basal diet, except for formaldehyde (Hou and Hsiao, 1979), was adopted from Hsiao and Hou (1978). The dietary composition is shown in Table 1. Wheat germ was added to 5 ml distilled water and was then autoclaved at 121°C for 20 min. Cholesterol and linseed oil were dissolved in small quantity of ether, and were then added to the mixture of Alphacel, cabbage leaf powder, and heated wheat germ, stirring well to evaporate ether. This portion was then poured into a blender together with the mixture of sucrose, vitamin-free casein, methyl p-hydroxybenzoate, and Wesson's salt mixture in the melted agar solution (62 ml). Ascorbic acid, i-inositol, choline chloride, potassium hydroxide, vitamin-B solution, and aureomycin were immediately added to the blender with other solid ingredients, and were ground thoroughly. The complete diet was dispensed in sterile feeding vials (3 x 7 cm) stoppered with cotton. For fatty acid requirement tests, linoleic acid, linolenic acid, α-tocopherol (vitamin E), and cholesterol were dissolved in ether, defatted wheat germ was not heated.

Table 1. Composition of the semi-synthetic diet for *Plutella xylostella*

Ingredient	Quantities in 100 g diet
Casein, vitamin-free	3.500 g
Alphacel	0.500
Wesson's salt mixture	1.000
Sucrose	3.500
Wheat germ, heated	3.000
Methyl p-hydroxybenzoate	0.150
Choline chloride	0.100
Ascorbic acid	0.400
Aureomycin	0.015
Cabbage leaf powder	3.000
Cholesterol*	0.250
i-Inositol	0.018
Agar	2.500
Distilled water	87.000 ml
KOH (4M)	0.500
Vitamin-B solution	1.000
Linseed oil*	0.650

* Dissolved in ether.

Preparation of vegetable leaf powder and defatting procedures

Several cruciferous vegetables were used to make leaf powder. Leaves were washed thoroughly and were then dried at 50-60°C for 24 h in an oven. The dried leaves were ground using a blender. The resultant powder was screened through a fine sieve (ca. 150 mesh). Larval survival, pupation, adult emergence, etc. on each diet were recorded.

To determine the dietary requirements for fatty acids, wheat germ and leaf powder were defatted by extracting with a mixture of chloroform: methanol (2:1, v/v) in a Soxhlet apparatus for 5 h. Linseed oil was replaced by linoleic acid or linolenic acid, cholesterol was retained in the diet.

Feeding procedures

Adult females were confined in an oviposition cage after mating and were then allowed to lay eggs on cabbage leaves. The eggs were transferred onto a piece of paper for incubating. Thirty newly hatched larvae were transferred onto the diet placed in a vial. Each series of experiment was carried out in three replicates. The insects were maintained at 25± 1°C, 70% R. H. The diet was changed every 2 to 3 days.

RESULTS AND DISCUSSION

Rearing of diamondback moth on an improved diet

Table 2 shows that larvae fed on diet-I can grow and develop better than those on diet-II. Both larval survival and adult emergence were higher on diet-I than on diet-II. The diet-I was prepared by autoclaving wheat germ and by dissolving lipid-containing ingredients in ether before adding to other components. Obviously improvement in dietary preparations is beneficial to the insect growth and development. Lipke et al. (1954) reported that autoclaving of soybean meal could eliminate toxic factors which may deter growth of *Tribolium confusum* and *Tenebrio molitor*. Although wheat germ lacks toxic substances and feeding deterrents, heating or autoclaving may improve its nutritive value for insects and fish (Vanderzant, 1974). Heat treatment of wheat germ seems to decrease its fat content (Adkisson et al., 1960). Whether the dietary effect on *P. xylostella* by heating wheat germ is attributed to lowering its fat content or to increasing its phagostimulation is not known. However it is advisable to heat wheat germ before adding

Table 2. Growth and development of *Plutella xylostella* on two diets

Diet*	No. larvae reared	Larval survival (%)	Pupation rate (%)	Adult emergence (%)	Larval period (days) Mean±S.D.	Pupal wt. (mg) Mean±S.D.	Pupal duration (days) Mean±S.D.
I	83	84.34	84.34	84.34	14.5 ±0.2	5.4 ±0.5	7.8 ±0.6
II	88	54.55	54.55	52.27	13.8 ±1.6	4.4 ±0.2	8.5 ±0.7

* Diet-I: prepared by improved procedures

Diet-II: prepared according to Hsiao and Hou (1978)

to other dietary components while preparing the diets for this insect.

Natural food is usually added to a semi-synthetic diet to enhance its feeding stimulation. Leaf powder of host plants is always used for this purpose. Since the diamondback moth infests mainly cruciferous vegetables, we selected seven commonly attacked crucifers for testing their dietary effect on the insect rearing. Table 3 shows that common cabbage and kale are best, followed by Chinese cabbage, in promoting the insect development while others are not benficial. In particular, cauliflower was unusually poor for rearing insects. This result could be due to inadequate drying processes or improper storage of fresh leaves. It is also possible that various crucifers contain different secondary metabolites which are phagostimulative to diamondback moth. This view can be supported by the fact that cabbage verieties have significant differences in the amounts of glucosinolates and their derivatives (Van Etten et al 1976). Certain insect species, e.g., *Pieris brassicae*, could survive on the diets without leaf powder (David and Gardiner 1966), however, *P. xylostella* grew poorly on the leaf powder-deleted diet (Table 3). Most larvae died during the first or second instar. It is suggested to add leaf powder to the diets for rearing diamondback moths.

Table 3. Growth and development of *Plutella xylostella* fed on the diets containing different cruciferous leaf powders

Leaf powder	No. larvae reared	Larval survival (%)	Pupation rate (%)	Adult emergence (%)	Larval Period (days) Mean±S.D.	Pupal wt. (mg) Mean±S.D.	Pupal duration (days) Mean±S.D.
Cauliflower	81	16.05	12.35	8.64	20.9 ±3.0	3.0 ±0.3	7.9 ±2.7
Chinese cabbage	84	65.48	65.48	65.48	17.6 ±0.1	4.1 ±0.3	10.5 ±2.1
Common cabbage	83	84.34	84.34	84.34	14.5 ±0.2	5.4 ±0.5	7.8 ±0.6
Kale	81	76.54	76.54	76.54	14.1 ±0.3	5.0 ±0.4	8.6 ±0.9
Leaf mustard	82	63.41	63.41	41.46	15.0 ±0.9	3.8 ±0.3	9.1 ±0.4
Radish	89	51.69	51.69	32.58	13.5 ±1.1	2.9 ±0.7	8.3 ±0.9
Pe-tsai	83	46.99	46.99	26.51	16.2 ±3.4	4.0 ±0.5	7.3 ±0.4
Check*	83	19.28	19.28	19.28	18.4 ±0.5	3.6 ±0.6	8.7 ±0.3

* Reared on the diet without leaf powder.

Dietary requirements for fatty acids

Studies on dietary requirements of the diamondback moth for linoleic acid and linolentic acid were revised by feeding the larvae on an improved diet and comparing the results with those of Hou and Hsiao (1978). Table 4 shows that addition of different concentrations of linolenic acid to the defatted diet could meet nutritional requirements for larval survival and pupation, however, this fatty acid failed to support adult emergence although cholesterol was present in the diet. Similar results were obtained by incorporating linoleic acid and/or α-tocopherol in combination with linolenic acid into the diets as linoleic acid alone was unable to promote adult emergence (Table 5). Insects fed on these diets died mostly in the course of emergence. Few insects emerged

Table 4. Growth and development of *Plutella xylostella* fed on the diets containing different concentrations of linolenic acid

Conc. of linolenic acid (g/100g diet)	No. larvae reared	Larval survival (%)	Pupation rate (%)	Adult emergence (%)	Larval period (days) Mean±S.D.
0	82	29.27	29.27	0	15.9 ± 1.5
0.15	88	53.41	53.41	0	16.9 ± 2.1
0.25	86	77.01	77.01	5.81*	14.5 ± 0.9
0.50	86	58.14	58.14	1.16*	14.3 ± 1.2

* deformed adults

Table 5. Effect of linoleic acid, linolenic acid and α-tocopherol on growth and development of *Plutella xylostella*

Lipids added	No. larvae reared	Larval survival (%)	Pupation rate (%)	Adult emergence (%)		Larval period (days) Mean±S.D.
				Normal	Abnormal	
Linoleic acid 0.5 ml	89	78.65	78.65	0	0	15.4 ± 0.7
Linoleic acid 0.5 ml +α-tocopherol 0.1 g	86	77.91	68.60	2.32	20.81	15.0 ± 0.9
Linolenic acid 0.25 g +α-tocopherol 0.1 g	81	35.80	35.80	0	0	20.5 ± 1.5
Linoleic acid 0.25 ml +linolenic acid 0.25 g +α-tocopherol 0.1 g	86	73.26	70.93	0	3.49	16.6 ± 1.7

but had curly wings. Thus, linoleic acid or linolenic acid cannot be a sole fatty-acid source for rearing diamondback moth. Vitamin E is not effective in enhancing the moth's development. Hou and Hsiao (1978) reported compensation of fat-free diets by linolenic acid in *P. xylostella*. The present study apparently contradicts our previous results. The main reason for this difference could be the variations in procedures for defatting. In this study we defatted wheat germ and leaf powder by using chloroform: methanol (2:1) in a Soxhlet apparatus whereas in our previous study we washed both ingredients by the same solvent system without using any extracting apparatus.

Therefore, some minor lipid components which would be nutritionally required for normal development or are phagostimulative to insect feeding were possibly still remaining in washed wheat germ and leaf powder in our previous study but were eliminated by exhaustive extraction in a Soxhlet apparatus.

Although linoleic acid and linolenic acid have been known as vital dietary components for normal development, especially adult emergence and wing formation, in lepidoterans (Turunen 1974, Sivapalan and Gnanapragasam 1979), the presence of other minor lipids which cannot be substituted by these fatty acids is essential for insect development in *P. xylostella* as revealed in this study.

Alpha-tocopherol is a strong antioxidant and is dietarily required for insect reproduction (Vanderzant and Richardson 1964). Turunen (1976) stated that vitamin E is capable of increasing linolenic acid accumulation in fat body, midgut and other tissues when added to the diets of *P. brassicae*. But our results did not show any appreciable benefit of this vitamin on the insect development. Therefore, it seems that nutritional requirements of diamondback moth for lipids are rather complicated and that there could be some bulk and trace requirements for different lipids which are not physiologically interconvertible.

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飼養小菜蛾之改良飼料及其對脂肪酸之需求

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