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An artificial diet for the swallowtail butterfly, *Papilio xuthus*

Seonghyun Kim*, Seongjin Hong, Haechul Park, Youngbo Lee, Kwanho Park, Wonho Choi, Namjung Kim

[1]*Department of Agricultural Biology, National Academy of Agricultural Science, Suwon 441-100, Korea.*

Abstract

The effect of an artificial diet on developmental rate, a life history parameter, was examined for the swallowtail butterfly *Papilio xuthus*. Artificial insect diets are an essential component of many insect rearing systems that produce insects for research purposes. Complex agar-gelled diets are generally prepared in large batches and used shortly after preparation because the degradation of perishable diet ingredients, such as vitamins and fatty acids, can adversely affect insect quality (Brewer 1984). However, the timing of diet preparation may be inconvenient, and large batches wasteful, if the unused excess is discarded. The percentage of pupation varied considerably, with no significant differences among diets, on which a maximum pupation percentage of 83% was observed. Pellet-type diets were investigated with the aim of developing a more easily prepared diet. The extrusion of the artificial diet under high temperature and pressure may induce desirable chemical and physical changes in the extruded product. The purpose of the present study was to develop an artificial diet for rearing *P. xuthus*.

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Introduction

Papilio xuthus is one of the swallowtail butterfly species commonly encountered in South Korea. The majority of swallowtail butterflies in the genus *Papilio* in South Korea use rutaceous plants as hosts, and most of these butterflies are oligophagous (Honda and Hayashi, 1995). The development of artificial diets and culturing techniques for rearing large numbers of lepidopterous insects has proliferated over the last 15 years and has produced a large body of literature, establishing diets for more than 250 species (King and Hartley, 1992, Singh, 1977). The cabbageworm butterfly (*Pieris rapae*) (Webb and Shelton 1988) and the painted lady butterfly (*Vanessa cardui*) have been successfully reared on artificial diets. Artificial insect diets are an essential component in many insect rearing systems that produce insects for research purposes. Complex agar-gelled diets are generally made in large batches and used shortly after preparation because the degradation of perishable diet ingredients such as vitamins and fatty acids can adversely affect insect quality (Brewer, 1984). Extrusion is a processing technology used to produce foodstuffs such as cereals, snack foods, and pet food. Food ingredients can undergo physical and chemical changes if variables such as temperature, moisture, and retention time are controlled in the extruder. The extrusion processing of ingredients can affect their carbohydrate composition,

***Corresponding author.**
Seonghyun Kim
Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-100, Korea.
Tel: +82-31-290-8560 / FAX: +82-31-290-8543
E-mail: ichibbang@korea.kr

namely, the starch and fiber fractions. The cooking of foods at high temperatures for a few seconds generally has favorable effects in terms of maintaining the properties of food components and active ingredients, while markedly reducing or completely eliminating microorganisms that are present in the starting material. Therefore, the final extruded artificial diet, with its low moisture content, is considered a shelf-stable product. The most common processing steps in the extruder-cooker are gelatinizing, dissolving, denaturing, roasting, mixing, shaping, and expanding (Wiedman and Strobel, 1987). Ready-to-eat extruded snack products are very attractive because of their convenience, textural attributes, shelf stability, and enhanced flavor. Additionally, the nutritional appeal of a high-protein, high-nutritional, low-calorie diet is a value-added attribute of extruded diets originating from plant materials. The manipulation of processing conditions in extrusion results in the gelatinization of starch. Thus, the extruded pellets are more water-stable than other dietary products (Stickney, 1979). This study was conducted to determine the physical quality of the diet pellets produced by feed processing technology and the physiological response of *Papilio xuthus* larvae to such pellets in terms of their growth and survival. As mentioned above, the use of plant materials is expensive and labor intensive, and their partial or complete replacement would be beneficial to the insect industry. This study examines the use of a broad variety of artificial diets for *P. xuthus.*

Table 1. The composition of the diets for Papilio xuthus

	A	B	C	D	E
Trifoliate orange leaf powder	47.5 g	40 g	32.5 g	25 g	35 g
wheat germ (Bio-Serv, Inc.)	120 g	90 g	60 g	30 g	0 g
high-nitrogen casein (Bio-Serv, Inc.)	34 g	34 g	34 g	34 g	68 g
Wesson salt mixture (Bio-Serv, Inc.)	8 g	8 g	8 g	8 g	16 g
vitamin premix[1]	10 g	10 g	10 g	10 g	20 g
sorbic acid (Bio-Serv, Inc.)	2 g	2 g	2 g	2 g	4 g
methylparaben (Bio-Serv, Inc.)	1 g	1 g	1 g	1 g	2 g
agar (Bio-Serv, Inc.)	15 g	15 g	15 g	15 g	30 g
distilled water	475 mL	400 mL	325 mL	250 mL	350 mL

[1]Vitamin premix composition = vitamin A 5 g , vitamin D3 1 g, tocopherol acetate 0.001 g, fursultiamine hydrochloride 1.3 g, riboflavin 2 g, pyridoxine hydrochloride 1 g, cyanocobalamin 3.3 g, ascorbic acid 2 g, folic acid 0.1 g, nicotinic acid 5.3 g, DL-methionine 2 g

Materials and Methods

Experimental insects

A colony of *Papilio xuthus* was founded from females collected at the National Academy of Agriculture Science (NAAS). The host plant was collected from the NAAS and maintained in containers and small outdoor plots. Adults were allowed to lay eggs on the leaves of the living host plant, and newly hatched first instars were removed and placed on rearing diets. Neonates were reared on trifoliate orange leaves (*Citrus trifoliata*) in an environmentally controlled room (25±1°C, 40 ± 10% RH and LD 16:8 h).

Preparation of artificial diets

The standard diet (Table 1) used was slightly modified from the Webb diet for the cabbageworm butterfly, *Pieris rapae* (Webb and Shelton, 1988). Trifoliate orange leaves (*Citrus trifoliata*) were grown in NAAS. The leaves were blended to a uniform consistency in a paddle-type mixer, then powdered in a disc attrition mill (PL-BM10L; Swon, South Korea) at 3,600 rpm. The leaf flour and certified flour were mixed in various proportions. A twin-screw extruder equipped with a 5-hp motor and corotating intermeshing screws, with three pairs of right-handed kneading blocks, was used at a constant screw speed of 200 rpm. Three extruder-barrel sections were electrically heated and air-cooled. The temperature profile selected for this study was 115, 80, and 115°C, with the first temperature corresponding to the feed barrel section and the last to the die section. Water was measured into the first barrel section using a variable-stroke piston pump to adjust the moisture content of the flour undergoing extrusion. The extruded artificial diet was collected in plastic trays, cooled to room temperature, bagged in zip-lock plastic bags, and stored under refrigeration (5°C).

Rearing on artificial diets

One day after hatching, first-instar larvae that had completely eaten their own eggshells were introduced into Petri dishes (6 × 1.5 cm) with one cake of the dietary material. The larvae were maintained in the dish during the first to fifth instars. The diets were changed every 2 d for the first to fourth instars and every other day for the fifth instar. The larvae were reared under long-day conditions (25°C, LD 14:8 h). The pupal weights 3 d after pupation were used as an indicator of diet suitability.

Statistical analysis

Differences in development were tested with an analysis of variance (ANOVA). If significant differences were detected, multiple comparisons were performed with a Tukey HSD multiple range test.

Results and Discussion

Five different diets (diets A to E) were prepared by formulating variations of the standard diet using various proportions of dried trifoliate orange powder and wheat germ (Table 1). The larvae were reared on these diets under long-day conditions (25°C, 14 L). A large number of artificial diets suitable for rearing many lepidopteran species can be found in the literature (Vanderzant, 1967; Bergomaz, and Boppre, 1986; Morton, 1979). The growth and survival of the larvae were examined by rearing groups on each of the artificial diets. The larvae reared from hatching on diet C completed larval development in 25.5 d, and adult emergence occurred on day 10.8. An analysis of variance showed no significant differences among the five diets in the mean number of days required for larval development and in the mean weight of adults. By the fourth instar, larval mortality did not differ significantly among the artificial diets (Fig. 1). The highest mortality, 75%, was recorded for the fifth instar on diet A. The percentage of pupation varied considerably, with no significant differences among diets except for diet C. The maximum pupation, 83%, was observed on diet C ($F_{4,9}$ = 65.596, $p < 0.0001$). Significant differences in the percentage of pupation were observed between diet C and the other diets. The highest adult emergence was 50%, also on diet C. This value differed significantly from the values for the other diets ($F_{4,9}$=53.024, $p < 0.001$). The percentage of adult emergence on the other diets was less than 33%. This low value was primarily due to high mortality in the fifth larval instar. Modification of the nutrient balance in an artificial diet is an effective approach for

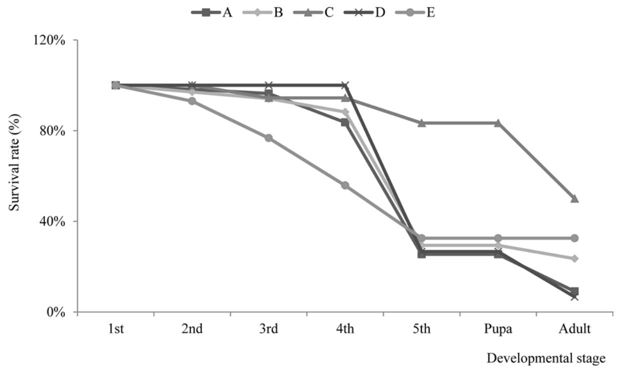

Fig. 1. Effect of artificial diets on the survival of *Papilio xuthus* at various developmental stages.

Table 2. Development of Papilio xuthus on artificial diets

	Larval duration (days)					Larvae to Pupa	Pupa
	1st	2nd	3rd	4th	5th		
A	6.2b	4.1	5.8	6.3	8.3	26.8	11.6
B	4.0a	4.0	7.0	6.7	5.8	24.0	10.0
C	3.8a	4.3	5.9	4.7	7.6	25.5	10.8
D	7.6c	3.7	5.0	7.2	6.8	24.1	10.9
E	5.9b	3.6	6.3	6.2	9.3	29.2	13.4

Hatching larvae of *P. xuthus* fed various artificial diets (A, B, C, D, or E). Values followed by the same letters within a column do not differ significantly (Tukey test following ANOVA, $P > 0.05$)

improving rearing efficiency, as has been shown in the development of artificial diets for Lepidoptera. In this study, we developed an artificial diet for the swallowtail butterfly *P. xuthus*, which could be reared on diet C to the adult stage. Diet C provides an effective tool for studying physiological parameters, such as the nutritional requirements of larvae. This study presents the first example of the successful rearing of *P. xuthus* on a pellet-type diet. Before a diet is adopted, it should be evaluated for several generations to determine the maintenance of the biological parameters vital for survival, reproduction, and behavior (Cohen, 2001). This study demonstrated that as many as 50% of the *P. xuthus* larvae successfully emerged as adults if individually reared on an artificial diet. Although this result was achieved with individual rearing, it represents a valuable step toward the establishment of a mass-rearing technique for *P. xuthus* using an artificial diet. In the present study, the emergence rate of *P. xuthus* adults increased as a result of appropriate modifications of the components of the artificial diet. This diet would, therefore, be potentially useful as an artificial diet for rearing several

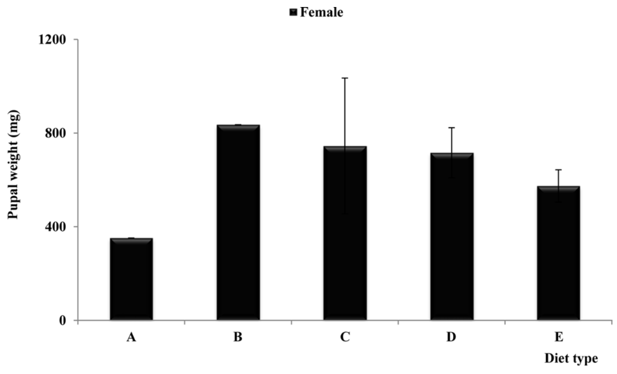

Fig. 2. Means of female pupal weights (mg) at 3 d of age from larvae reared in Petri dishes on a variety of artificial diets.

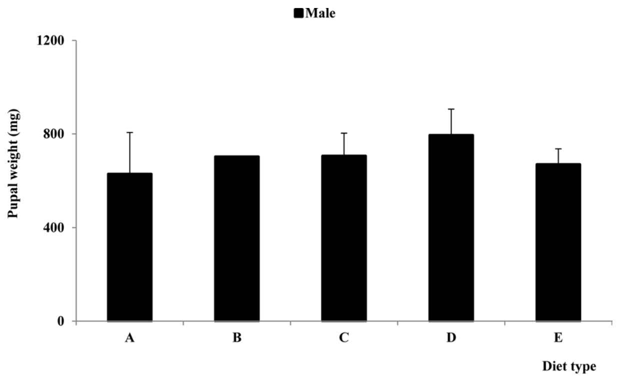

Fig. 3. Means of male pupal weights (mg) at 3 d of age from larvae reared in Petri dishes on a variety of artificial diets.

other economically important species of Lepidoptera. Further improvements in the formulation and production of the artificial diet are feasible. It is probable that these improvements would facilitate the labor-effective use of the diet for the mass rearing of *P. xuthus*.

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