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Nutrient Self Selection by the Armyworm, *Spodoptera exempta* WALKER (Lepidoptera: Noctuidae) Larvae

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Abstract: Last instar larvae of the armyworm, *Spodoptera exempta* WALKER were given the opportunity to self-select from two defined diets, both complete except that one contained protein (casein) but no digestible carbohydrate (sucrose) and the other contained carbohydrate but no protein. The larvae ate the protein and carbohydrate diets in a ratio of 80:20. In general, the growth and efficiency of food use of the larvae were not significantly different between the self-selectors and that of the controls. When the larvae were provided with a nutritionally complete diet with a protein:carbohydrate (casein:sucrose) ratio of 80:20, 50:50 , or 20:80, they performed best in 80:20 diet, "the self-selected ratio" as compared with the 50:50 diet. The larvae grew very poorly in 20:80 diet, with all nutritional indices significantly inferior as compared to those of 80:20, or 50:50 diets.

Key words: *Spodoptera exempta*, self selection, feeding behaviour

Introduction

Some animals are known to eat two or more foods in proportions that yield a more favorable balance of nutrients than will any single food or arbitrary mixture. This behaviour is usually called dietary "self-selection" (Richter *et al*., 1938). Waldbauer and Friedman (1988) restated the definition as the ability of an animal "... to eat two or more foods in proportions that yield a more favorable balance of nutrients than any of these foods alone or an inseparable and arbitrarily selected mixture of them."

The ability of insect to self-select their food in nature is suggested by many reports, e.g. certain predatory insects (Coccinellidae) require more than one species of aphids for their growth and reproduction during one season of the year but not in other (Takeda *et al.*, 1964). Herbivorous insects, such as grasshoppers, have been observed to feed selectively on more than one genus of grasses (Bernays and Barbehenn, 1987).

Despite the fact that certain insects may self-select their diet in nature, until recently, few laboratory investigations have been carried out to study this phenomenon. Self-selection has been observed in several species including the confused flour beetle, *Tribolium confusum* (Waldbauer and Bhattacharya, 1973), the two-striped grasshopper, *Melanoplus bivittatus* (MacFarlane and Thorsteinson, 1980), the corn earworm, *Heliothis zea* (Waldbauer *et al.*, 1984), the brown-banded cockroach, *Supella longipalpa* (Cohen *et al.*, 1987), *Locusta migratoria* (Chyb and Simpson, 1990) and *Ceratitis capitata* (Fernandes-Da-Silva and Zucoloto, 1993; Cangussu and Zucoloto, 1995), *Manduca sexta* (Ahmad, 1999).

Waldbauer and Bhattacharya (1973) published the first demonstration that insects can self-select their diet and obtain benefit from this behaviour. They reported that when *T. confusum* larvae, were given a 1:1:1 mixture of wheat bran, germ and endosperm particles, the larvae fed on the mixture and selected a mean of 81 germs, 17 % endosperm, and 2 % bran. This mixture was utilized more efficiently for growth by self-selecting larvae than any other single fraction fed to other larvae.

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 invertebrates), some possible mechanisms have been proposed by Waldbauer *et al.* (1991) which suggested a "malaise" hypothesis. They proposed that when an insect feeds on a nutritionally inadequate diet, there is metabolic feedback that stimulates the insects to move from the food being eaten and subsequently select a different food source. Further observation by, Friedman *et al.* (1991) and Ahmad *et al.* (1993), suggested that both metabolic feed back and chemosensory cues are involved. Unfortunately, how these two factors interact to govern self-selection is not clear. We conducted a series of studies to further examine the behaviour and possible physiological mechanisms that mediate food choice in insects by examining the ability of an insect with feeding habits different from those of the temperate species that have already been reported to self-select. Here the tropical Armyworm, *Spodoptera exempta,* was tested for its ability to self-select during the 5th instar. In nature the larvae of *S.exempta* feed on various wild and cultivated Graminae. The larvae mostly feed on foliage of paddy, maize and sugar cane (Kalshoven, 1981).

Materials and Methods

Experimental insects: Larvae of *S. exempta*, were obtained from a laboratory colony maintained at Inter University Center for Life Sciences 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). Larvae were kept at 24-25°C, 70 % RH and 12 h light:12 h dark cycle.

All experiments began with newly molted, un-fed, 5th-instar larvae and ended when these insects had become pharate pupae

Artificialy 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, 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. 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 24 ± 2 °C under a 12:12 light:dark photo period. 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.

Experiment I: Self-selection: This experiment was designed to determine the ability of last instar larvae to self-select their food intake from a choice of two defined diets. Larvae were placed individually in arenas containing two blocks of diet, located on diametrically opposite sites of the arena. Experimental larvae were given one diet containing protein (casein) but lacking digestible carbohydrate (sucrose), and a second containing digestible carbohydrate (sucrose) and lacking protein (casein). The missing nutrient in each diet was replaced by the same amount of the other nutrient resulting in a doubling of the concentration of each nutrient compared to the control diet. Control larvae were given two nutritionally complete diets, each with a casein:sucrose ratio of 50:50.

Experiment II: Food consumption and growth rate on diet with **pre-determined Protein:carbohydrate ratio:** This experiment was conducted to determine whether the self-selected ratio of the larvae found in experiment I was, in fact, optimum for meeting the nutritional needs of the larvae. The procedures of this experiment were similar to those of experiment I, except that a single block of diet, placed in the center of the arena, was given to each larva. Four formulations of nutritionally complete diet were prepared by varying the protein:carbohydrate ratio. Four groups of larvae were used and each group was presented a single diet, in a no-choice situation, with a casein:sucrose ratio of 50:50, 80:20 or 20:80.

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 °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 dry weight basis in the usual way (Waldbauer, 1968). 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).

Results

Protein:carbohydrate selection: When last instar *S.exempta* larvae were given the opportunity to self-select between two diets that lacked either only protein (casein) or only carbohydrate (sucrose) , the larvae selected and regulated their casein and sucrose intakes. When food intake through the instar was summed, last instar larvae ate the casein and sucrose diets in the ratio of 80:20 (Table 1) . Control larvae, which were given two identical and nutritionally complete diets tended to eat mostly one or the other of them (either from the "A" or "B" diet).

Self-selecting larvae ate significantly less amounts of food (400 mg) as compared to controls (438 mg) . These findings proved that last instar *S. exempta* larvae were able to self-select

Growth and the efficiency of food use: Compared with the controls, the self-selecting larvae did not differ significantly in all values of Growth and efficiency of food use measured, i.e. Weight gain, Instar period, CR (Consumption rate), GR (Growth rate) , AD (approximate digestibility), ECI (efficiency of conversion of ingested food) and ECD (efficiency of conversion of digested food into biomass (Table 2).

Food consumption and growth rate on diet with pre-determined Protein:carbohydrate ratio: Table 3 shows the food consumption and utilization parameters of 5th-instar *S. exempta* larvae on diets containing casein and sucrose in various ratios. When larvae were fed the 80:20 diet, the protein:carbohydrate (casein:sucrose) ratio self-selected by the larvae, they ate significantly less food as compared to those feeding on 50:50 diet, but similar amount of food to those feeding on 20:80 diet. The weight gain, CR, GR and ECI were not significantly different between the 80:20 and 50:50 diets, but these values were significantly higher than those larvae fed on 20:80 diet.

On 80:20 and 50:50 diets, weight gain reached an average of 70.5 mg within 8 days, whereas the 20:80 diet gave significantly less weight gain (54 mg), which is about 23 % lower than that of 80:20 and 50:50 diets. The time needed to reach pupation was also longer i.e. 10 days, which make the growth rate 40 % lower than other two diets.

The value of ECD was 45 % for 80:20 diets, which is higher than the other two diets. The AD value that is correlated with ECD, showed that in order to get a better GR the larvae only needed to have lower AD (39%) as compared to other two diets. The ECI which is the efficiency of conversion of ingested food to biomass was significantly higher on 80:20 and 50:50 diet as compared to 20:80 diet (Table 3). Based on the values of amount of food eaten and ECD, we have reason to believe that the 80:20 diet was indeed optimum for the insect in question to meet their nutritional requirement.

Discussion

The results of self–selection experiments clearly showed that 5th-instar *Spodoptera exempta* larvae, given the opportunity to self-select between two defined diets differing only in macronutrient content (casein and sucrose), were able to select and regulate their protein (casein) and carbohydrate (sucrose) intake. Such self-selecting larvae achieved rates of growth comparable to those of control larvae fed on a nutritionally complete diet.

Fifth-instar larvae self-selected a mean 80:20 casein:sucrose

Table 1: Food intake of 5th-instar Spodotera exempta larvae when given a choice of either two nutritionally incomplete diet blocks or two nutritionally complete diet blocks.

Larvae offered	Total eaten (mg)	Intake from each diet block (mg)	
		Casein block	Sucrose block
A diet lacking casein and one lacking sucrose (self-selectors)	400±12[a]	320±10(80)	79a±5(20)
		Block A	Block B
Two nutritionally complete diet blcoks (control)	438±7[b]	232±15[b](54)	205±13[b](46)

Note: N = 20 for each treatment. All values are means±SE. Means within a column followed by the same superscript are not significantly different as compared by paired t-tests (P≤0.05). Values in parentheses are percent of intakes.

Table 2: Duration, growth and utilization parameters of the 5th-instar Spodoptera exempta larvae described in Table 1.

Treatment	Instar duration (d.)	Weight gain (mg)	CR (mg/day)	GR (mg/day)	ECI(%)	AD(%)	ECD(%)
Self-selectors	8.24a	69.00a	48.00a	8.00a	17.85a	38.53a	47.65a
	±0.13	±2.00	±1.00	±0.00	±0.93	±1.80	±2.56
Control	8.37a	70.00a	53.00a	8.00a	16.72a	44.02a	42.46a
	±00.23	±2.00	±3.00	±0.00	±0.86	±3.02	±3.77

Note: N = 20 for each treatment. All values are means±SE. Means within a column followed by the same superscripts are not significantly different as compared by paired t-test (p≤0.05).

Table 3: Food consumption and utilization parameters of fifth-instar Spodoptera exempta larvae on diets containing various ratios of casein to sucrose.

Casein: Sucrose Ratio	Total etan (mg)	Weight gain (mg)	Duration (d)	CR (mg/day)	GR (mg/day)	ECI (%)	AD (%)	ECD (%)
80:20	414.00a	71.00a	8.10b	52.00a	9.00a	17.29a	39.38a	44.79a
	±4.00	±1.00	±0.20	±1.00	±0.00	±0.35	±1.11	±1.81
50:50	446.00b	70.00a	8.00b	54.00a	8.00a	15.68a	43.20b	36.45b
	±9.00	±2.00	±0.20	±2.00	±0.00	±0.31	±0.55	±0.95
20:80	393.00a	54.00b	10.70b	37.00b	5.00b	13.71b	50.12c	27.43c
	±6.00	±0.00	±0.20	±0.00	±0.00	±0.17	±0.76	±0.45

Note: N = 20 for each treatment. All values are means±SE. Means within a column followed by the same superscript are not significantly different (ANOVA followed by SNK test, p≤0.05).

ratio. The finding that *S. exempta* larvae eat more protein than carbohydrate is consistent with the results of previous studies that involved different species of lepidopterous larvae such as *Heliothis zea* (Waldbauer *et al.*, 1984) and *Spodoptera littoralis* (Simpson *et al.*, 1988). However, immature insects from other Orders, a locust, *Locusta migratoria* (Simpson *et al.*, 1988) and a cockroach, *Supella longipalpa* (Cohen *et al.*, 1987), self-selected much less protein and more carbohydrate, with protein:carbohydrate ratios of 46:54 and 16:84, respectively. It is believed that metabolic feedback and chemosensory cues are involved in the mechanisms of control self-selection (Friedman *et al.*, 1991). Interestingly Ahmad *et al.* (1993) showed that, the ablation of all the chemoreceptors on the maxillae of *Manduca sexta* does not significantly perturb its ability to self-select. This suggests other chemosensilla in the pre-oral cavity or on the structure other than the maxillae (perhaps the epipharynx) may control food selection in the absence of the maxillae. Therefore, the present results showed that *S. larvae* were able to self-select, support the hypothesis that the self-selection is controlled by both metabolic feedback and chemosensory cues. Both the levels of metabolic feedback and sensitivity of peripheral gustatory receptors are correlated with changing patterns of protein and carbohydrate ingestion during the 5th-instar period which led the insect to compensate the nutritional deficiency in any diet by selecting alternative food and regulating the amount of food, ingested. Comparison of nutritional indices between the self-selecting and control 5th-instar larvae (Table 2) showed that, the self-selecting larvae were similar to the control larvae. There was no significant difference in nutritional parameters between controls and self-selecting larvae. The reason for the similar growth was apparently the fact that the control and the self-selected ratio of 80:20 and 50:50 diets was in fact not very different nutritionally. Different results as expected can be seen when the larvae were given the 20:80 diets (Table 3).

Food consumption and growth rate on diet with predetermined casein:sucrose ratio: The data from experiments with pre-determined protein (casein) and carbohydrate(sucrose) ratio (Table 3) reveal that 5th-instar larvae grow variably but reasonably well when fed on diets containing 80:20 or 50:50 protein:carbohydrate (casein:sucrose) ratios, indicating an ability to maintain growth rate within a fairly narrow range of protein:carbohydrate ratios. There was difference in the efficiency of food use.

The high ECD and low AD observed in 80:20 larvae is not unexpected, based upon the fact that 5th-instar larvae self-selected this protein:carbohydrate ratio (Table 1). The results are similar to the findings presented by Waldbauer *et al.* (1984), who found last instar larvae of the temperate corn earworm, *Heliothis zea*, self-selected about an 80:20 protein:carbohydrate ratio. When this 80:20 ratio was incorporated into a single diet, they found that larvae fed on this diet were superior in overall food utilization (ECI) and the efficiency of conversion of digested food to biomass (ECD) to larvae on all other protein:carbohydrate ratios (100:0; 50:50 and 20:80). Although the 80:20 and 50:50 larvae had similar weight gain and growth rate (GR), the 80:20 larvae ate significantly less food and managed to utilize the food, they ate more efficiently for growth.

When the ratio was changed to 20:80, in general, the larvae suffered inferior food utilization efficiencies. The high AD in 20:80 diet is not unexpected since the diet had a large sugar content and that was apparently very easy for larvae to digest. This finding is similar to that of Waldbauer *et al.* (1984), who showed that fifth-instar larvae of *H. zea* similarly increased their AD in response to increasing sugar content of their diet. The authors suggested that this increase in AD, which was accompanied by a decrease in ECD, might have been due to larvae's excessive intake of sucrose, a consequence of ingesting a larger amount of diet in order to increase protein consumption. Excess sucrose 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.

From the values obtained, it is probable that reduced growth of 5th-instar larvae on 20:80 diet is due to lower food consumption efficiency and digestibility, and a higher metabolic cost associated with diet processing.

Furthermore, it is probable that the poor performance of 5th-instar larvae on 20:80 diet (Table 3), on which growth rate and nutritional indices were significantly lower than all other diets, is due to its gross imbalance between protein and carbohydrate.

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