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# Consumption and Utilization of Complete Defined Diets Covarious Carbohydrates by *Spodoptera exempta* (Lepidoptera: Noctuidae)

## (Konsumsi dan Penggunaan Makanan Buatan Lengkap yang Mengandung Berbagai Jenis Karbohidrat oleh *Spodoptera exempta* (Lepidoptera:Noctuidae))

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***Abstrak***
*Pengukuran pengaruh makanan buatan lengkap yang mengandung berbagai jenis karbohidrat (sukrosa, glukosa, fruktosa, mannitol, dan maltosa) terhadap kinerja instar lima Spodoptera exempta (Lepidoptera : Noctuidae) telah dilakukan. Larva akan memakan lebih banyak makanan pada makanan buatan lengkap yang mengandung perangsang makan jika dibandiingkan dengan larva yang memakan makanan tanpa perangsang makan.. Hasil ini mengindikasikan bahwa kemampuan merangsang makan dari karbohidrat besar peranannya. Walaupun makanan buatan yang mengandung mannitol, karbohidrat yang tidak mempunyai kemampuan merangsang makan ,dimakan dalam jumlah yang sama jika dibandingkan dengan makanan yang mengandung glukosa dan tanpa karbohidrat, makanan ini dapat dipergunakan dengan baik untuk pertumbuhan larva. Hasil ini mengindikasikan bahwa pengaturan makan dipengaruhi oleh umpan balik metabolik dan juga memperlihatkan bahwa perangsang makan bukanlah faktor utama dalam pengaturan makan. Makanan yang mengandung sukrose terbukti yang terbaik dalam menunjang efieisnsi makanan yang dimakan (ECI) maupun efisiensi makanan yang dicerna dan dikonversikan ke biomassa (ECD). Makanan buatan yang mengandung maltose ternyata merupakan makanan yang terburuk dalam menunjang hampir semua parameter penggunaan makanan, terutama rendahnya nilai ECI dan ECD, yang memperlihatkan besarnya usaha metabolik yang harus dilakukan oleh serangga dalam rangka memproses makanannya. Hasil ini memperihatkan bahwa maltose kemungkinan besar tidak dapat dipergunakan oleh larva untuk menunjang pertumbuhannya*

*Kata kunci:* Spodoptera exempta*, karbohidrat, penggunaan makanan, perangsang makan*

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## Introduction

Many immature phytophagous insects live in a nutritionally heterogeneous environment. They are faced with variations in the amounts and proportions of nutrients (e.g., protein, lipids, carbohydrate, elements and vitamins) in their food that could directly affect their growth and reproduction (Tabashnik and Slansky, 1987). Thus, because growth and development are of primary importance to all animals, insects may respond to such variations in two ways. First, by dietary self-selection (i.e., adjustment of the balance of their intake by selecting among different available foods (Waldbauer and Friedman, 1991). Second, by adjusting the amount of food eaten (Scriber and Slansky, 1981).

Recently, Ahmad *et al.,* (2001) investigated dietary self-selection in the last instar of the Armyworm *Spodoptera exempta* larvae, fed on defined artificial diets. They showed that if *S. exempta* larvae were given the opportunity to self-select between two nutritionally incomplete but complementary diets one which lacked only sucrose (the only digestible carbohydrate) and another one which lacked only casein (the only protein), they were able to self-select. They ate these diets in a mean ratio of 80 % casein: 20 % sucrose. And, when the larvae were provided with a nutritionally complete diet with a casein:sucrose ratio of 80:20, 50:50, or 20:80, they performed best in 80:20 diets, as compared with the 50:50 or 20:80 diets.

The work of previous workers, including Waldbauer *et al.,* 1984 (*Heliothis zea*), Ahmad 1999 (*Manduca sexta*) and Ahmad *et al.,* 2001 (*Spodoptera exempta*) have restricted their studies of dietary self-selection to sucrose as the source of carbohydrate. The reason for this has been the fact that except for sucrose, relatively little is known about the nutritional value of other carbohydrates to lepidopterous larvae. Therefore, it would be of interest to know whether *Spodoptera exempta* larva would thrive when allows to feed on complete defined diets which contained various carbohydrates.

In nature, *Spodoptera exempta* larva is a generalist feeder that feeds on various wild and cultivated Graminae (Kalshoven 1981). Generally, these plants may contain different sugar and carbohydrates, among others, starch, glucose, sucrose and fructose (Creech, 1968)

To support this study, Kamal (1999) tested several carbohydrates for their ability to stimulate last instar larvae of *Spodoptera exempta* to bite, he showed that sucrose, fructose, glucose, and maltose strongly stimulated biting, while mannitol did not stimulate biting. His findings and previously by Schiff *et al*., 1989, which showed that mannitol is nutritive but not phagostimulatory in *Heliothis zea,* open the way to study the effects of feeding on diet which contained carbohydrate which is phagostimulatory but not nutritious and not phagostimulatory but nutritious.

The present study was conducted to determine the nutritional value of several carbohydrates to support the growth and food utilization of the last instar *Spodoptera exempta*, as well as providing support to the hypothesis that feeding regulation is based in metabolic feedback and that of chemosensory may play an important but secondary role (Cohen *et al.,* 1987).

**Materials and Methods**

Larvae of *S. exempta*, were obtained from a laboratory colony maintained at our Laboratory, Department Biology Institut Teknologi Bandung. The animals were reared on their natural diet, the mustard green, *Brassica juncea* (L.). Methods used to rear larvae and adults were essentially as described by Patana (1985). All animals were kept at 24-25$^{o}$ C, 70 % RH and 12 h light:12 h dark cycle.

The diets used in all experiments were based on the defined diet developed by Ahmad *et al.,* (1989), which contained a 48:52 protein (casein and ovalbumin):carbohydrate (sucrose) ratio. This basic ratio as well as the source of carbohydrate for the complete diet was modified in accordance with the designated experiments. Except where noted, all diets contained similar concentrations of casein, vitamins, minerals, lipids, salts, agar, cellulose powder and antibiotic substances.

The experiments were performed in circular arenas; 17-cm diameter petri dishes lined with water-saturated Whatman no. 1 filter paper. One last instar larva per arena with diet of known quantity was placed in the arena on a small strip of aluminum foil. This experiment included six groups of larvae that received complete diets, with 80:20 protein:carbohydrate ratio. Each group containing the same source of protein (casein) but different source of carbohydrates (i.e., sucrose, glucose, fructose, mannitol, and maltose) and a control group (no carbohydrate, only protein). Sucrose, glucose, fructose, and maltose are known for their ability to stimulate biting (phagostimulatory), whereas mannitol is known to be tasteless for *S.exempta* (Kamal, 1999). The experiment began with newly molted, unfed, 5th-instar larvae and ended when these insect had become pharate pupae

The experiments were run in a laboratory room at 24 $\pm$ 2 $^{o}$ C under a 12:12 light: dark photoperiod. The RH within the arenas was approximately 90 %. The arenas were checked every 12-h for larvae that had stopped feeding and then were transferred for pupation to a plastic container filled with moist sawdust.

*Food consumption and growth*

The gravimetric method described by Waldbauer (1968) was used to determine food consumption and growth parameters of all experiments. The initial mean dry matter of larvae was estimated by weighing and then killing ten caterpillars from the group used in an experiment, oven-drying them at 60$^{o}$ C for 6 days, and re-weighing them. Thus, the initial dry weight of each larva was calculated from its fresh weight and the mean percent dry matter of an aliquot of similar larvae. The initial dry weights of diet(s) were measured by taking ten aliquots of each diet and oven-drying them to constant weight to establish the average percent dry weight of the diet. The dry weights fed to the larvae were determined by multiplying the fresh weight of fed diet(s) by this constant

*Nutritional indices*

Nutritional indices are calculated according to Waldbauer (1968) and Scriber and Slansky (1981):

CR = consumption rate
GR = growth rate
AD = approximate digestibility
ECD = efficiency of conversion of digested food to biomass
ECI = efficiency of conversion of ingested food to biomass

These indices are calculated on a dry weight basis as follows:

$$CR = \frac{\text{Wt. food eaten}}{\text{duration of exp. (days)}}$$

$$GR = \frac{\text{Wt. Gain}}{\text{duration of exp. (days)}}$$

$$ECI = \frac{\text{Wt. gain}}{\text{Wt. food eaten}} \times 100$$

$$AD = \frac{\text{Wt. food eaten - wt. Feces}}{\text{wt. food eaten}} \times 100$$

$$ECD = \frac{\text{Wt. gain}}{\text{Wt. food eaten - wt. feces}} \times 100$$

Weight gain was calculated by subtracting the final dry weight of the pupa from the initial dry weight of the larva; mean weight of an insect during the feeding period was calculated to be one-half the sum of its initial and final weights (Waldbauer, 1968).

The data were analyzed using ANOVA followed by Duncan test (Zar, 1984).

## Results and discussion

The Table 1. Shows the food consumption and utilization parameters of fifth instar *S. exempta* larvae on complete defined diets containing casein and one of the carbohydrates (*i.e.,* sucrose, glucose, fructose, mannitol, or maltose).

*Food consumption, growth rate and weigh gain*

In general, the results show that different carbohydrate content of the diets led to an either increase or decrease in the amount of food eaten. The food eaten were similar when larvae were fed the casein-sucrose, casein-fructose, or casein-maltose diets, on these diets they ate significantly more food than when fed the other diets.

The insects ate the least amount of diet on no carbohydrate diet (control)

The ability of various carbohydrates *i.e.,* sucrose, glucose, fructose and maltose to stimulate feeding has been studied in several lepidopterous larvae, including *S. littoralis* (Meisner *et al*., 1972), *Heliothis zea* (Schiff *et al.* 1989), and *S. exempta* (Kamal, 1999). Therefore, considering the levels of sucrose, glucose, fructose and maltose given in the experiment (16 g/liter water) , it is likely that the larvae were able to taste the carbohydrate in the complete diet, thus increasing the amount of food eaten significantly as compared to those larvae feeding on complete diet containing non phagostimulatory carbohydrate (mannitol) or control (without carbohydrate).

Following with the results reported by Kamal (1999) which showed that mannitol was not a feeding stimulant for *S. exempta*, one might expect a lower food consumption in the complete diet which contained mannitol as compared to the diet which contained feeding stimulant (glucose). Unfortunately, this is not true, since there was no significant difference in the amount of food eaten between larvae feeding on casein-glucose and casein-mannitol diets. This finding indicates that the larvae regulate their intake of food upon the levels of both protein and carbohydrate in the diet regardless whether the carbohydrate being eaten is phagostimulatory or tasteless. With this finding, we have supported the malaise hypothesis (Cohen *et al.*, 1987) which proposes that dietary feeding is primarily based on metabolic feed back from ingested nutrient rather than on the innate abilities of the insect to taste the food being eaten; Had the larvae relied only upon the taste of the food, they would have eaten more food on casein-glucose diets.

The importance of the carbohydrate source on growth of the larvae is made apparent by comparison of the instar duration, GR's and weigh gains on all treatments. Both GR and weight gain of larvae fed on diet casein-sucrose was significantly higher than for larvae fed on the other diets. In particular weigh gain and GR were reduced to a greater degree for the larvae fed on casein-maltose diet, which is similar to that of controls. This finding suggests that maltose can not be used by the larvae as the source of utilizable carbohydrate to support their growth.

Even tough instar duration, GR and weight gains were significantly affected by the carbohydrate content of the diet (see Table 1). It was apparent from the experiments that the larvae could survive on a defined diet that contains only protein (casein) and lacks carbohydrate (control) , or mannitol (probably non utilizable carbohydrate). The results, in general, indicate that they do not have an absolute requirement for dietary carbohydrate. Nonetheless, our finding suggest that several carbohydrates i.e., sucrose, glucose, fructose and mannitol, which might not be essential nutritionally, did make possible optimal growth.

As expected, larvae that fed on the casein-sucrose diets were superior to all others in overall food utilization (ECI) and the efficiency of conversion of digested food to biomass (ECD), although digestibility (AD) was lower than that of the larvae that fed on casein-maltose diets, but similar to those larvae that fed on casein-glucose, casein-fructose, and casein-mannitol diets.

Considering the ECI and ECD, the values show that last instar larvae of *S. exempta* grow to pupation equally well but variably when fed on a single diets containing casein-sucrose, casein-glucose, casein-fructose, and casein-mannitol, but rather poorly when fed on a casein-maltose or carbohydrate free diets.

The maltose and carbohydrate free diets were inferior in almost all respect as compared to the complete diets containing sucrose, glucose, fructose, and mannitol. It is probable that the poor performance of the last instar *S. exempta* on the maltose and carbohydrate free diets, on which growth rate and nutritional indices were almost all significantly lower than on the other diets is due to lacks of utilizable carbohydrates.

The finding that the ECD of maltose diet is significantly lower than that of carbohydrate free diet, suggest that not only maltose can not be utilized for energy by the insect's metabolic pathways, but also indicates that handling maltose entails an additional metabolic cost. However, it is the fact that the AD is significantly higher on maltose diet than on the rest of the diets. Suggests that the insects did not have any problem in digesting the maltose . However, excess mannitol digestion does not lead to increased mass, and , in fact, there is a measurable metabolic cost (therefore, decreased ECD) associated with its catabolism and excretion.

In conclusion, our studies show that sucrose, glucose, fructose, and mannitol are carbohydrates that are nutritionally utilizable by *S. exempta* larvae. Whereas maltose, albeit its ability to stimulate feeding, is apparently a non-utilizable carbohydrate for *S. exempta* larvae. Our studies also show that complete diet containing mannitol a tasteless carbohydrate was almost equally eaten and well utilized by *S.exempta* larvae, which suggest that feeding regulation is influenced by metabolic feedback and that taste or feeding stimulant is not a primary factor in feeding regulation.

Table 1. Mean dry weight food consumption and utilization parameters of last instar *Spodoptera exempta* larvae on complete diet containing 64 g protein (casein) and 16 g carbohydrate (either, sucrose, glucose, fructose, mannitol, or maltose) per liter water or an otherwise identical diet lacking carbohydrate (control)

| | Sucrose | Glucose | Fructose | Mannitol | Maltose | Control |
|---|---|---|---|---|---|---|
| Amount eaten (mg) | $68.06^{a} \pm 2.93$ | $58.81^{b} \pm 2.52$ | $70.77^{a} \pm 3.02$ | $54.65^{bc} \pm 2.31$ | $73.83^{a} \pm 3.63$ | $48.81^{c} \pm 2.29$ |
| Weight gain (mg) | $16.49^{a} \pm 1.19$ | $11.16^{b} \pm 0.70$ | $13.30^{b} \pm 0.92$ | $11.16^{b} \pm 0.65$ | $8.29^{c} \pm 0.35$ | $6.06^{c} \pm 0.33$ |
| Instar duration (d) | $2.8^{a} \pm 0.02$ | $3.2^{b} \pm 0.04$ | $3.03^{b} \pm 0.04$ | $3.55^{c} \pm 0.12$ | $3.78^{d} \pm 0.08$ | $3.98^{e} \pm 0.03$ |
| CR (mg/d) | $24.15^{a} \pm 1.12$ | $18.47^{cd} \pm 0.86$ | $23.49^{a} \pm 1.16$ | $16.09^{d} \pm 0.48$ | $19.69^{d} \pm 1.08$ | $12.50^{e} \pm 0.57$ |
| GR (mg/d) | $5.86^{a} \pm 0.44$ | $3.51^{c} \pm 0.24$ | $4.40^{b} \pm 0.31$ | $3.22^{c} \pm 0.23$ | $2.21^{d} \pm 0.11$ | $1.53^{d} \pm 0.09$ |
| ECD (%) | $51.25^{a} \pm 3.33$ | $39.94^{b} \pm 2.70$ | $39.64^{b} \pm 2.88$ | $39.40^{b} \pm 2.63$ | $22.20^{d} \pm 2.78$ | $31.09^{c} \pm 1.50$ |
| ECI (%) | $24.31^{a} \pm 1.60$ | $19.04^{b} \pm 0.91$ | $18.99^{b} \pm 1.28$ | $20.12^{b} \pm 1.49$ | $11.77^{c} \pm 1.45$ | $12.54^{c} \pm 0.59$ |
| AD (%) | $47.86^{b} \pm 2.08$ | $48.93^{b} \pm 1.78$ | $48.31^{b} \pm 1.56$ | $51.48^{b} \pm 2.00$ | $57.38^{a} \pm 2.77$ | $40.52^{c} \pm 1.40$ |

N = 15 per treatment. All values are Means $\pm$ SE. Means within a row followed by the same superscript are not significantly different (ANOVA followed by Duncan's test, $p<0.05$).

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