Jurnal Perlindungan Tanaman Indonesia, Vol. 7, No. 2, 2001: 81–92.

DIETARY COMPENSATORY FEEDING IN *MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE) LARVAE

KOMPENSASI MAKAN PADA LARVA MANDUCA SEXTA (LEPIDOPERA: SPHINGIDAE)

Intan Ahmad
Department of Biology, Institut Teknologi Bandung
E-Mail: intan@itb.ac.id

INTISARI

Penelitian ini bertujuan untuk memperoleh informasi lebih lanjut tentang sifat dan faktor pengendali dari kemampuan melakukan kompensasi makan, dalam hal ini ingin diketahui apakah salah satu sumber bahan makanan merupakan faktor penentu yang membuat serangga melakukan kompensasi makan. Untuk itu telah diukur respons larva instar IV Manduca sexta *terhadap bahan makanan protein atau karbohidrat yang dikurangi konsentrasinya. Dalam penelitian ini, rasio protein dan karbohidrat yang dipilih beserta konsumsi makan dan efisiensi penggunaannya adalah parameter-parameter yang diukur.*

Larva instar empat diberi kesempatan untuk melakukan seleksi makanan dari dua paket makanan, salah satu paket makanan mengandung konsentrasi protein yang berbeda diantara perlakuan, sedangkan yang lainnya mengandung jumlah karbohidrat standar dan tidak berbeda kandungannya antara satu dengan perlakuan lainnya.

Sebagai respons terhadap pengurangan konsentrasi protein, larva melakukan kompensasi dengan memakan lebih banyak makanan yang mengandung baik protein maupun karbohidrat. Pada umumnya, kompensasi yang dilakukan cukup untuk mempertahankan pertumbuhan yang normal, walau nilai-nilai AD, ECI dan ECD semuanya menurun sesuai dengan pengurangan konsentrasi protein dalam makanannya.

Sewaktu larva melakukan pemilihan makanan dari makanan protein standar yang dipasangkan dengan makanan karbohidrat yang mengandung salah satu dari enam konsentrasi sukrosa, larva memberikan respons dengan memakan lebih banyak makanan yang mengandung protein seiring dengan pengurangan konsentrasi sukrosa di makanan yang mengandung karbohidrat. Dari semua perlakuan, pertumbuhan dan efisiensi penggunaan makanan hanya terpengaruh sedikit. Walau pengaturan memakan karbohidrat terindikasikan tidak sekuat pengaturan mengkonsumsi protein; nampaknya, sebagai respons terhadap pengenceran bahan makanan (nutrisi), larva melakukan pemilihan makan pada tiap konsentrasi dengan melakukan rasio pemilihan makan protein:karbohidrat yang paling sesuai untuk mereka.

Kata kunci: Manduca sexta, pemilihan makan, kompensasi makan

ABSTRACT

The aim of the experiments reported in this article is to gain further insight into the nature and control of dietary compensatory feeding: *i.e.*, to investigate whether one or another nutrient is more important in eliciting compensatory behavior. The response of 4th-instar *Manduca sexta* to dilution of either protein or carbohydrate nutrient in the diet was measured. In these experiments, ratio of protein and carbohydrate selected, food consumption and utilization parameters were measured.

Fourth instars were given the opportunity to self-select between two defined diets, one which differed between treatments in protein content and a complementary diet, which contained the standard amount of carbohydrate and did not vary from treatment to treatment.

In response to protein dilution, the larvae compensated by consuming more of both the protein-containing and carbohydrate-containing diets. In most of the cases, this compensation was sufficient to maintain normal growth, although AD, ECI, and ECD declined with the reduction of protein in the protein-containing diet.

When larvae self-selected from a standard protein diet that was paired with a carbohydrate diet with one of six concentrations of sucrose, they responded by eating more from the protein-containing diet as the sucrose content of the carbohydrate diet declined. The growth and efficiency of food use was little affected over the entire range of carbohydrate diets tested. Although apparently the regulation of carbohydrate intake is not as strong as the regulation of protein intake, it seems that in response to dilution, the larvae select at each concentration a protein:carbohydrate ratio that is favorable to them.

Key words: *Manduca sexta*, self-selection, compensatory feeding

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 (Slansky & Scriber, 1985). Thus, because growth and development are of primary importance to all animals, including insects, they 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 & Friedman, 1991)]; and second, by adjusting the amount of food eaten (Scriber & Slansky, 1981). For example, 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 diet, as compared with the 50:50 or 20:80 diets.

The evidence that insects are able to compensate for dilution of a complete diet is extensive. In general, insects respond to dietary dilution by adjusting the amount of food intake. Such studies demonstrate the quantitative feeding behavior of insects but do not shed light on qualitative feeding behavior (requirements for the relative proportions of nutrients). For example, Chyb & Simpson (1990) found compensatory feeding for protein and carbohydrate during the first 15 days of the adult life of a locust. Male and female *Locusta migratoria* were given a choice of two diets, one of which lacked carbohydrate but contained one of a graded series of protein concentrations (10, 20 or 28% protein) and another that lacked protein but contained one of a series of carbohydrate concentrations (10, 20 or 28% carbohydrate). During the first 5 days of adult life, they did not find any evidence of compensatory feeding with respect to variation in the concentrations of either protein or carbohydrate in the two diets. However, after this, the insects compensated by eating more from the diluted diets. As a result, the ratio of protein to carbohydrate eaten remained relatively constant. The authors suggested that this selection behavior indicated a response by the insect to current nutritional requirements.

The evidence that insects of various orders are capable of self-selecting a nutritionally favorable balance of nutrients when the nutrients are separately presented to them suggests some type of post-ingestive feedback loop. Although the actual mechanism(s) that control self-selection are not well understood (not only in insects but also vertebrates), some possible mechanisms have been proposed. For example, Waldbauer and his colleagues (Waldbauer & Friedman, 1991) suggested a "malaise hypothesis". They proposed that when an insect feeds on a nutritionally inadequate diet, there is a metabolic feedback that stimulates the insect to move from the food being eaten and subsequently select a different food source. Furthermore, Friedman *et al*. (1991) proposed that dietary self-selection behavior is ultimately controlled by an interaction between blood sugar and amino acid levels.

While some investigators tend to emphasize the role of metabolic feedback in controlling dietary self-selection behavior (Friedman *et al*., 1991; Ahmad *et al*., 1993) others emphasize the role of sensory stimuli (Abisgold & Simpson, 1988; Simpson & White, 1990). Nevertheless, both metabolic feedback and chemosensory cues are certainly involved.

The aim of the experiments reported in this article was to gain further insight into the nature and control of dietary compensatory feeding: *i.e*., to investigate whether one or another nutrient is more important in eliciting compensatory behavior. The response of 4th-instar *Manduca sexta* to dilution of either protein or carbohydrate nutrient in the diet was measured.

MATERIALS AND METHODS

Experimental insects. All experimental larvae of *Manduca sexta* were obtained from a laboratory colony reared on wheat germ diet. Methods used to rear larvae and adults were essentially as described by Bell & Joachim (1976). Larvae and adults were kept at 26^{o}C, 60–65% RH and under non diapause-inducing conditions (16hL:8hD cycle under fluorescent lighting).

All experiments began with newly molted, unfed, 4th-instars and ended when these insects had become pharate 5th instars. Preceding the experiments, pharate 4th-instar were collected from the colony. They were held individually without food in moist filter paper-lined petri dishes until ecdysis. On the day of ecdysis, they were then weighed and used for the experiments within 6 h.

Artificial defined diets. 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 was modified in accordance with the designated experiments. Except where noted, all diets contained similar concentrations of vitamins, minerals, lipids, salts, agar, cellulose powder and antibiotic substances.

Experimental arenas and conditions. All experiments were performed in circular arenas, with one 4th-instar per arena. Plastic petri dishes (14 cm diameter × 1.5 cm deep) lined with 2 sheets of 12.5 cm water saturated Whatman no. 1 filter paper served as the arenas.

The appropriate diet of known quantity was placed in the arena on a small strip of aluminum foil. The experimental arenas were checked every 8 h to determine the end of feeding period which is shown by the slippage of the head capsule.

To account for any possible effects of uneven illumination and location, the position of diet(s) was altered between arenas. All experiments were run in a laboratory room at 25 $\pm$ 2^{o}C and under a 16:8 L:D photo period. The RH within the arenas was approximately 90%.

The objective of the experiments was to evaluate self-selection by 4th-instar when concentrations of either protein or carbohydrate were varied.

Protein dilution experiment. In this experiment, the carbohydrate diet (the standard sucrose diet of 63% sucrose by dry weight, referred to as S) was paired with one of five protein diets (referred to as P): 4.5%, 9%, 19%, 37% or 63% (% dry weight). The diets were offered in a choice situation to five diets of larvae. The protein-containing diets were adjusted to constant bulk by the addition of cellulose, which is indigestible to *M. sexta* larvae (Timmins *et al*., 1988). The diets in these experiments were placed touching on the edge of the arena and touched to one another.

Carbohydrate dilution experiment. The design of this experiment is the converse of that of protein dilution experiment. The standard protein diet (referred to as P; 63% protein dry weight) was paired with each of six carbohydrate diets (referred to as S) with concentrations of sucrose: 0.0 M, 0.001 M, 0.01 M, 0.1 M, 0.5 M or 1.0 M, and offered in a choice situation to six diets of larvae. The concentration of sucrose in the carbohydrate diet was estimated from the amount of sucrose in the bulk of diet (% dry weight excluding cellulose and other dry weight ingredients). Such a calculation produced a corresponding value (sucrose as a percentage of the dry weight of the diet) of 0.0%, 0.3%, 3%, 23%, 60% or 75%. All diets contained similar amounts of cellulose.

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 an aliquot of ten caterpillars from the diet 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 fed 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. Amount of food eaten was calculated by subtracting the dry weight of the uneaten portion from the estimated dry weight of fed diet. Upon completion of the experiments, experimental larvae were frozen and then dried in the oven to determine final weight.

Nutritional indices. Nutritional indices were calculated according to Waldbauer (1968) as modified by Scriber & Slansky (1981). The indices are:

CR = Consumption rate (mg/day)
GR = Growth rate (mg/day)
AD = Approximate digestibility (%)

ECD = Efficiency of conversion of digested food to biomass (%)
ECI = Efficiency of conversion of ingested food to biomass (%)

RESULTS

Protein dilution experiment. There was no mortality among larvae self-selecting from diet choices: 63% P + S, 37% P + S or 19% P + S. However, as the concentration of protein was further reduced to 9.0% and 4.5%, mortality occurred. There was 33% mortality in the 9% P + S diet and 100% mortality in the 4.5% P + S diet. Diet 4.5% P + S as well as the larvae that were dead in the 9% P + S diets were not included in the statistical analysis. If survival of the insects on a diet can be used as one of the indications of the nutritional adequacy of the diet in question, the results indicate that the 19% P + S, 37% P + S and 63% P + S diets are probably nutritionally adequate for the larvae to satisfy their nutritional needs by the means of self-selection. Mean feeding duration varied significantly among the four diets (Table 2). Mean feeding periods varied from 70.2 h on the 63% P + S diet to 107.6 h on the 9% P + S diet, which had the lowest protein content on which survival was possible.

In response to protein dilution in the protein diet, the larvae compensated by consuming more of both the protein and sucrose containing-diets. The amount of food eaten was such that more protein than sucrose diet was always selected (Table 1). However, comparing the ratios of protein:sucrose diets selected among treatments with ANOVA showed that the mean ratios were not significantly different (Table 1).

Although the larvae appeared to be trying to ensure themselves of an adequate intake of protein (dry weight, excluding cellulose and other dry diet ingredients), as shown by a progressive increase in the consumption rate (CR) with decreasing protein concentration, the compensation was not complete. Thus, when larvae self-selected from diet pairs with a 37 % protein level or less, a high proportion of sucrose to protein was always selected. This contrasts sharply with the 63% P + S diet, in which intake of protein was higher than that of sucrose. When larvae self-selected from 37% P + S diets, their consumption rate (CR) was significantly higher than that of larvae fed on 63% P + S diets. Being 1.4 time higher, this CR was apparently sufficient to maintain enough protein and sucrose intake. As a result they grew at similar rates. (Table 2). When the protein content in the protein diet was further reduced to 19% (19% P + S diet), they increased their CR to the highest level. However, despite this, the larvae actually had a reduced protein intake because most of their intake came from the sucrose rather than the protein. As a result, they grew at a lower rate than those of the 37% P + S and 63% P + S diets. Larvae fed on the 9% P + S diet did not increase their food consumption as the 19% P + S larvae did. Their CR was not significantly greater than that of either the 37% P + S or 63% P + S diets. As a result their growth rate was severely reduced.

All nutritional indices showed significant differences among treatments (Table 2). The values of AD, ECD and ECI all declined steadily with decreasing protein content and at the same time increasing cellulose content in the diet choices.

Table 1. Ratio of protein and carbohydrate selected (dry weight of diet and dry weight of nutrient) by 4th-instar *Manduca sexta*

	Concentration of protein in the protein diet (% d.w)			
	9 (10)	19 (14)	37 (15)	63 (15)
Protein:carbohydrate (diets) Average Ratio	64:36	62:38	56:44	63:37

Note: The larvae were given a choice of two diets; a protein diet with one of four concentrations and a standard carbohydrate diet (63% by d.w). Average ratios sharing a common underlining do not differ significantly (Log. transformation followed by ANOVA and Tukey's HSD test, $p < 0.05$). Numbers in parentheses are the number of larvae in each treatment.

Table 2. Food consumption and utilization parameters of 4th-instar *Manduca sexta* self-selecting from a protein diet consisting one of four concentrations of protein (% d.w.) paired with a standard sucrose diet. Abbreviations: S = Carbohydrate (sucrose) diet; P = protein diet (%).

Dietary treatment	N	Weight gain (mg)	Duration (h)	GR (mg/day)	CR (mg/day)	ECI (%)	AD (%)	ECD (%)
9% P + S	10	50 ± 5^{b}	107.60 ± 3.38^{a}	111 ± 9^{c}	166 ± 10^{bc}	6.69 ± 0.35^{d}	26.73 ± 1.70^{c}	26.64 ± 3.14^{d}
19% P + S	14	127 ± 4^{a}	90.00 ± 2.59^{b}	343 ± 2^{b}	283 ± 12^{a}	12.18 ± 0.26^{c}	30.21 ± 0.71^{c}	40.80 ± 1.66^{c}
37% P + S	15	131 ± 5^{a}	79.87 ± 4.13^{c}	404 ± 2^{a}	198 ± 13^{b}	20.86 ± 0.86^{b}	41.62 ± 1.36^{b}	50.87 ± 2.59^{b}
63% P + S	15	124 ± 4^{a}	70.20 ± 2.98^{c}	430 ± 2^{a}	145 ± 6^{c}	30.11 ± 0.69^{a}	52.54 ± 1.23^{a}	57.76 ± 1.87^{a}

Note: Five larvae died on 9 % P + S diet, but no mortality was found in other treatments. All values are mean ± SE. Means within a column followed by the same superscripts are not significantly different (ANOVA followed by Tukey's HSD test, $p < 0.05$).

Among the four diets of larvae, the nutritional indices were highest in larvae self-selecting from diet pairs which contained the highest concentration of protein (63% P + S diet) and lowest in the larvae fed on the 9% P + S diet.

Carbohydrate dilution experiment. In the face of decreasing concentrations of sucrose in the carbohydrate diet, the value of consumption rate (CR) showed no significant differences among treatments. When sucrose was present in the second diet, the proportion of food selected from each of the two diets varied considerably, depending upon the concentration of sucrose in the sucrose diet. These results clearly show that 4th-instar were able to self-select for protein and carbohydrate adequately from a choice of two defined diets, one which contained the same protein content from treatment to treatment and a complementary diet, which differed between treatments in sucrose content. As the concentration of sucrose was increasingly diluted, the larvae fed selectively by increasing protein intake and at the same time reducing the sucrose intake (Table 3).

While CR did not differ among treatments, the ratio of protein to carbohydrate diets eaten varied as the sucrose diet was increasingly diluted. It was close to 50:50 in larvae self-selecting from diet pairs with the highest concentration of sucrose (P + 75% S diet) but rose significantly to about 80:20 in the diets fed either from P + 0.0% S, P + 0.3% S or P + 3% S diets (Table 3). Neither CR nor GR changed as the sucrose content of the carbohydrate diet was reduced (Table 4); nevertheless, weight gain was significantly lower in larvae fed on diets with little or no sucrose (P + 0.0% S, P + 0.3 % S and P + 3% S diets) than in larvae fed on either the P + 60% S or P + 75% S diets (Table 4).

The value of AD, did not differ significantly between P + 60% S and P + 75% S diets. But, as the sucrose content was further reduced to 23% and below, a significant decrease in AD was observed (Table 4). Significant differences in the values of ECD among treatments were observed, with trends down on both sides of P + 3% S and P + 23% S. The highest ECD values were observed in larvae fed on P + 3% S and P + 23% S diets; nonetheless these were significantly higher only than values obtained in the larvae self-selecting from P + 60% S diets. ECI was higher for larvae fed on P + 23% S, P + 60% S and P + 75% S diets than for larvae fed on other combinations. However, the only significant difference in ECI values was between the highest value of ECI, in the P + 75% S diet, and the P + 0.0% S, P + 0.3 S and P + 3% S diets.

Table 3. Ratio of protein and carbohydrate selected (dry weight of diet and dry weight of nutrient) by 4th-instar *Manduca sexta*

	Concentration of sucrose in the carbohydrate diet (% d.w)					
	0.0 (16)	0.3 (16)	3.0 (16)	23 (16)	60 (16)	75 (16)
Protein:carbohydrate (diets) Average Ratio	86:14	87:13	80:20	71:29	63:37	57:43
	______	______	______	______		
			______	______	______	
					______	______

Note: The larvae were given a choice of two diets: a standard protein diet (63% d.w) and a sucrose diet with one of six concentrations. Average ratios sharing a common underlining do not differ significantly (Log. transformation followed by ANOVA and Tukey's HSD test, $p < 0.05$). Numbers in parentheses are the number of larvae in each treatment.

Table 4. Food consumption and utilization parameters of fourth-instar *Manduca sexta* self-selecting from a standard protein diet paired with a carbohydrate diet containing one of six concentrations of sucrose. Abbreviations: P = protein diet; S = Carbohydrate (sucrose) diet (% dw).

Dietary Treatment	N	Weight gain (mg)	Duration (h)	CR (mg/day)	GR (mg/day)	ECI (%)	AD (%)	ECD (%)
P + 0.0 % S	16	91 ± 4^{b}	72.00 ± 1.98^{a}	137 ± 55^{a}	31 ± 2^{a}	22.69 ± 0.73^{b}	40.97 ± 0.73^{b}	55.51 ± 2.57^{ab}
P + 0.3 % S	16	96 ± 2^{ab}	74.00 ± 2.99^{a}	133 ± 69^{a}	32 ± 2^{a}	24.19 ± 0.72^{b}	42.23 ± 0.90^{b}	57.69 ± 2.07^{ab}
P + 3.0 % S	16	93 ± 4^{ab}	67.50 ± 2.06^{a}	142 ± 64^{a}	34 ± 2^{a}	23.89 ± 0.83^{b}	39.04 ± 0.67^{b}	61.26 ± 1.96^{a}
P + 23.0 % S	16	104 ± 3^{ab}	65.50 ± 2.61^{a}	152 ± 9^{a}	39 ± 2^{a}	26.02 ± 0.55^{ab}	42.75 ± 1.13^{b}	61.51 ± 2.13^{a}
P + 60.0 % S	16	105 ± 3^{a}	68.00 ± 2.33^{a}	148 ± 6^{a}	37 ± 1^{a}	25.62 ± 0.86^{ab}	50.58 ± 1.35^{a}	51.30 ± 2.37^{b}
P + 75.0 % S	13	106 ± 19^{a}	69.46 ± 2.95^{a}	139 ± 10^{a}	38 ± 2^{a}	27.97 ± 1.24^{a}	51.60 ± 1.15^{a}	54.64 ± 1.24^{ab}

Note: All values are mean ± SE. Means within a column followed by the same superscript are not significantly different (ANOVA followed by Tukey's HSD test, $p < 0.05$).

DISCUSSION

The results obtained in these two experiments clearly show the ability of *M. sexta* larvae to adapt their self-selection capability to varying concentrations of dietary macronutrients. These experiments also permit the exploration of the interaction of protein and carbohydrate dietary levels in self-selection.

In the protein dilution experiment, as the protein concentration in the protein diet decreased, the larvae increased their consumption rate by eating more of both the protein and carbohydrate diets. This observation suggests that the larvae compose their diet in order to obtain a suitable combination of protein and carbohydrate. When the protein concentration fell to 9% they compensated less well; the intake of protein and carbohydrate were both reduced as compared to the 19% protein diet (Table 2). Although these larvae had the longest feeding period as compared to the other treatments (Table 2), their CR was similar to the standard diet (63% P + S). This suggests that the 9% protein diet was so poor nutritionally (actually five larvae died) that it precluded adequate compensation by these larvae under the conditions of this experiment. As a result their weight gain and GR was severely affected (Table 2).

The fact that these larvae were able to maintain constant weight gain when their protein diet was diluted to 19% (Table 2) suggests that they were able to compensate by increasing CR and adjusting the relative proportions of each diet so as to eat sufficient protein and carbohydrate. As the protein concentration was reduced, they ate more carbohydrate in an apparent attempt to reach the "proper" weight. In the more diluted protein diet (19% d.w.), these larvae needed to both prolong feeding period and increase CR in order to achieve the "proper” weight. Nonetheless, it seems obvious that there are limits to the larval ability to compensate for changing relative availabilities of protein and sucrose.

There have been many reports indicating the importance of dietary protein to insects’ performance and control of feeding (Scriber 1984; Ahmad & Kamal 2001). Compensatory feeding in response to dietary dilution has been seen in a number of other studies, including not only *M. sexta* larvae (Timmins *et al.*, 1988) but also many others species, *i.e.*, *Melanoplus sanguinipes* (McGinnis & Kasting, 1967), *Spodoptera eridania* (Peterson *et al.*,1988), *Anticarsia gemmatalis* (Slansky & Wheeler, 1989; 1991), *Spodoptera frugiperda* (Wheeler & Slansky, 1991). However, most of these studies were conducted in no-choice experiments. For example, Timmins *et al*. (1988) demonstrated that dilution of 5th instar *M. sexta* artificial diet (wheat germ diet) with various degrees of cellulose led to an increase in consumption rate. However, in most of the cases, this compensation was insufficient to maintain normal growth. For example, when the diet was diluted by one-half (50% cellulose), the insects significantly increased their CR (Consumption Rate) to about 1.5 times that of controls on undiluted diet, but the insects grew at only 74% of the control rate. A further dilution to 1/4 of the control diet (75% cellulose), elicited an increase in CR of about 2.25 times, but worse than before, they grew at a significantly slower rate, 43% of the control rate. When diet concentration was further reduced to 1/10 that of the control diet (90% cellulose), the larvae compensated less well and there was very poor growth.

In contrast with the result of the protein dilution experiment, consumption rate was unaffected in the carbohydrate dilution experiment. As well, Abisgold & Simpson (1985) showed no compensation for carbohydrate dilution.

The ability of sucrose to stimulate biting has been studied in a large number of insects (Ahmad & Kamal 2001), including *M. sexta* larvae (Städler & Hanson, 1978). For example, Städler & Hanson (1978), using feeding discrimination studies showed that sucrose is stimulatory to fifth-instar *M.sexta* at a concentration from 0.003 M to at least 0.1 M. Thus, considering the levels of sucrose given in the carbohydrate dilution experiment, it is unlikely that larvae feeding on the most diluted carbohydrate diets [(P + 0.0% S (0.0 M sucrose) and P + 0.3% S (0.001 M sucrose) diets] were able to taste the sucrose in the diet.

The finding that the protein: carbohydrate ratio selected by P + 75% S larvae was significantly different from the ratio selected by P + 23% S larvae (Table 3), suggests that the levels of sucrose in the diet could influence or modify the establishments of dietary self-selection. However, the observation that there were no significant differences in the ratio of protein to carbohydrate diets selected among the P + 0.0% S, P + 0.3% S, P + 3% S and P + 23% S diets (Table 3) indicated that taste of sucrose alone was not the sole determinant in dietary self-selection.

These studies suggest that *M. sexta* larvae regulate their intake of food based upon the levels of both protein and carbohydrate in the diet. Regulation for protein seems to be stronger than that for carbohydrate, the disappearance of carbohydrate being compensated for by increasing the protein level. This regulation apparently works to maintain appropriate quantities of protein and carbohydrate. The results, in general, indicate that protein is more limiting than carbohydrate to *M. sexta* larvae.

Actually 4th-instar *M. sexta* feeding on the P + 0% S diet (Table 4) and final-instar *H. zea* larvae (Waldbauer *et al.*, 1984) can survive on a defined diet that contains only protein and lacks carbohydrate, indicating that they do not have an absolute requirement for dietary carbohydrate.

The efficiency of food use. As expected, the AD of self-selecting larvae in the protein dilution experiment decreased significantly as the cellulose content of the protein-containing diet was increased. Decreased digestibility is expected, because cellulose is indigestible by Lepidoptera larvae in general and to *M. sexta* larvae in particular (Timmins *et al.*, 1988). Reduced AD as a function of increased cellulose in artificial diets has been reported by several other authors (Slansky & Wheeler, 1991; Wheeler & Slansky, 1991).

In the protein dilution experiment the ECD declined as the amount of protein in the protein-containing diet was reduced. This decrease in ECD was particularly noticeable when the larvae self-selected from 19% P + S and 9% P + S diets. These reduced ECD values may reflect higher metabolic feeding costs resulting from the greater intake of the low-protein diets. The value of ECD decreased as AD decreased, suggesting that a greater proportion of the assimilated food was used for energy metabolism rather than converted into insect biomass. This was expressed earlier by Timmins *et al.* (1988), who found a similar relationship between reduced food quality and low ECD with 5th-instar *M. sexta* larvae given a single wheat germ diet diluted with cellulose. They suggested that ECD was low because the poor quality diet required that a greater amount of time and energy be devoted to feeding. Although the factors leading to increased metabolic costs are unknown in this experiment, it is certain that the decline in ECI simultaneously with the increasing concentration of cellulose in the protein-containing diet was due to the combination of reduced AD and ECD.

Similar to the results of the protein dilution experiment, reducing the sucrose content of the carbohydrate-containing diet elicited a decrease in AD (Table 4). The reason for reduced AD on larvae self-selecting from either P + 23% S, P + 3% S, P + 0.3% S or P + 0.00% S diets is most likely due to the reduced content of easily digested sucrose. The finding that there is no compensatory feeding no doubt accounts for the fact that the decrease in AD was much less than in the variable protein experiment.

In general, the larvae were able to obtain sufficient nutrients and maintain a similar growth rate over the entire range of carbohydrate diet tested. The larvae compensated for reduced carbohydrate by eating more from the protein-containing diet. The fact that CR (Consumption Rate) was similarly high in all treatments (Table 4), suggested that the larvae did not experience reduced food quality. Likewise, the values of AD and ECD were little affected, indicating that a greater proportion of assimilated food was converted into insect biomass. The ECI, an index of overall food utilization, being the product of AD and ECD, generated similar trends to the values of AD and ECD found in this experiment. This finding suggests that carbohydrate is not necessarily a required nutrient for 4th-instar *M. sexta* larvae.

ACKNOWLEDGMENTS

I wish to thank my former advisors Dr. G.P. Waldbauer and Dr. S. Friedman for their valuable suggestions and discussions on the manuscript, and to Dr. Carl Bouton for his helpful comments.

LITERATURE CITED

Abisgold, J.D., & S.J. Simpson. 1987. The Physiology of Compensation by Locusts for Changes in Dietary Protein. *J. Exp. Biol.* 129: 329–346.

Abisgold, J.D. & S.J. Simpson. 1988. The Effect of Dietary Protein Levels and Haemolymph Composition on the Sensitivity of the Maxillary Palp Chemoreceptors of Locusts. *J. Exp. Biol.* 135: 215–229.

Ahmad, I., G.P. Waldbauer & S. Friedman. 1989. A Defined Artificial Diet for the Larvae of *Manduca sexta*. *Ent. exp. & Appl.* 53: 189–191.

Ahmad, I., G.P. Waldbauer & S. Friedman. 1993. Maxillectomy Does Not Disrupt Self-Selection by Larvae of *Manduca sexta* (Lepidoptera: Sphingidae) Ann. Entomol. Soc. Am. 86 (4): 458–463

Ahmad, I., S. Hariyadi & T. Anggraeni. 2001. Nutrient Self Selection by the Armyworm, *Spodoptera exempta* WALKER (Lepidoptera: Noctuidae) Larvae. *Pakistan Journal of Biological Sciences 4(6): 684-687.*

Ahmad, I. & M. Kamal. 2001. Consumption and Utilization of Complete Defined Diets Containing Various Carbohydrates by *Spodoptera exempta* (Lepidoptera: Noctuidae). Submitted to *Biota.*

Bell, R.A. & F.G. Joachim. 1976. Techniques for Rearing Laboratory Colonies of Tobacco Hornworms and Pink Bolworms. *Ann. ent. Soc. Am.* 69: 365–363.

Chyb, S. & S.J. Simpson. 1990. Dietary Selection in Adult *Locusta migratoria. Ent. exp. & appl.* 56: 47–60.

Friedman, S., G.P. Waldbauer, J.E Eermoed, M. Naeem & A.W. Ghent. 1991. Blood Trehalose Levels Have a Role in the Control of Dietary Self-Selection by *Heliothis zea* Larvae. *J. Insect. Physiol.* 37(12): 919–928.

Gelperin, A. & V.G. Dethier. 1967. Long Term Regulation of Sugar Intake by the Blowfly. *Physiol. Zool.* 40: 218–228.

Martin, M.M. 1983. Cellulose Digestion in Insects. *Comp. Biochem. Physiol.* 75A:

313–324.

McGinnis, A.J. & R. Kasting. 1967. Dietary Cellulose Effect on Food Consumption and Growth of a Grasshopper. *Can. J. Zool.* 45: 365–367.

Peterson, S.S., J.M. Scriber & J.G. Coors. 1988. Silica, Cellulose and their Interactive Effects on the Feeding Performance of the Southern Armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). *J. Kansas. Ent. Soc.* 61: 169–177.

Scriber, J.M. 1984. Host Plant Suitability, p. 159–202, *In* W.J. Bell & R.T. Carde (eds.), *Chemical Ecology of Insects*. Chapman & Hall, London.

Scriber, J.M. & F. Slansky Jr. 1981. The Nutritional Ecology of Immature Insects. *Ann. Rev. Entomol.* 26: 183–211.

Slansky, F.Jr. & G.S. Wheeler. 1989 Compensatory Increases in Food Consumption and Utilization Efficiencies by Velvetbean Caterpillars Mitigate Impact of Diluted Diets on Growth. *Ent. exp. & Appl.* 51: 175–187.

Slansky, F. Jr. & G.S. Wheeler. 1991. Food Consumption and Utilization Responses to Dietary Dilution with Cellulose and Water by Velvetbean Caterpillars, *Anticarsia gemmatalis. Physiol. Ent.* 16: 99–116.

Städler, E. & F.E. Hanson. 1978. Food Discrimination and Induction of Preference for Artificial Diets in the Tobacco Hornworm, *Manduca sexta*. *Physiol. Entomol.* 3: 121–133.

Timmins, W.K., A.J. Bellward, J. Stamp & S.J. Reynolds. 1988. Food Intake, Conversion Efficiency, and Feeding Behaviour of Tobacco Hornworm Caterpillars Given Artificial Diet of Varying Nutrient and Water Content. *Physiol. Ent.* 13: 303–314.

Waldbauer, G.P. 1968. The Consumption and Utilization of Food by Insects. *Advan. Insect Physiol.* 5: 229–288.

Waldbauer, G.P. & S. Friedman. 1991. Self-Selection of Optimal Diets by Insects. *Ann. Rev. Entomol.* 36: 43–63.

Waldbauer, G.P., R.W. Cohen & S. Friedman. 1984. Self-Selection of and Optimal Nutrient Mix from Defined Diets by Larvae of the Corn Earworm, *Heliothis zea* (Boddie). *Physiol. Zool.* 57: 590–597.

Wheeler, G.P. & F. Slansky, Jr. 1991. Compensatory Responses of the Fall Armyworm (*Spodoptera frugiperda*) when Fed Water and Cellulose-Diluted Diets. *Physiol. Entomol.* 16: 361–374.