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An Artificial Diet and the Rearing Method for the Asian Corn Borer, *Ostrinia furnacalis* (Guenee) (Lepidoptera; Pyralidae)

조명나방(*Ostrinia furnacalis* Guenee) (Lepidoptera; Pyralidae)의 인공사료와 사육법

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ABSTRACT This experiment was carried out to develop an artificial diet and a mass rearing system, which is essential in the pheromone research for *Ostrinia furnacalis*. Component deletion and addition methods were applied to define the nutritional importance of some components, especially yeast, Wesson's salt mixture and vitamin complex. Secondly we have developed a proper rearing system for *O. furnacalis*. and also tried to find out the factors affecting rapid sclerotization (dark-tanning) of pupae. Addition of yeast raised the pupal weight and pupation rate. However Wesson's salt mixture got the female pupae lighter. Vitamin complex as a substitue for yeast showed a good survivality in an early larval stage but pupal weight was lower than that of the individuals reared on the yeast diet. Also corrugated cardboard was found to be very good for pupation. And the rate of dark-tanning in pupae was higher as the period of larval development was shorter.

KEY WORDS *O. furnacalis*, artificial diet, yeast, Wesson's salt mixture, corrugated cardboard, dark-tanning

초 록 본 실험은 성페로몬 실험에 필수적인 조명나방의 인공사료와 대량사육 체제를 개발하기 위하여 수행되었다. 영양원으로서 효모, Wesson 염, 비타민 등의 중요성을 알아보기 위하여 그들을 사료에서 삭제 또는 첨가하였다. 또한 적절한 사육방법을 고안했으며 번데기의 암경화현상에 영향을 미치는 요인도 알아 보았다. 효모의 첨가는 용무게와 용화율을 높여 주었으나 Wesson 염의 첨가는 암컷 용의 무게를 감소시켰다. 효모의 대용으로 사용된 비타민은 어린 유충의 생존율을 높여 주었으나 용무게에서는 효모첨가사료의 경우 보다 가벼웠다. 용화장소로서 골판지는 매우 좋은 결과를 보였고, 용의 암경화율은 유충의 발육속도가 빠를수록 높았다.

검 색 어 조명나방, 인공사료, 효모, Wesson 염, 골판지, 암경화

The term of the artificial diet, when applied to insects, has been defined as any diet that is not their natural food. Artificial diets and the nutrition of insects have been studied over the last 70 years. The fact that insects eat the food which man grows clearly indicates that insects use some of the same nutrients as ours, such as the proteins, carbohydrates and fats. Lipid soluble substances also play a prominent role in the search for growth factors. For example,

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cholestrol which insects cannot synthesize, was discovered to be the substance that promotes growth (Chippendale 1971, Goodfellow et al. 1971). Polyunsaturated fatty acids are also needed for successful adult emergence without wing deformation in moths (Vanderzant 1968). Ascorbic acid or vitamine C is another substance which was found to be a feeding stimulant and eventually an essential nutrient for the growth, and especially for inhibiting the rapid sclerotization of larvae and pupae of moths (Navon 1978).

Diets for many insects could be made simply by combining substances of known nutritional value supplemented with some others such as yeasts and cereal grains found in the natural foods of the insects. Since the introduction of diets containing wheat germ, there has been a proliferation of reports of such diets (Beck et al. 1949, Beck & Stauffer 1950). Hundreds of insect species have been reared on diets containing wheat germ. The most important changes include the addition of other plant materials; supplimentation with more fatty acids. sterols and vitamins; adjustments in quantities of nutrients; and the addition of a wide variety of antimicrobial agents. Wheat germ contains all the minor nutrients with the possible exception of ascorbic acid. There are 18 ordinary amino acids included in several proteins, sugars, triglycerides, phospholipid(which include choline and inositol), B vitamines. tocopherols, carotenes, 21 mineral elements, and over 50 enzymes (MacMaster et al. 1971).

Soybeans (*Glycine max*) have also long been used as a diet component for insects. They contain good quality proteins except for sulfur amino acids which are low when compared with egg albumin. However, raw soybeans contain toxic substances, and the proteins are poorly utilized (Wolf & Coean 1971). Heat treatment, either dry or wet, improves the nutritional quality and destroys the toxic factors (Mickelson & Yang 1966, Lipke et al. 1954). Whole yeasts and yeast extracts from several species have been used frequently in insect diets to supply unidentified nutritional factors, vitamins, and/or the major nutrient. As an adjunct to vitamins, yeasts not only supply unidentified factors, but may also have a protective effect on the vitamins during heating (Vanderzant 1973).

A major problem in devising diets for chewing insects is to prepare a diet that is solid and has a high water content. Agar to form a rigid gel at low concentrations has been used for solid diets because it is inert nutritionally.

Mass production of insects on artificial diets during the last decades has greatly accelerated research on pest control methods. Insects have been available for extraction and identification of pheromones, release of irradiated insects, production of viruses, parasites and predators, and many other studies such as their biology and biochemistry.

We designed this experiment to develop an artificial diet for *Ostrinia furnacalis*. In Japan, a kidney-wheat germ diet was used for rearing this insect for a decade (Saito & Nakayama 1981). That diet was not a new one but was one which had been used for mass production of tobacco cutworm (*Spodoptera litura*). And the necessity of certain components was not explained. In the present study, diet deletion and addition strategy has been used to define the nutritional importance, especially of yeast, vitamin complex and salt mixture in development of *O. furnacalis*. We also compared developmental performance between group-and individual-rearing systems and designed a new rearing system. Finally we tried to find out the factors

affecting the rapid sclerotization (abnormal phenomenon in development)in pupae (dark-tanned pupae).

MATERIALS AND METHODS

Insect

O. furnacalis larvae were collected from corn fields around the College of Agriculture and Life Sciences, Seoul National University in Suwon. The insectary light condition under which the collected larvae were reared was 16 hours of light and 8 hours of darkness (16L : 8D). But, temperature was variable depending on experiments.

Artificial diets

An artificial diet (A-1) was prepared with the components which were selected from artificial diet components for other Pyralidae (Singh 1977). Another (B-1) was based on the diet of the tobacco cutworm (*S. litura*) (Saito & Nakayama 1981). From the experimental results conducted on these two diets, more artificial diets were tested. The amount of yeast and salt mixture in the diet A-1 was made variable to result in 3 different versions (A-2~4). Also the diet B-1 had 4 more subcategories (B-2~5) depending on the amount of yeast, corn oil, vitamine, salt mixture, cystein hydrochloride, sorbic acid, soybean or kidney bean (Table 1). The diet C is similar to the diet A-1, except for putting the corn powder instead of soybean powder.

Table 1. Composition of artificial diets for *Ostrinia furnacalis*.

(unit : g)

Diets / Components	A−1	A−2	A−3	A−4	B−1	B−2	B−3	B−4	B−5	C
Corn powder	−	−	−	−	−	−	−	−	−	40
Soybean	40	40	40	40	100	100	100	−	−	−
Kidney bean	−	−	−	−	−	−	−	100	100	−
Yeast	−	40	20	20	40	40	40	40	−	−
Agar	15	15	15	15	12	12	12	12	12	15
Sucrose	30	30	30	30	−	−	−	−	−	30
Cellulose	20	20	20	20	−	−	−	−	−	20
Casein	30	30	30	30	−	−	−	−	−	30
Corn oil	−	−	−	−	−	−	−	4	4	−
Vitamine	−	−	−	−	−	−	−	−	10	−
Ascorbic acid	6	6	6	6	4	4	4	4	4	6
Wheat germ	20	20	20	20	100	100	100	100	100	20
Salt mixture	7	7	7	−	7	−	−	−	−	7
Cholesterol	0.4	0.4	0.4	0.4	−	−	−	−	−	0.4
Cystein HCl	0.6	0.6	0.6	0.6	0.4	0.4	−	−	−	0.6
Cholin Cl	1	1	1	1	−	−	−	−	−	1
Sorbic acid	2.2	2.2	2.2	2.2	−	2	2	2	2	2.2
M P H	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Form.(35%)(ml)	3	3	3	3	3	2	2	2	2	3
D. W.(ml)	1000	1000	1000	1000	700	700	700	700	700	1000

HCl : Hydrochloride, Cl : Chloride, MPH : Methyl *p*-hydroxybenzoate, Form.:Formalin, D.W : Distilled water

Rearing system

Sixty or more larvae of lst instar were applied to a petridish (10ϕ × 2cm) containing artificial diets just after hatching, and then their subsequent third instar larvae were individually moved to a plastic case (4.5ϕ × 1.5cm) to observe the developmental performance depending on the artificial diet composition. But in comparison experiment of density effect on larval development, some of the third instar larvae were reared in a petridish (10ϕ × 2cm) containing twenty to thirty individuals.

They were allowed to pupate in a petridish (10ϕ × 2cm) or a small plastic case (4.5ϕ × 1.5cm), or in the large plastic case (25 × 25 × 25cm) with corrugated cardboard. Pupation efficiency was checked in each pupation system. A new rearing system was also designed according to the results of the basic rearing method, and the developmental performance of *O. furnacalis* in this system was compared.

Attractiveness of some artificial diets to the first and third instar larvae

Thirty larvae of lst or 3rd instar were placed at the center of a petridish containing artificial diets(A-3, B-4 or B-5)(Fig. 1) which were relatively good for larval development. Another experiment was carried out by application of thirty larvae of lst instar on the top of the artificial diets in direct contact. In this case, the petridish also had 3 different diets and consequently 90 larvae. Attractiveness was defined by the number of larvae located on each diet one day after application.

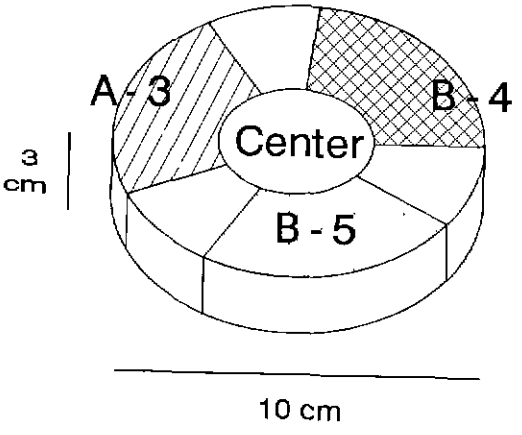

Fig. 1. System for testing the attractiveness of *O. furnacalis* larvae to artificial diets. Thirty larvae of 1st or 3rd instar were applied at the center or on the top of artificial diet in direct contact.

Determination of factors inducing dark-tanning in pupae

Pupal color was divided into three categories depending on the amount of melanin pigments (Table 2) after pupal tanning when they were reared in the laboratory. Field collected larvae showed their pupal color more or less similar to the category 1 from laboratory colony.

Table 2. Designation of laboratory-reared *O. furnacalis* pupal color

No.	Description
1	No melanin pigment
2	Partly melanized
3	Wholly melanized

To examine the effect of diets on dark-tanning of pupae, the protein contents of pupal exuviae which were categorized just after pupal tanning and collected after adult emergence were determined by Lowry method (Lowry et al. 1951). Pupal exuviae of four categories were dryed for 24hrs. at 60℃ and then were ground with sea sand to powder. Protein extraction was done with 2% SDS for 30 min. at a room temperature (Anderson 1975). To detect the relationship between larval developmental rate and dark-tanning, pupae from larvae fed on the diets A-3, B-4 or B-5 were weighed

within 10hrs. after pupation and the dark-tanned pupae (second and third categories) were seperated, depending on the larval period.

RESULTS

Artificial diet

Pupae from larvae reared on the diet A-1 in which yeast was absent, had lower weight and pupation rate than those on the diet B-1 (Table 3). But when 40 g yeast was added to the diet A-1 (the diet A-2), pupal weight, larval period and pupation rate increased and this phenomenon was more apparent in females than males. In the diet A-3 containing 20 g yeast, larval period was similar to but pupal weight of female was still heavier than those in the diet B-1. In the growing 5th generation larval, we deleted Wesson's salt mixture from the diet A-3 to make the diet A-4 and from the diet B-1 to make a diet B-2 and found that pupal weight was similar in males between the two diets but became heavier in females in the case of new versions (Table 3). Larval period in the diet A-4 was similar to that in A-3 but longer by about 2 days in the diet B-3 than that in the diet B-1. Pupation rate was higher in both diets containing Wesson's salt mixture, and such a result was clearer in the diet B versions.

Table 3. Performance of *O. furnacalis* fed on eight different artificial diets under three different temperature conditions

Generation (temp.)	Diet		No. larvae	Larval period (day)	Pupation rate(%)	Pupal weigh (mg)	Pupal period (day)	Emerg. rate(%)	Sex. ratio (%)	No.malformed adult
3rd (26±1℃)	A-1	♀	150	–	50.0	84.3±12.2	7.2±0.7	92.7	51.6	4
		♂				63.8±11.7	7.8±0.7		48.4	4
	B-1	♀	150	–	61.3	98.4±13.2	7.4±0.8	98.0	56.3	2
		♂				74.7±10.2	7.0±0.5		43.7	2
	C		60*	–	–	–	–	–	–	–
4th (28±1℃)	A-2	♀	160	17.7±1.2	55.7	128.3±15.3	9.7±0.8	93.1	38.3	0
		♂		16.2±1.8		89.4± 9.6	10.0±0.9		61.7	2
	B-1	♀	100	19.2±2.0	48.0	117.2±12.2	9.5±1.1	96.0	50.0	1
		♂		18.1±1.6		86.9± 7.4	9.8±0.7		50.0	1
5th (26±1℃)	A-3	♀	60	21.6±2.2	65.0	88.3±13.8	7.3±0.8	90.0	54.3	0
		♂		20.8±2.1		68.2± 8.5	7.4±0.9		45.7	1
	A-4	♀	60	22.1±2.5	63.3	105.0±15.7	7.5±0.5	89.5	35.3	1
		♂		20.3±2.7		69.4± 6.5	7.8±0.5		64.7	1
	B-1	♀	60	22.2±2.8	71.1	81.1±21.3	7.5±0.9	79.1	50.0	0
		♂		21.6±2.9		64.8±10.5	7.8±0.8		50.0	0
	B-2	♀	60	24.4±3.7	32.1	84.4±19.3	7.5±0.7	94.4	70.6	1
		♂		21.4±2.3		66.6± 7.6	7.8±0.4		29.4	0
	B-3	♀	60	24.5±2.5	49.0	90.4±13.7	7.9±0.6	84.5	52.3	1
		♂		21.4±2.6		67.8±10.6	7.7±0.5		47.7	1

* : All larvae were dead by the 2nd larval instar stage.

Temperature was also found to be a factor in larval performance in artificial diets. For example, higher temperature shortened the larval

period, increased the pupal weight, and lowered the pupation rate noticeably. And the heavier the pupal weight was, the longer the pupal period was. Larvae fed on the diet B-1 showed the lower pupal weight and lower rate of emergence at the 5th generation than that of the 3rd generation.

We wanted to know the field population's performance for the comparison with the population we reared in the laboratory. The weight of female pupae obtained from collected larvae was more or less similar to that of the third generation population fed on the diet B-1. But they were heavier than any of the fifth generation except for the one fed on the A-4 (Tables 3, 4). And no population fed on an artificial diet for the population reared at 28±1℃, was heavier than the collected population in male pupal weight.

Table 4. Performance of *O. furnacalis* collected as hibernating larvae in a corn field(April 21, 1992)

Pupation rate(%)	Larvae parasited(%)	Pupal weight (mg)		Pupal period (day)	Emergence rate(%)	Sex ratio (%)	No.malformed adult
		♀	♂			♀/♂	0
52	48	98.6±19.8	77.7±16.5	10.3±1.1	100	52/48	

Lee et al. (1980) reported that *O. furnacalis*, reared on corn plant, underwent six or seven instars, but the population we reared with artificial diets had just five instars (Table 5). Headwidth of the first instar larvae fed on artificial diets was smaller than that of larvae reported by Lee et al. (1980) and our fifth instar larvae showed the remarkably smaller size than the seventh instar but larger than the sixth instar larvae reported.

Table 5. Head width(mm) of *O. furnacalis* larvae fed on a host plant(corn)(A) and on an artificial diet(B)

Diet\Instar	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th
A	0.32±0.01	0.49±0.04	0.76±0.09	1.16±0.13	1.36±0.07	1.50±0.08	2.42±0.25
B	0.27±0.02	0.47±0.02	0.73±0.04	1.17±0.07	1.61±0.08		

A : headwidth reported by Lee *et al.*(1980)

B : headwidth reared on an artificial diet at 26±1℃ in this study

Designing a new rearing system

It is very laborious to rear a lot of larvae individually. So we tried to rear a group of larvae in a petridish (10ϕ × 2cm). Twenty to thirty larvae of 3rd instar were transferred to a new petridish containing an artificial diet and maintained there until pupation. Larval period tend to be longer in group-rearing system but pupal period was shorter than that from individual-rearing system. But the difference was not so great except for a case of the diet A-3 (Table 6). Pupae obtained from larvae reared individually generally showed much heavier weight and higher rate of emergence than those of pupae reared in groups (table 6). But pupation rate was not consistent in any rearing system.

A proper pupation place had to be deviced, and corrugated cardboard was found to be a good candidate because of crevices on both

Table 6. Performance of *O. furnacalis* fed on three different artificial diets in group and individual rearing systems(24±1℃)

Diet	Rearing density	Survival rate after 2nd instar(%)	Larval period (day)	Pupation rate(%)	Pupal weight(mg) ♀	Pupal weight(mg) ♂	Pupal period (day)	Emergemce rate(%)	No.malformed adult
A−3	G	33	33.0±3.9	23	69.5± 7.2	54.5±3.5	5.4±0.8	57.1	1
	I	62	25.2±1.6	60	101.8±9.9	67.7±9.8	7.0±0.7	91.6	1
B−4	G	85	25.6±2.6	65	77.7± 9.9	52.3±5.6	6.6±0.6	60.0	2
	I	73	23.9±1.5	63	95.5±14.6	64.2±8.5	7.1±0.6	86.8	0
B−5	G	89	26.2±3.0	86	83.0±16.1	60.6±7.3	6.3±0.7	88.3	2
	I	68	24.0±1.6	43	91.3±15.4	66.2±9.6	7.7±0.7	92.3	1

G : Reared in groups of 20~30 larvae in a petridish(10ϕ × 2cm).

I : Reared individually in a small plastic case(4.5ϕ × 1.5cm).

sides. Several sheets of corrugated cardboard were piled up on one side of a plastic case(25 × 25 × 25cm) which could accomodate more than a hundred of last instar larvae, and the artificial diet was placed on the other side. Fifty last instar larvae which had fed on the diet for 3 or 4 days were transferred to that case, and another group was maintained in the group-rearing system in a petridish. Last instar larvae needed shorter time for pupation in the corrugated cardboard than in the petridish group-rearing system. Rates of pupation and of emergence were also clearly higher in the corrugated cardboard system (Table 7).

Table 7. Advantages of corrugated cardboard(CC) as a pupation site for *O. furnacalis*

Pupation place	Pupation duration*	Pupation rate(%)	Emergence rate(%)	No. malformed adults
C C	5.4±2.5	92.0	91.3	3
Petridish	12.7±3.7	41.7	60.0	5

* : Period(days) from application of fully grown-larvae to pupation.

Rate of pupation was greatly influenced by the number of larvae survived until the 3rd instar (Table 6) since the mortality during the 4th and 5th instar stages was negligible. The first instar larvae, transferred to the diet A-3, often showed a high mortality. So we assumed that there might be no attractant or stimulant for feeding of the first instar larvae in the diet A-3. When 3 different diets were compared, the diet B-4 was much more attractive than the diet A-3 to the first instar larvae (Table 8). Such a phenomenon was also observed when

Table 8. Attractiveness of artificial diets to the first and third instar larvae* of *O. furnacalis* applied at the center of the petridish

Larvae\Diets	Mean rate (%) A−3	B−4	B−5	Others[a]
1 st	15.8±5.8	56.8±6.4	22.7±9.3	5.1±1.7
3 rd	22.2±6.5	31.3±7.1	33.1±8.0	13.3±9.0

* : Thirty larvae were applied in each of 4 replicates.

a : Individuals that strayed.

the first instar larvae were applied in direct contact with each diet (Table 9). But the third instar larvae, placed at the center of the petridish, did not show any preference (Table 8). All these figures were tabulated 24 hours after introduction of larvae into the petridish.

Table 9. Attractiveness of artificial diets to the first instar larvae of *O. furnacalis* when applied in diret contact with the diets

Diets	A−3	B−4	B−5	Others[b]
Rate(%)[a]	15.5±3.5	69.0±9.0	15.5±3.5	2.5±0.8

a : Total mean value of two replicates in which thirty individuals were applied to each diet
b : individuals that strayed

From the results of these rearing methods, we found and corrected a few faults to design a new rearing system, in which the mixed diet of A-3 and B-4 (1 : 1, v/v) was used instead of a single diet because the diet A-3 frequently showed a high mortality in the first and second instar larvae, and the diet B-4 was usually contaminated within a few days. And to reduce the excess stagnation of water evaporated from the diet, kitchen paper towel was put down under the mixed diet. After 10 days (at 3rd instar stage) from the application of 1st instar (26±1 ℃), all larvae were transferred to the other larger case (15×15×10cm) with the new diet and a glass plate was used as a lid. Fully grown 5th instar larvae were transferred to the pupation place (plastic case of 25×25×15cm) with corrugated cardboard at one side and the new mixed diet at the other side. In this system, developmental performance was good (table 12).

Factors inducing dark-tanning of pupae

Some of the pupae reared on artificial diets showed a very blackish color as a result of melanin precipitation on the cuticle. But there were no individuals showing darkly tanned color in pupae obtained from field-hibernated larvae (Table 10). Therefore, this situation must be considered as an abnormal phenomenon.

Cuticle of darkly tanned pupae was usually sclerotized rapidly and so fragile that we had to be careful in their handling. When protein content of exuviae was examined (Fig. 2), it decreased with the degree of tanning. Such an abnormal phenomenon could affect the rate of emergence and the female adult longevity. The emergence rate of dark-tanned pupae was about half that of normal pupae on the diets A−3 and B−4, and the female adult longevity, which is defined as the number of days from

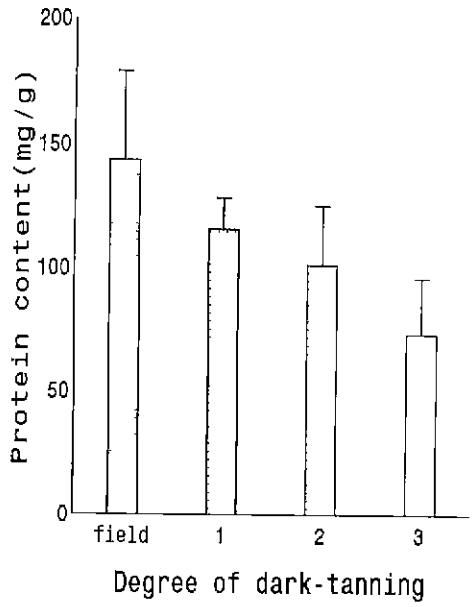

Fig. 2. Protein contents of pupal exuviae showing different degrees of blackening.

adult eclosion to losing the ability of flight, was shorter by about 3 days (Fig. 3).

We checked the effects of growing temperature and larval period on the pupal color. Generally, pupae reared on the diet As showed more dark-tanning at any temperature. And

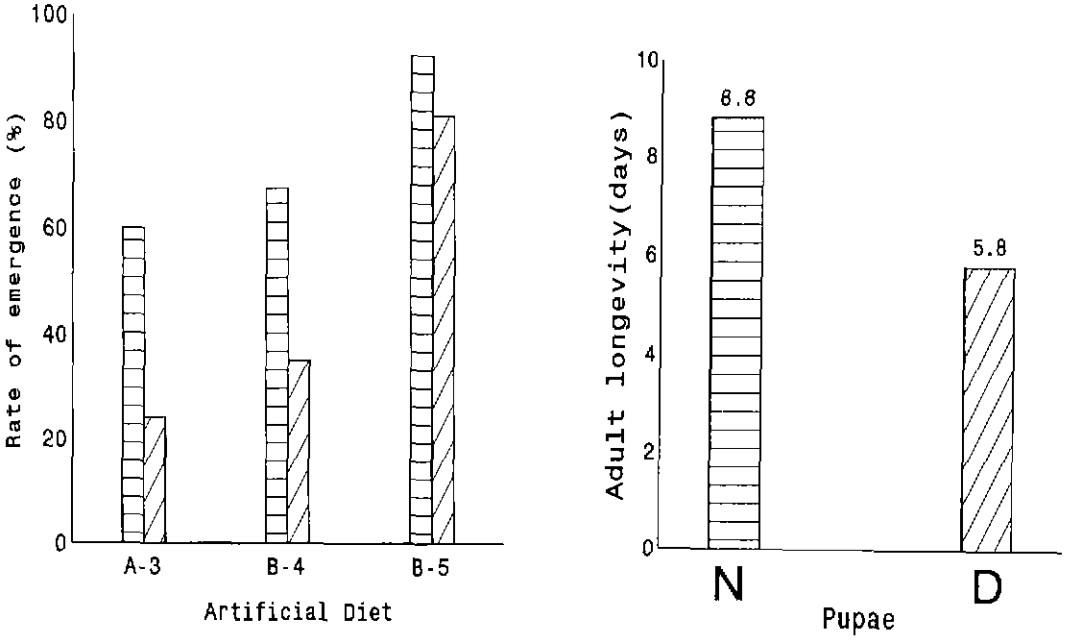

Fig. 3. Emergence rate and female adult longevity of normal(▤, N) and dark-tanned(▨, D) pupae.

the higher the temperature was, the higher the rate of dark-tanning on each diet was. Pupae fed on the diet A−4 which had no Wesson's salt mixture showed a higher rate than those from the diet A−3 with the salt mixture but Wesson's salt mixture did not affect the pupal dark-tanning in diet Bs (Table 10). The production rate of dark-tanned pupae also seemed to be closely related to the developmental period. For example, the diet A−2 produced the highest rate of dark pupae (Table 10), and also showed shorter larval period than that in the diet B−1 at 28±1 ℃ (Table 11). And even at 24±1℃, the shorter the larval period was, the

Table 10. Rate(%) of dark-tanned pupae reared on artificial diets *in three different temperature conditions*

Temperature (℃)	Diet						
	A−2	A−3	A−4	B−1	B−2	B−4	B−5
24		51				14	10
26		57	80	32	36		
28	98				50		

Table 11. Developmental period(days) of *O. furnacalis* larvae fed on two different artificial diets(28±1 ℃)

Diet	Instar					
	1 st	2 nd	3 rd	4 th	5 th	Total
A−2	3.1±0.2	2.3±0.2	2.4±0.6	3.0±0.7	5.7±0.6	16.5
B−1	3.5±0.7	2.5±0.4	2.5±0.7	3.5±1.0	6.5±0.9	18.5

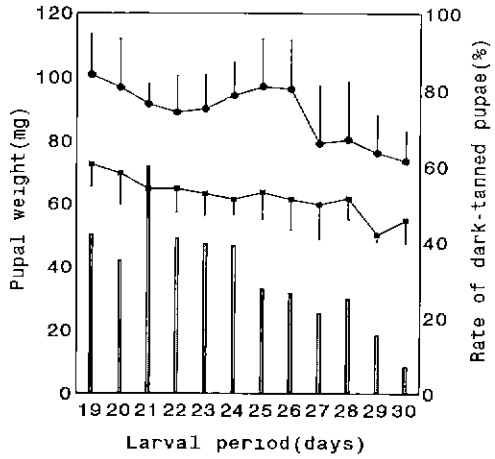

Fig. 4. Male(■) and female (●) pupal weight and production rate of dark-tanned pupae(bar) vs. larval developmental period.

more dark-tanned pupae appeared. The pupal weight was also lighter as the larval period was prolonged (Fig. 4). Then pupae obtained from the larvae reared in a new rearing system showed few individuals tanned darkly (Table 12) though the larval developmental rate was similar with that of larvae in the old rearing methods.

DISCUSSION

The diet A−1 had soybean (22.5%), wheat germ (11.3%) and casein (16.9%) as protein, lipid and carbohydrate sources. Larvae fed on the diet A−1, which lacked yeast, showed a low level of pupal weight and pupation rate (Table 3), but those levels increaed as yeast added to the diet. Yeast might supply such nutrients as amino acids, nucleic acids, vitamines, phospholipids, glycerides or sterols (mostly ergosterol) which are known to be rich in them (Reinecke 1985) for a sound growth of *O. furnacalis*. Yeast also would play a major role in attracting the first instar larvae (Table 8, 9). The reason that larval period in the diet A−2 was shorter than that in the diet B−1 was due to the difference (about one day) in the last larval period (table 11). Such a rapid development induced the pupae to be tanned darkly and to have a heavier weight (Tables 3, 10).

The rapid development of larvae fed on diet As might be caused by the high quantity of protein supplied from casein which was absent from diet Bs. Casein contains about 15 amino acids (with about 0.8% each of bound phosphorous and sulphur), but may be lack, or have insufficient amounts of certain amino acids (e.g. cystein, glycine, and glutamic acid)

Table 12. Performance of *O. furnacalis* (6th generation) fed on the artificial diet mixture[a] under the new rearing system[b] at 26±1 ℃

No. larvae	Larval period (day)	Pupation rate (%)	Pupal weight (mg)	Pupal period (day)	Emerg. rate (%)	Sex ratio (%)	No. malformed adult	No. dark-tanned pupae
♀	21.8±2.2		95.4±15.9	7.5±0.7		60	1	2
100		66.0			91.5			
♂	20.9±2.6		69.8± 9.0	7.3±0.6		40	0	1

a : The artificial diet A−3 and B−3 were mixed in about 1 : 1(v/v) ratio.

b : First instar larvae were applied to the mixed diet in a petridish(10 × 2cm) containing kitchen towel on the bottom. After 10 days(at 3rd instar), all larvae were transferred to other case(15 × 15 × 10cm) with the new fresh diet and a glass plate was used as a lid. Fully grown 5th instar larvae were transferred to the pupation place(plastic case of 25 × 25 × 25cm) with corrugated card boards at one side and the new mixed diet at the other side.

(Vanderzant and Reiser 1956). Vitamines are also needed for a good survivality (Table 6). Though the level of pupal weight reared on diet B−5 was lower than those on diets B−4 and A −3, survivality was higher (Table 6). Wesson's salt mixture reduced the weight of female pupae, but not of male in both diets (Table 3).

How could Wesson's salt mixture induce such a result? We may list the reason in three respects (1) the composition of salts (Na^+.K^+, etc.) in the Wesson's salt mixture was not adequate to *O. furnacalis* larval development; (2) some important ions might be absent from the Wesson's salt mixture; (3) the quantity was over the optimum concentration in the diet for this insect. Although most insects tolerate the mamalian salts, studies showed that such salts were ill balanced with respect to salt ratios obtained from analysis of host material. Plants tend to be high in potassium, phosphate and magnessium, but the mamalian salt mixtures are comparatively high in sodium, calcium and chloride. In some instances where the elements had been adjusted to better represent a more natural element ratio growth was enhanced (Beck 1968). In these repects the first possibility listed above could explain the some adverse facts of the Wesson's salt mixture. But, Wesson's salt mixture remains for more tests in terms of its quantity and ratio of certain salts.

Dark-tanning of pupae can be caused by precipitation of melanin which is synthesized from polymerization of quinones. Then, dark-tanning may take place when there are more quinones than proteins which will be used for β-tanning, or proteins are lacking (Kayser 1985), or the *O* -diphenoloxidase acts rapidly in the pupal cuticle during the pupal sclerotization (Navon 1987). Other factors affecting concentration of MRCH (melanization and reddish coloration hormone) (Raabe 1989) or PMRF (pupal melanization reducing factor) (Boo 1989) could be involved in dark-tanning of pupae. Raabe (1989) reported that the MRCH concentration rose when the rearing temperature was over 30 ℃ or CO_2 concentration rose in the environment, and then the pupal color become blackish. Environmental color when pupation occured also affected pupal color (Maish and Buckmann 1987). Therefore we could conclude that, since the darkening of pupal cuticle affected the rate of emergence and the adult longevity (Fig. 3) the rate of dark-tanned pupae could be an indicator for determining the usefulness of diets or rearing conditions. And the understanding of pupal dark-tanning needs more sophisticated research in analysing environmental factors.

Now we are rearing *O. furnacalis* successfully in the following system. About one hundred larvae of the first instar are transferred just after hatching to a petridish (10ϕ × 2cm) containing the mixed artificial diet (A−3 and B−4) on the kitchen paper towel. and reared until the third instar (about 10 days). The population of third instar larvae is wholly transferred to another larger case (15 × 15 × 10cm) containing a new diet, and when they mature in about a week, healthy fifth instar larvae are tranferred to the pupation place with corrugated cardboards and a new diet. After a week, the pupae are collected from corrugated cardboard. This rearing system enable us to reduce the amount of the artificial diet and labor.

LITERATURES CITED

Anderson, S. D. 1973. Comparison between the sclerotization of adult and larval cuticle in *Schistocerca gregaria*. J. Insect Physiol. 19: 1063 ~1614.

Beck, S.D. & J.F. Stauffer. 1950. An aseptic method for rearing European corn borer larvae. J. Econ. Entomol. 43: 4~6

Beck, S.D., G.M. Chippendale & D.E. Swinton. 1968. Nutrition of the European corn borer, *Ostrinia nubilalis*. VI. A larval rearing medium without crude plant fractions. Ann. Entomol. Soc. Am. 61: 459~462.

Beck, S.D., J.H. Lilly & J.F. Stauffer. 1949. Nutrition of the European corn borer *Pyrausta nubilalis*(Hubn). I. Development of a satisfactory purified diet for larval growth. Ann. Entomol. Soc. Am. 42: 483~96.

Boo, K. S. 1989. 곤충생리학. 618 p. 집현사. 서울.

Chippendale, G. M. 1971. Lipid requirements of the angoumois grain moth, *Sitotroga cerealella*. J. Insect Physiol. 17: 2169~77

Goodfellow, R.D., F.J. Barnes & W.J. Graham. 1971. Dietary sterol effects on mevalonate kinase activity in axenic *Sarcophaga bullata*. J. Insect Physiol. 17: 1625~35.

Lee, Y.B., C. Y. Hwang, K. M. Choi & J. Y. Shim. 1980. Studies on the Bionomics of the Oriental cornborer, *Ostrinia furnacalis* (Guenee). Korean J. Pl. Prot. 19: 187~192.

Lipke, H., G. S. Fraenkel & I. E. Liener. 1954. Effect of soybean inhibitors on growth of *Tribolium confusum*. J. Agr. Food. Chem. 2: 410~14.

MacMacters, M.M., J.J.C. Hinton & D. Bradbury. 1971. Microscopic structure and composition of the wheat kernel. In Wheat Chemistry and Technology, ed. by Y. Pomeranz, Vol 3.pp51~113. St. Paul. Minn: Am. Assoc. Cereal Chemists. 821pp.

Maisch, A. & D. Buckmann. 1987. The control of cuticula melanin and lutein incorporation in the morphological color adaptation of a nymphalid pupa *Inachis io* L. J. Insect Physiol. 33: 393~402.

Mickelsen, O. & M.G. Yang. 1966. Naturally occurring toxicants in foods. Fed. Proc. 5: 104~23.

Navon, A. 1987. Effects of dietary ascorbic acid on larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. J. Insect Physiol. 24: 39~44.

Raabe, M. 1989. Recent developments in insect neurohormones. 503pp. Plenum Press. New York.

Reinecke, J. P. 1985. Nutrition: Artificial diets. In Comprehensive insect physiology, biochemistry and pharmacology, Eds. by G.A. Kerkut & L. I. Gilbert. Vol. 4. pp391~419. Pergamon Press. Oxford.

Saito, O. & I. Nakayama. 1981. A simple rearing method for Oriental corn borer, *Ostrinia furnacalis*(Guenee)(Lepidoptera, Pyralidae). Bull. Tohoku Natl. Agric. Exp. Stn. 63: 243~247.

Sign, P. 1977. Artificial diets for insects, mites, and spiders. 594p. IFI/Plenum.

Vanderzant, E.S. 1968. Dietary requirement of the bollworm, *Heliothis zea*, for lipids, cholin, and inositol and the effect of fats and fatty acids on the composition of the body fat. Ann Entomol. Soc. Am. 61: 120~25.

Vanderzant, E. S. 1973. Improvements in the rearing diet for *Chrysopa carnea* and the amino acid requirements for growth. J. Econ. Entomol. 66: 336~38.

Vandezant, E. S. & R. Reiser. 1956. Studies of the nutrition of the pink bollworm using purified casein media. J. Econ. Entomol. 49: 454~458.

Wolf, W. J. & J. C. Cowan. 1971. Soybean as a food source. CRC Crit. Rev. Food Techol. 2: 81~158.

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