The Journal of Experimental Biology 208, 611-623
Published by The Company of Biologists 2005
doi:10.1242/jeb.01404

Nutrition interacts with parasitism to influence growth and physiology of the insect *Manduca sexta* L.

S. N. Thompson*, R. A. Redak and L.-W. Wang

Department of Entomology, University of California, Riverside, California 92521, USA

*Author for correspondence (e-mail: nelsont@ucr.edu)

Accepted 24 November 2004

Summary

The influence and interaction of dietary protein:carbohydrate balance and parasitism by *Cotesia congregata* on nutrient intake and growth were examined over the last two larval stadia of *Manduca sexta*. Effects of nutritional status on host blood metabolite concentrations were also determined. Six fat-free chemically defined diets were tested, each having the same total level of casein and sucrose, but with casein to sucrose ratios varying from low protein/high carbohydrate to equal levels of both nutrients through to high protein/no carbohydrate. Nutrient ratio and parasitism each affected nutrient consumption and growth. Feeding responses differed between normal and parasitized larvae, as illustrated by nutrient arrays, two-dimensional plots of protein and carbohydrate consumption on diets having different nutrient ratios. Normal larvae consumed more nutrients and took longer to develop as dietary nutrient ratio was displaced from equal levels of both nutrients. Except on the diet having the same amount of protein and carbohydrate, parasitized larvae consumed less nutrients than normal larvae, although on all diets parasitized larvae took longer to develop. When the contribution of parasite biomass was excluded, parasitized larvae showed lower mass gain than normal larvae on all diets. Total mass gain by normal and parasitized larvae with parasite biomass included, however, was similar on diets having intermediate nutrient ratios. Differences in mass gain between diets relative to nutrient consumption were evident from multi-dimensional representations of mass gain with protein and carbohydrate consumption. Three-dimensional plots and contour maps of normal and parasitized larvae were different. When differences in nutrient consumption between diets were taken into account, protein consumption had a greater effect on growth than carbohydrate consumption and normal larvae generally displayed greater mass gain than parasitized larvae on the same diets. Utilization efficiency, the efficiency of conversion of ingested food to body mass, was, therefore, generally reduced in parasitized insects. Concentrations of blood protein, total free amino acids and trehalose were each influenced by dietary nutrient ratio and parasitism. Concentrations of protein and free amino acids generally increased and trehalose concentration decreased as dietary protein increased and carbohydrate decreased. The opposite was the case as dietary carbohydrate increased and protein decreased. Dietary nutrient ratio, however, affected normal and parasitized larvae differently. Parasitized larvae had higher overall trehalose concentrations while normal larvae had higher protein and total free amino acid concentrations. When differences in nutrient consumption between diets were accounted for, protein consumption had a greater effect on blood protein and free amino acid concentrations than did dietary nutrient ratio or parasitism. Protein consumption, however, did not affect trehalose concentration. Carbohydrate consumption had no effect on the concentration of any of the metabolites after differences in nutrient consumption were taken into account. Effects of nutrient consumption on trehalose concentration, therefore, were due to dietary nutrient ratio and parasitism. The potential relevance of the above findings to the biology of parasitized *M. sexta* larvae is discussed.

Key words: insect, growth, nutrition, diet, parasitism, *Manduca sexta*, *Cotesia congregata*.

Introduction

Nutrition is the essential connection between an animal and its environment, providing the energy and the chemicals necessary for survival. Animals acquire nutrients through an integration of behavior and physiology that is considered the subject of nutritional ecology (House, 1977; Slansky, 1982). This integration ensures that an optimal quantity and balance of the required nutrients are consumed and available for growth, development and reproduction (Simpson and Raubenheimer, 1993a; Raubenheimer and Simpson, 1999). During multitrophic interactions, nourishment of a predator or parasite is achieved by prior provision of nutrients to herbivorous hosts and prey. Additionally, nourishment of

many parasites requires a living host. The host must therefore consume nutrients in support of parasite growth and development and ensure its own survival during that period. In these cases, dramatic alterations of host behavior, physiology and development accompany parasitism and these alterations often appear beneficial for parasite success (Thompson, 1993; Quickie, 1997; Beckage and Gelman, 2001). Little, however, in known of the roles of nutrition and of nutritional interaction occurring between the host and parasite that may be essential for parasite success. Important questions remain. How does parasitism influence host nutrition and physiology? How do parasitism-induced alterations of host nutrition influence parasite development? This study addresses the first question, specifically the effects of parasitism by a gregarious braconid wasp, *Cotesia congregata*, on the feeding and physiological responses of last instar larvae of the Sphingid moth *Manduca sexta*, to variations in dietary composition. These responses by parasitized host larvae will ultimately define the nutritional conditions under which *C. congregata* develops.

Previous studies suggest that parasitism alters the normal feeding responses of *M. sexta* larvae (Thompson et al., 2001). Normal, unparasitized larvae given a choice of two diets, one containing casein but lacking carbohydrate and a second containing sucrose but lacking protein, feed in a ratio of 2 parts of the protein diet to 1 part carbohydrate diet. Parasitized insects given the same choice consume equal amounts of each diet. This difference in diet consumption is principally due to lower consumption of the protein diet by parasitized larvae, which explains in part the slow growth of parasitized insects. When fed individual diets having different ratios of casein and sucrose, those parasitized larvae feeding on a diet with equal amounts of protein and carbohydrate produce the greatest number and biomass of parasites. This suggests that altered host nutrient intake may be important for optimizing parasite growth and development. Lacking, however, is precise information on the influence of dietary nutrient composition on nutrient consumption, growth and development by normal and parasitized larvae.

Knowledge of information on host blood metabolite concentrations is essential for understanding the impact of host response to dietary nutrient ratio and parasitism because these metabolites provide the nutrients on which *C. congregata* feed, and their concentrations play a role in regulating feeding and food choice by the host (Simpson and Raubenheimer, 1993b). Nutrients digested and absorbed by the gut and metabolites synthesized from nutrients and released by tissues are transported directly in the open circulation. They occur at variable and often high concentrations depending on nutrient intake. The concentrations of circulating metabolites provide a continuous reading of the insect's nutritional and metabolic state, information that serves as a basis for maintaining nutritional homeostasis through regulation of feeding (Schiff et al., 1989; Simpson and Raubenheimer, 1996). Studies with several species of lepidopteran larvae, including *M. sexta*, demonstrate that the concentration of trehalose, the blood sugar of insects, correlates positively with carbohydrate consumption. Carbohydrate intake and blood sugar level, in turn, affect the subsequent consumption of dietary carbohydrate (Simpson et al., 1988; Friedman et al., 1991; Thompson and Redak, 2000). Food choice is a dynamic process. Many insects offered nutritionally unbalanced foods continuously adjust their feeding, selecting a combination of foods that ultimately results in an intake of required nutrients that is optimal for growth and development (Waldbauer and Friedman, 1991; Simpson and Raubenheimer, 1993a; Raubenheimer and Simpson, 1999).

The present study establishes the relationship between dietary nutrient ratio, nutrient consumption and growth of normal unparasitized *M. sexta* larvae and larvae parasitized by *C. congregata.* We fed larvae a series of chemically defined diets all containing the same total level of casein and sucrose but with variable ratios of these nutrients. We also examined the effects of dietary nutrient ratio and nutrient intake on the blood concentrations of protein, total free amino acids and trehalose. Based on the results of earlier studies described and cited above, we predicted that both dietary nutrient level and parasitism would affect diet and nutrient consumption. Further, we predicted that the levels of blood metabolites would reflect the intake of casein and sucrose by larvae fed the various diets. Last, we consider how the effects and interactions of nutrition and parasitism may influence the feeding behavior and ecophysiology of *M. sexta* larvae. In a subsequent study we will examine how these effects of parasitism directly influence parasite growth and development.

Materials and methods

Insect culture

Stock colonies of *Manduca sexta* L. were reared on a semi-defined artificial diet containing wheat germ, Torula yeast, linseed oil, Wesson's inorganic salt mixture, ascorbic acid and B-complex vitamins (Bell and Joachim, 1976). Following egg hatching, first instar larvae were housed individually in multi-well tissue culture plates and transferred to larger plastic containers as they grew and developed. Upon completion of larval development, fifth or terminal stage larvae were removed from the diet containers and placed in holes bored in small wooden blocks where they pupated. Adult moths mated and laid eggs on greenhouse tomato plants.

Mixed-sex populations of approximately 100–150 *Cotesia congregata* Say adult parasitoids were housed in 3 l glass jars. *M. sexta* larvae in the second instar were placed in the bottom of the jars and superparasitized two to three times. The parasitized host larvae were removed, placed on artificial diet, and treated in the same fashion as normal larvae described above. Parasitized host larvae did not pupate and parasitoids emerged from hosts in the terminal stadium housed in 160 ml plastic cups. Parasitoid cocoons were carefully removed from the surface of hosts and placed in 30 ml plastic cups, approximately 150 per cup. Cups were then individually placed in 3 l glass jars for emergence. After eclosion adult parasitoids

were fed honey and supplied with water from dental cotton wicks in small test tubes.

Insects were maintained in a Precision Scientific incubator at 28°C with a 16 h:8 h light:dark non-diapausing, long-day photocycle.

Experimental rearing protocol

Normal and parasitized larvae were fed a chemically defined artificial diet (Ahmad et al., 1989) immediately upon moulting to the fourth stadium. The diet contained casein and sucrose as digestible protein and carbohydrate, respectively. The stock diet formulation contained 90 g l^{-1} of each nutrient. In addition, the diet consisted of linseed oil, Wesson's salts, ascorbic acid and B vitamins. Nutrients were principally obtained from Nutritional Biochemicals (Cleveland, OH, USA) and Bioserve (Frenchtown, NJ, USA). Experiments were conducted with a series of six diets, each with an equivalent amount of combined protein and carbohydrate, but with the following ratios of casein to sucrose: 0.125C:1.875S, 0.25C:1.75S, 0.5C:1.50S, 1.0C:1.0S, 1.5C:0.5S and 2.0C:0S. The values are relative to the amount of casein and sucrose in the stock formulation, that is 1C:1S is equivalent to 90 g l^{-1} casein and 90 g l^{-1} sucrose. The nutrient content of the diets were equivalent in mass, and because carbohydrate and protein have similar caloric value the diets were approximately equal in energy content.

Non-feeding pharate fourth instar larvae were removed from the rearing diet and superparasitized two to four times, as described by Alleyne (1995). This produced high parasite burdens or maximal numbers of parasites developing within individual host larvae. Larvae were synchronized as described by Baker et al. (1987). In our studies, however, parasitized insects were not starved for 10-12 h after moulting, but immediately placed on the experimental diets. Groups of ten randomly selected parasitized larvae were maintained on each of the experimental diets for the feeding studies described below. Normal fourth instar larvae at the equivalent stage of development served as controls. All larvae were housed individually in 30 ml plastic cups during the fourth stadium, and pharate fifth instar larvae were transferred to 160 ml cups for the duration of the experiment.

Insects were maintained at 28°C with a long-day photocycle as described above.

Feeding studies

Larvae were fed the various experimental diets throughout the fourth stadium until the end of the fifth stadium. In the case of normal larvae, the experiments were discontinued once after approximately 25% of the larvae had stopped feeding and entered the wandering phase in preparation for pupation. Normal larvae wander within 6–12 h of each other and development time on the various diets was measured in full day increments. At this point, all larvae had reached a fresh mass of approximately 8–10 g with a total development time between 7 and 15 days, depending upon diet. In the case of parasitized larvae, cessation of feeding occurred over a longer time interval, up to 24 h. The time also varied with diet, between 12 and 15 days.

Determination of nutrient consumption and larval growth

Diet consumption was measured as the difference between the total amount of diet offered to larvae and the amount remaining in the diet cups at the end of the experiment together with undigested diet remaining in the gut. Dry mass of the diet remaining in the cups was determined by drying the diet in an oven at 100°C for 24 h and weighing on a microbalance. Initial dry mass of the diet offered to the insects was estimated from the known ratio of wet/dry mass. Protein and carbohydrate consumption were estimated based on the composition of each diet. Fecal material, collected at the end of the experiment, was also dried and weighed. The amount of food assimilated was calculated as the difference between the food consumed and the amount of frass produced on the different diets.

The guts of normal and parasitized larvae were dissected at the end of the experiment and the diet remaining in the gut was removed. This diet was added to that remaining in the diet cups, for estimation of diet consumption. The insect carcasses, including the gut, were dried as described above, after which they were removed and weighed. Initial dry mass of larvae was estimated from the wet mass and the wet/dry mass ratio of several larvae that were dried at the beginning of the experiments.

Analysis of host blood metabolites

Analyses were conducted to determine the effects of dietary nutrient ratio and parasitism on blood concentrations of trehalose, protein and total free amino acids. Groups of five larvae were maintained on each of the diets until approximately two days before feeding stopped. The time chosen was based on the results for the feeding studies described above. We did not wait until feeding had stopped, in order to avoid difficulties in collecting blood at the later time, rapid blood coagulation in the case of normal larvae and interference from parasites in parasitized larvae. Blood was collected from small incisions made with microscissors in one or more prolegs. To remove blood cells and tissue debris, whole blood was centrifuged at 3000 ***g*** for 3 min in a refrigerated Beckman microfuge.

Trehalose was determined by ^{13}C nuclear magnetic resonance (NMR) spectroscopy following deproteination of the cell-free supernatant by addition of perchloric acid to 3.5%. Following centrifugation as above, blood plasma was neutralized with 2 M K_2CO_3. Samples were refrigerated for approximately 12 h and then centrifuged to remove $KClO_3$. An internal standard, 3-(trimethylsilyl)-1-propane sulfonic acid, was added to the supernatant to 20 mmol l^{-1}. Deuterium oxide, added to 15%, served as a field frequency lock. ^{13}C NMR analysis was conducted under non-saturating conditions at 75.48 MHz in a Varian Inova 300 spectrometer as described previously (Thompson et al., 2002). Spectra were generated from 4000 data acquisitions and referenced to the internal standard at approximately – 2.67 ppm. Trehalose concentration

was calculated by comparing the signal intensity of trehalose C1 at 93 ppm with the intensity of the internal standard.

For analysis of protein and amino acids, blood plasma was deproteinized by addition of sulphosalicylic acid to 2%. After centrifugation, the protein pellet was dried for 2 h at 100°C and the crude protein determined gravimetrically with a Sartorius M2P electronic microbalance (Goettingen, Germany). Amino acid analysis was conducted with a Beckman 6300 automated amino acid analyzer (Fullerton, CA, USA), equipped with a lithium ion exchange column. Samples were diluted 50 fold with Beckman Li-S® high performance lithium citrate buffer containing 50 µmol l^{-1} aminoethyl cysteine as an internal standard. Sample aliquots of 50 µl were loaded into the analyzer. Amino acid separations were achieved by stepwise pH elution with lithium citrate buffers: pH 2.92, 3.65 and 3.75 (Li292®, Li365® and Li375®, respectively; Pickering Laboratories, Mountain View, CA, USA). A variable temperature program of 32°, 50° and 70°C, respectively, was employed upon addition of each elution buffer. A ninhydrin reaction was used for detection. Total free amino acid concentration was calculated by summing the concentrations of the individual amino acids.

Concentrations of all blood metabolites are reported in mg ml^{-1} blood plasma.

General statistical analyses

Two-way analysis of variance (ANOVA) principally was used to analyze data from the feeding experiments to establish the effects of dietary nutrient ratio and parasitism on host growth (mass gain) and blood metabolite levels. Owing to potential variation in initial mass between treatments, we first analyzed the growth data by analysis of covariance (ANCOVA), using host initial mass as the covariate (Raubenheimer and Simpson, 1992; Horton and Redak, 1993). There was no initial mass covariate effect, and data were subsequently examined by ANOVA, with ANCOVA applied in particular cases where described. Normality and homogeneity of variance were established with the Shapiro-Wilk 'W' test and normal probability plots. All data met the assumptions of analysis of variance.

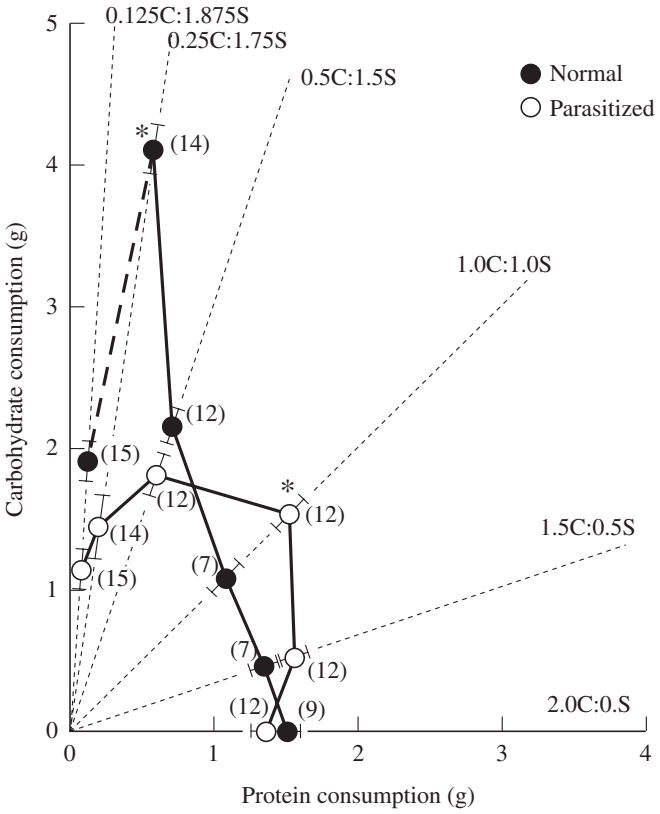


Fig. 1. Two-dimensional representation of nutrient intake by normal unparasitized *M. sexta* larvae (filled circles), and larvae parasitized by *C. congregata* (open circles), maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. Dotted lines are nutrient rails representing the trajectory of nutrient intake expected for larvae feeding on the individual diets. Nutrient intake points indicate actual nutrient intake (± S.E.M.). Significant differences (P<0.05) in nutrient intake between normal and parasitized larvae on the same diet are indicated by an asterisk. The lines through individual intake points for normal and parasitized larvae are the intake arrays. Development times for each dietary nutrient ratio are shown in parentheses. Dietary nutrient ratios are shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation where 1.0=90 g l^{-1}.

Results

Effects of dietary nutrient ratio and parasitism on nutrient consumption and development time

Dietary nutrient ratio and parasitism each affected nutrient consumption. Because intake of protein and carbohydrate varied in fixed proportions equal to nutrient ratio, we examined nutrient intake, according to the method of Raubenheimer and Simpson (1999, 2003). Using this approach, a bivariate plot simultaneously illustrates protein and carbohydrate consumption (Fig. 1). A single point in two-dimensional space indicates the intake of the two nutrients by larvae on each diet. Each point lies on a line or nutrient rail with a rail angle (slope of the line) equivalent to the nutrient ratio of that diet. The relationship between the intake points for all diets is an intake array reflecting the feeding response to variations in nutrient balance as well as any differences in development time. The nutrient 'intake target' is the amount and balance of nutrients required by the insect for optimal growth and development over the feeding period investigated. Owing to post-ingestive effects, that is, physiological mechanisms involved in processing nutrients after ingestion, insects may display near optimal growth and development under conditions where the nutrient intake target is not met (Raubenheimer and Simpson, 1999; see below).

Normal and parasitized *M. sexta* larvae displayed distinct intake arrays over the fourth and fifth stadia (Fig. 1). Differences in development time between diets, described below, contribute greatly to the overall nutrient intake patterns for both normal and parasitized insects. The intake array of

parasitized larvae was non-linear with the ends curved sharply inwards demonstrating reduced nutrient intake at more extreme dietary nutrient ratios. The nutrient intake array for normal larvae generally formed a linear configuration. The intake point for the 0.125C:1.875S diet, however, was radically displaced from the overall array. Clearly, larvae were unable to adjust their feeding response to accommodate the low protein and nutrient imbalance of this diet and, therefore, the diet can be considered a 'pathological food' (Raubenheimer and Simpson, 1999).

Total nutrient intake for the various diets was measured by the distance from the origin of the intake points along the nutrient rail of each diet calculated from protein and carbohydrate consumption using Pythagoras's theorem (Raubenheimer and Simpson, 2003).

Differences in nutrient intake between normal and parasitized larvae were established using *t*-tests (assuming unequal variances), comparing the distances along the individual nutrient rails. Normal larvae consumed significantly more of the nutrients on the 0.25C:1.75S diet than did parasitized larvae (t=7.86, P<0.0001), while parasitized larvae consumed more nutrients on the 1.0C:1.0S diet (t=–3.97, P=0.0019; Fig. 1).

Dietary nutrient ratio affected development time (Fig. 1). Normal larvae displayed longer development times at the extreme dietary nutrient ratios, especially on diets having lesser amounts of protein. A statistical analysis was not possible because feeding of all larvae on the individual diets terminated at the same time. Exempting the 0.125C:1.875S diet, increased development time was associated with increased nutrient consumption by normal larvae over the last stadia. Parasitized larvae showed a similar but less severe trend for development time. Nutrient consumption for parasitized larvae on the higher protein diets increased sharply when compared with normal larvae. In contrast to the results with normal larvae, however, higher nutrient consumption by parasitized larvae was not apparent with increased development time on lower protein diets.

Table 1. *ANOVA summary demonstrating the effects of dietary nutrient ratio and parasitism by* Cotesia congregata *on host growth (mass gain), over the fourth and fifth stadia of* Manduca sexta *larvae maintained on a chemically defined artificial diet*

Dependent variable	d.f.	Mean square	*F* value	Probability
Total host mass gain				
Dietary nutrient ratio	5	2.0131	65.89	<0.0001
Parasitism	1	1.1002	36.01	<0.0001
Interaction	5	0.2955	9.67	<0.0001
Error	70	0.0306		
Host mass gain (parasites excluded)				
Dietary nutrient ratio	5	1.3658	71.12	<0.0001
Parasitism	1	3.1820	165.69	<0.0001
Interaction	5	0.1807	9.41	<0.0001
Error	70	0.0192		

Effects of dietary nutrient ratio and parasitism on host growth and utilization efficiency

Larval mass gain, with or without parasite biomass included, was affected by dietary nutrient ratio and parasitism (Table 1). Mass gain was greatest on the 0.5C:1.5S, 1.0C:1.0S and 1.5C:0.5S diets, and was less on the other diets having more extreme nutrient ratios (Fig. 2). There were significant interactions between dietary nutrient ratio and parasitism, demonstrating that dietary nutrient ratio affects mass gain differently in normal and parasitized larvae (Table 1). When parasite biomass was included as mass gain, there was no difference in mass gain between normal and parasitized larvae on the 1.0C:1.0S and the 1.5C:0.5S diets, but on the other diets, mass gain was significantly less for parasitized insects (Fig. 2).

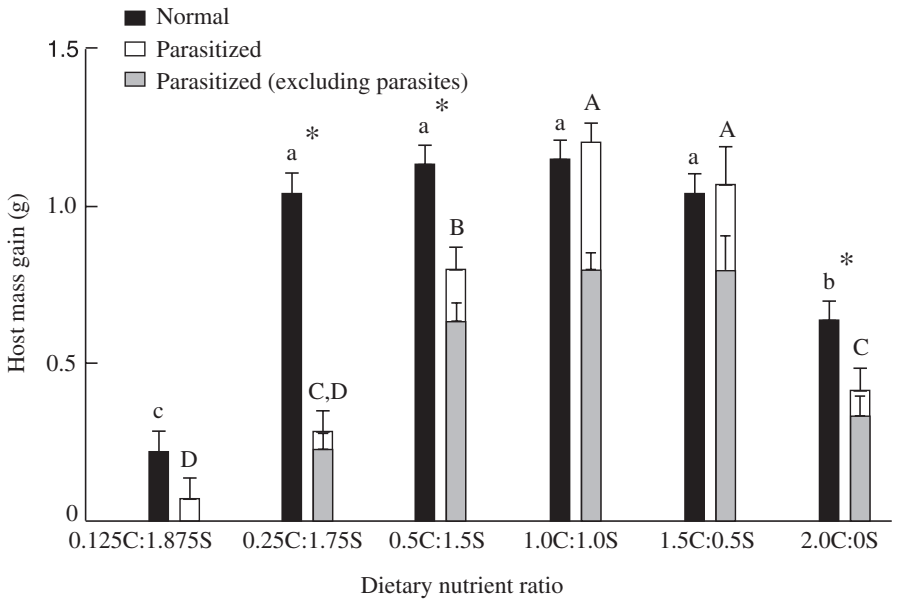


Fig. 2. Effects of dietary nutrient ratio and parasitism by *C. congregata* on growth (mass gain) of *M. sexta* larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. Bars show means ± S.E.M. Significant differences (P<0.05) among normal larvae are indicated by different lowercase letters and among parasitized larvae (including parasite biomass) by different upper case letters. Significant differences between normal and parasitized (including parasite biomass) larvae on the same diet are indicated by an asterisk. Dietary nutrient ratios are shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation where 1.0=90 g l^{-1}.

Excluding the parasite contribution, the mass gain by parasitized insects was less than that of normal larvae on all the diets (Fig. 2); an effect most pronounced on the most suitable diets above. Further, parasitism had a much greater impact on host mass gain than did dietary nutrient ratio when parasite biomass was excluded (as estimated by mean square values; Table 1).

To establish whether dietary nutrient ratio and parasitism affected mass gain independent of nutrient consumption, we conducted an analysis of mass gain by two-way ANCOVA using protein and carbohydrate consumption as joint covariates. The analysis confirmed the importance of protein and carbohydrate for mass gain, as there was a significant effect of each nutrient covariate (Table 2). Protein consumption had the greatest influence on total mass gain that included parasite biomass. Without parasite biomass considered, parasitism had the largest effect on mass gain, but of the two nutrient covariates, protein had a greater effect than carbohydrate. There were no interactions involving either nutrient covariate with dietary nutrient ratio or parasitism. Having accounted for differences in nutrient consumption, the effects of dietary nutrient ratio and parasitism on host mass gain are due to altered utilization efficiency, the efficiency of conversion of food consumed to body mass. There was also an interaction between dietary nutrient ratio and parasitism on mass gain, demonstrating that the effect of dietary nutrient ratio on mass gain differs between normal and parasitized larvae.

Table 2. *ANCOVA summary demonstrating the effects of dietary nutrient ratio and parasitism by* Cotesia congregata *on host growth (mass gain), over the fourth and fifth stadia of* Manduca sexta *larvae, after accounting for differences in nutrient consumption between diets*

Dependent variable	d.f.	Mean square	*F* value	Probability
Total host mass gain				
Dietary nutrient ratio	5	0.4373	63.14	<0.0001
Parasitism	1	0.2109	30.45	<0.0001
Interaction	5	0.0331	4.78	=0.0008
Covariate (protein consumption)	1	0.7947	114.73	<0.0001
Covariate (carbohydrate consumption)	1	0.1246	17.99	<0.0001
Error	68	0.0069		
Host mass gain (parasites excluded)				
Dietary nutrient ratio	5	0.2989	60.22	<0.0001
Parasitism	1	0.9464	190.65	<0.0001
Interaction	5	0.1177	23.72	<0.0001
Covariate (protein consumption)	1	0.3974	80.05	<0.0001
Covariate (carbohydrate consumption)	1	0.1247	25.11	<0.0001
Error	68	0.0050		

Dietary nutrient ratio and parasitism were main treatment effects and protein and carbohydrate consumption were joint covariates.

Parasitized larvae displayed lesser total mass gain, and thus lower utilization efficiency, than normal larvae on all diets except the 0.125C:1.875S and 0.25C:1.75S diets where the mass gain of normal and parasitized larvae was similar (Fig. 3). When parasite biomass was excluded, parasitized larvae grew less than normal larvae on all diets except the 0.125C:1.875S diet. With or without parasite biomass considered, the analysis accounted for approximately 97% of the variation within the data.

Multi-dimensional profiles showing the relationship between mass gain and nutrient intake indicate how nutrient consumption affects growth of normal and parasitized *M. sexta* larvae (Fig. 4). Three-dimensional plots of mass gain with protein and carbohydrate consumption were modeled using SAS (PROC GRID followed by PROC RSREG and PROC G3D. SAS version 8.02, 2001, SAS Institute Inc. Cary, NC, USA). This methodology uses a least-squares approach to fit a quadratic response surface regression model utilizing protein and carbohydrate consumption as independent variables and larval mass gain as a dependent variable (mass gain = protein consumption^2 + carbohydrate consumption^2 + [protein consumption×carbohydrate consumption] + protein consumption + carbohydrate consumption). Additionally, contoured surface maps were generated (PROC GCONTOUR) from a 4000 point matrix created by interpolating a simple linear function for the relationship between dietary nutrient intake and estimated mass gain by normal and parasitized larvae (PROC G3GRID). For the analysis of parasitized insects on the 1.5C:0.5S diet, we deleted the result for one larva that had a biomass twice that of the others in this group. The contribution of this larva resulted in a standard error for mass gain of twice that of any other diet group (Fig. 2).

Three-dimensional models show the nutrient space supporting growth of normal and parasitized insects (Fig. 4AC). The model for normal unparasitized larvae accounted for approximately 96%, and for parasitized larvae 95%, of the variation within the data. Growth of normal larvae is greater over a broader range of nutrient intake. The effects of nutrient consumption are generally consistent with mass gain (Fig. 2) and nutrient consumption (Fig. 1) by larvae on the various diets. Although growth of both normal and parasitized larvae is very low at low levels of protein consumption (Figs 1, 2), the model incorrectly suggests growth of parasitized larvae in the absence of protein consumption. The models, however, confirm that maximal growth of normal and parasitized larvae occur when protein and carbohydrate are consumed in equal amounts, approximately 2 g of each nutrient. The three-dimensional model for parasitized larvae was similar whether parasite biomass was included or excluded (not shown) from the analysis, except mass gain was lower without parasite contribution. The models are only predictive within the range of actual nutrient consumption approximately 0.1–2.0 g casein and 0–4.6 g sucrose for normal larvae and 0.1–1.5 g casein and 0–2.9 g sucrose for parasitized larvae. The contour maps demonstrate more precisely the growth expected for nutrient intake by larvae on specific diets. It is clear that the maximal growth of normal larvae occurs on the 0.25C:1.75S, 0.5C:1.5S, 1.0:1.5S and 1.5C:0.5S diets, while growth of parasitized larvae

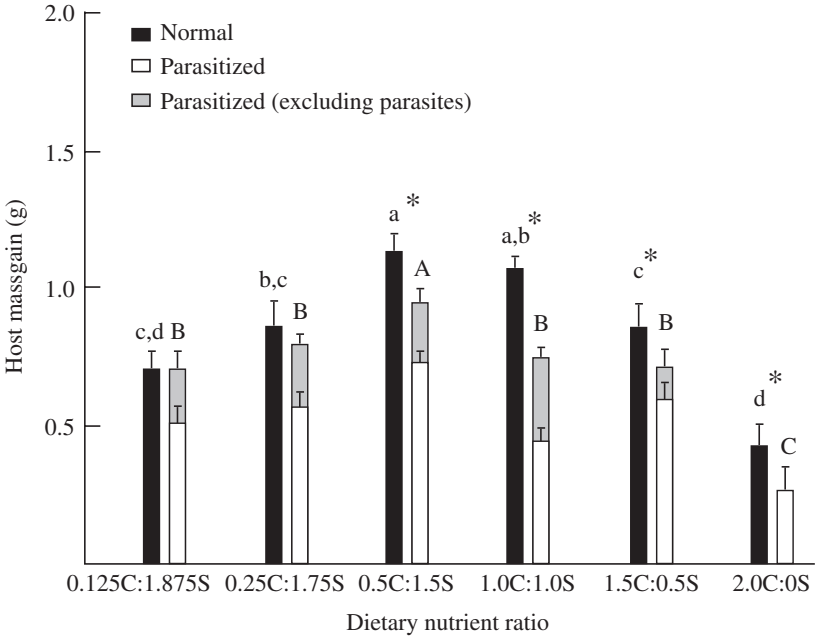


Fig. 3. Effects of dietary nutrient ratio and parasitism by *C. congregata* on growth (mass gain) of *M. sexta* larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose, after accounting for differences in protein and carbohydrate intake between diets. Bars show least-square means ± S.E.M. Differences between diets and between normal and parasitized larvae are due to differences in utilization efficiency. Significant differences (P<0.05) among normal larvae are indicated by different lowercase letters and among parasitized larvae (including parasite biomass) by different upper case letters. Significant differences between normal and parasitized (including parasite biomass) larvae on the same diet are indicated by an asterisk. Dietary nutrient ratios are shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation where 1.0=90 g l^{-1}.

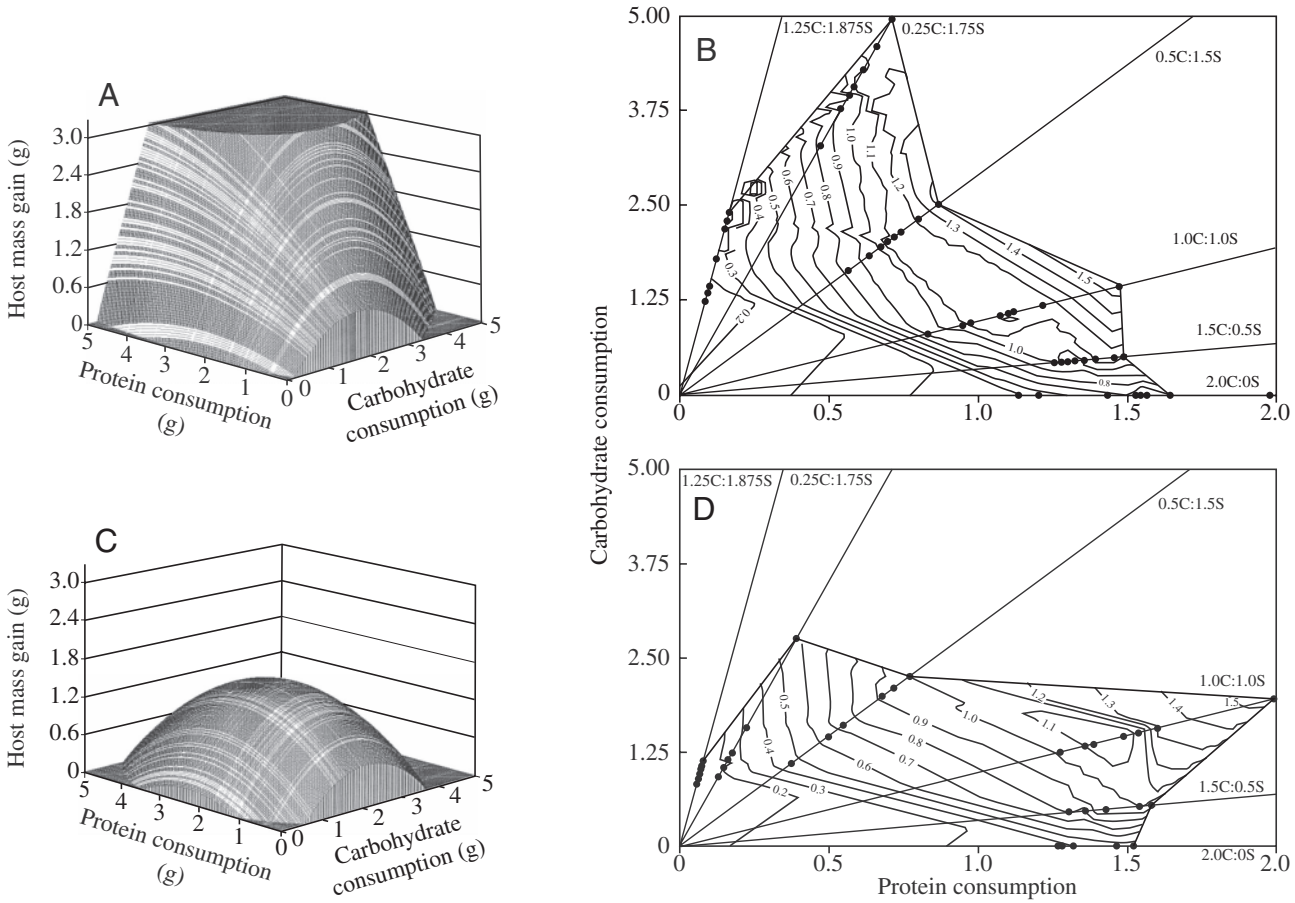


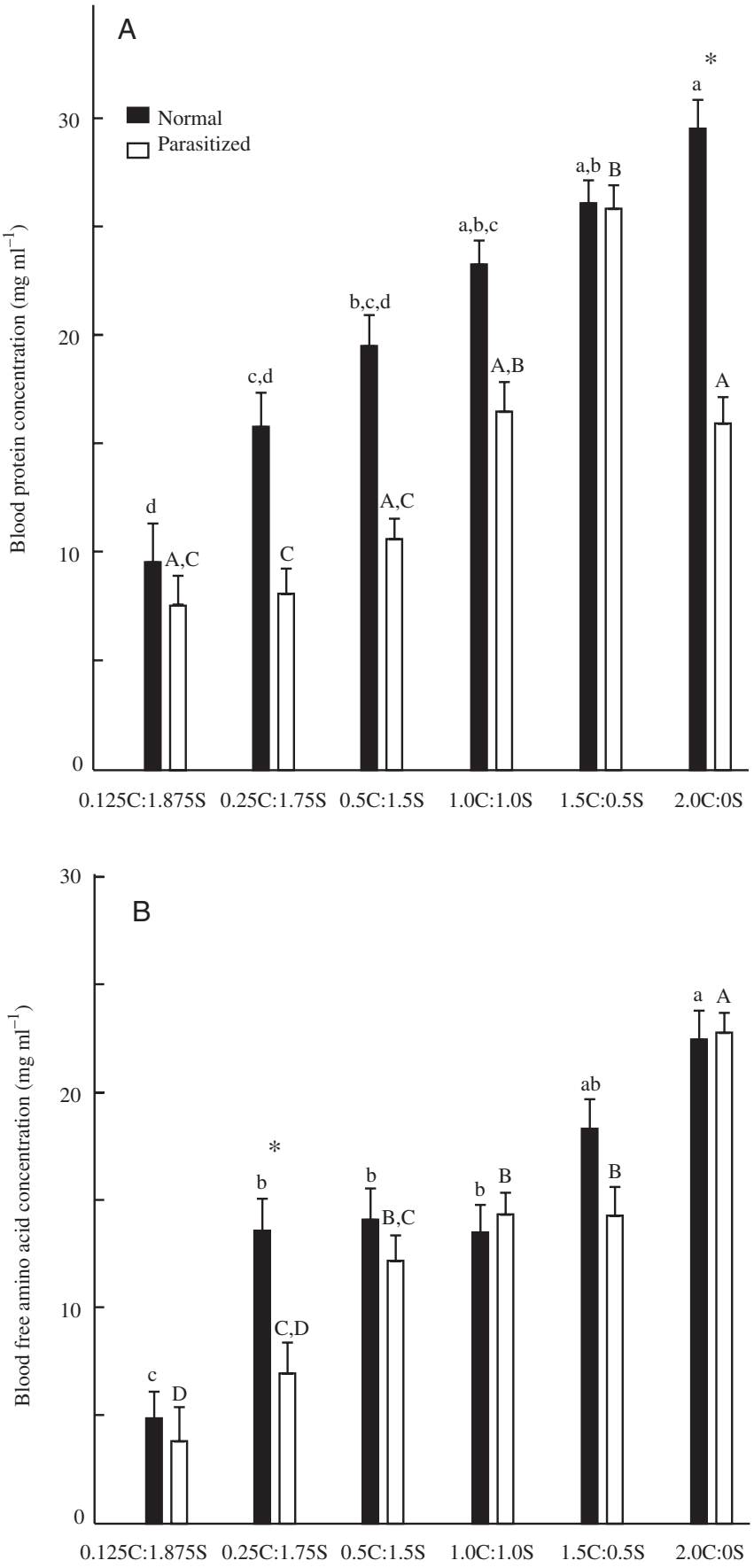
Fig. 4. Multi-dimensional profiles illustrating the effects of nutrient consumption on growth (mass gain) by normal unparasitized *M. sexta* larvae and larvae parasitized by *C. congregata*, maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. (A,C) Three-dimensional models for normal and parasitized larvae. (A) Normal: F=49.04, P<0.0001, r^2=0.9491; (B) parasitized: F=148.38, P<0.0001, r^2=0.9624. (B,D). Contour maps for normal and parasitized larvae, respectively. Nutrient rails shown for the individual diets. Actual mass gain by individual larvae indicated by filled circles.

is maximal on the 1.0C:1.5S diet and to a lesser degree the 1.5C:0.5S and 0.5C:0.15S diets.

Effects of dietary nutrient ratio and parasitism on blood metabolite concentrations

Dietary nutrient ratio and parasitism each affected the concentrations of blood protein, free amino acids and trehalose (Table 3). In the cases of free amino acids and trehalose there were significant interactions between dietary nutrient ratio and parasitism, indicating that dietary nutrient ratio affects the concentration of these metabolites differently in normal and parasitized larvae. Trehalose concentration decreased as dietary nutrient ratio shifted from the high carbohydrate to low carbohydrate diets (Fig. 5). Only on the 1.0C:1.0S diet was there a significant difference between the normal and parasitized larvae, with parasitized insects having a higher concentration. Over all diets, parasitized larvae had a significantly higher trehalose concentration than normal larvae, 25.21±0.94 and 20.38±0.97 mg ml^{-1}, respectively (P=0.0009). In contrast, free amino acids and protein concentration generally increased as dietary nutrient ratio shifted from low protein to high protein diets. Concentration of free amino acids was higher in normal larvae than in parasitized larvae on the 0.25C:1.75S diet and protein concentration was higher in normal larvae than in parasitized larvae on the 2.0C:0S diet (Fig. 5). However, protein concentration, over all diets, was higher in normal than in parasitized larvae, 20.49±0.79 and 14.30±0.79 mg ml^{-1}, respectively (P<0.0001). Free amino acid concentration was also higher overall in normal larvae, 14.48±0.49 and 12.27±0.50 mg ml^{-1}, respectively (P=0.0028).

Contour maps illustrated the effects of protein and carbohydrate consumption on blood concentrations of protein, free amino acids and trehalose (Fig. 6). Profiles for nitrogen metabolites in parasitized and normal larvae were generally similar, but parasitized insects had lower concentrations at equivalent levels of nutrient consumption (Fig. 6A,D and B,E). This was most notable at high levels of protein consumption where parasitized larvae exhibited dramatically lower blood levels of both protein and free amino acids. Profiles for trehalose concentration were also similar in normal and parasitized larvae, but in this case, parasitized larvae generally had higher trehalose concentrations at equivalent levels of nutrient intake (Fig. 6C,F). The profiles for trehalose in



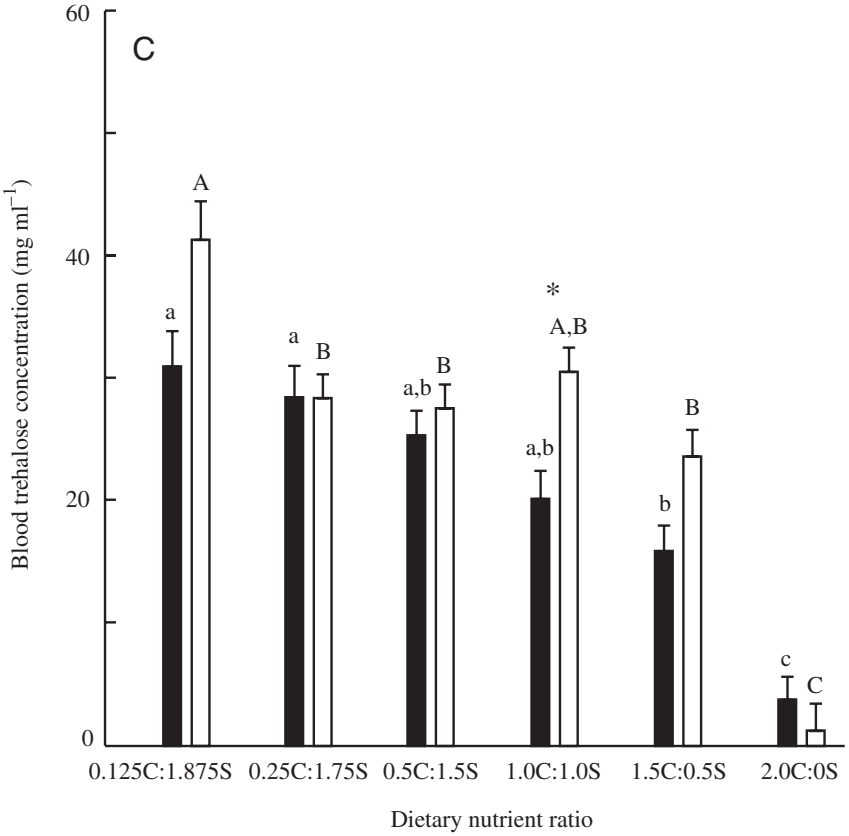


Fig. 5. Effects of dietary nutrient ratio and parasitism by *C. congregata* on blood concentration (mg ml^{-1}) of (A) protein, (B) free amino acids and (C) trehalose in fifth instar *M. sexta* larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. Bars show means ± S.E.M. Significant differences (P<0.05) among diets between normal larvae are indicated by different lowercase letters, and between parasitized larvae (including parasite biomass) by different uppercase letters. Significant differences between normal and parasitized (including parasite biomass) larvae on the same diet are indicated by an asterisk. Dietary nutrient ratios are shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation, where 1.0=90 g l^{-1}.

both normal and parasitized larvae were not as uniform as were those of protein and free amino acid concentrations. Trehalose maps displayed small concentration foci. These reflect the large variation in concentration among larvae. Consequently, plots for trehalose are probably less reliable for predicting trehalose concentrations at those levels of nutrient consumption.

ANCOVA analyses of metabolite concentrations using protein and carbohydrate consumption as joint covariates, as described for growth above, demonstrated significant effects of dietary nutrient ratio and parasitism in each case (Table 4). There were interactions between dietary nutrient ratio and parasitism, for trehalose and free amino acids concentration but not for protein concentration. There was a covariate effect for protein consumption in the cases of free amino acids and protein concentrations, and protein consumption had a greater effect on protein and amino acid concentration than did either dietary nutrient ratio or parasitism. No covariate effect of carbohydrate consumption was evident for any of the metabolites. Because of the absence of significant nutrient covariate effects for trehalose concentration, dietary nutrient ratio and parasitism, rather than differences in nutrient consumption, largely explain the effects of protein and carbohydrate consumption on trehalose. There were no interactions involving the nutrient covariates with parasitism and dietary nutrient ratio.

Discussion

This study demonstrates that dietary nutrient balance and parasitism interact to influence nutrient consumption and growth of *M. sexta* larvae. Differences in protein and carbohydrate intake between diets resulted from the nutrient ratios of the diets and differences in the amounts of diet consumed. Nutrient intake by normal *M. sexta* larvae on the various diets was such that increased consumption of one nutrient resulted in decreased consumption of the other nutrient, whether carbohydrate or protein. When linearized, this intake array has a negative slope <–1, demonstrating that larvae are much more sensitive to dietary imbalances (nutritional errors; Raubenheimer and Simpson, 1999) in protein than carbohydrate over the last two stadia. In this regard, the array is similar to that reported for complete larval development of another lepidopteran insect, *Spodoptera littoralis* (Lee et al., 2002) and nymphal development of two orthopteran species, *Locusta migratoria* and *Schistocerca gregaria* (Raubenheimer and Simpson, 2003).

Normal unparasitized larvae grow well on diets having a wide range of nutrient ratios where some diets have protein and others carbohydrate as the predominate nutrient. Of the diets tested, normal larvae had the greatest mass gains and shortest development times on the 1.0C:1.0S and 1.5C:0.5S diets, suggesting that the intake target for normal larvae lies on a nutrient rail between these two. This conclusion is consistent with studies of *Spodoptera littoralis* demonstrating that last instar larvae defend an intake target having a nutrient ratio of approximately 1.3 parts protein to 1 part carbohydrate (Lee et al., 2002). Larvae on diets having lower or higher protein increase their development time and adjust diet consumption in order to complete larval development at an appropriate size and with sufficient reserves to pupate.

Parasitism of *M. sexta* by *C. congregata* reduced host growth on most diets, and lower consumption of less suitable diets was a major contributor to this effect. The nutrient intake array of

parasitized larvae was sharply arced and did not approach linearity at the end of larval development as typically occurs with increased development times and nutrient consumption on unbalanced diets (Raubenheimer and Simpson, 1999). Insects were unable to adjust diet consumption as the dietary nutrient ratio deviated from 1.0C:1.0S where nutrient consumption was maximal. Thus, insects suffered a large shortfall of a deficient nutrient to avoid consuming a small surplus of an excessive nutrient. Parasitized larvae on unbalanced diets apparently decrease their rate of feeding over time.

Dietary nutrient ratio and protein consumption are the dominant factors explaining differences in host mass gain between normal and parasitized larvae. Utilization efficiency, however, also affects mass gain among diets, and after

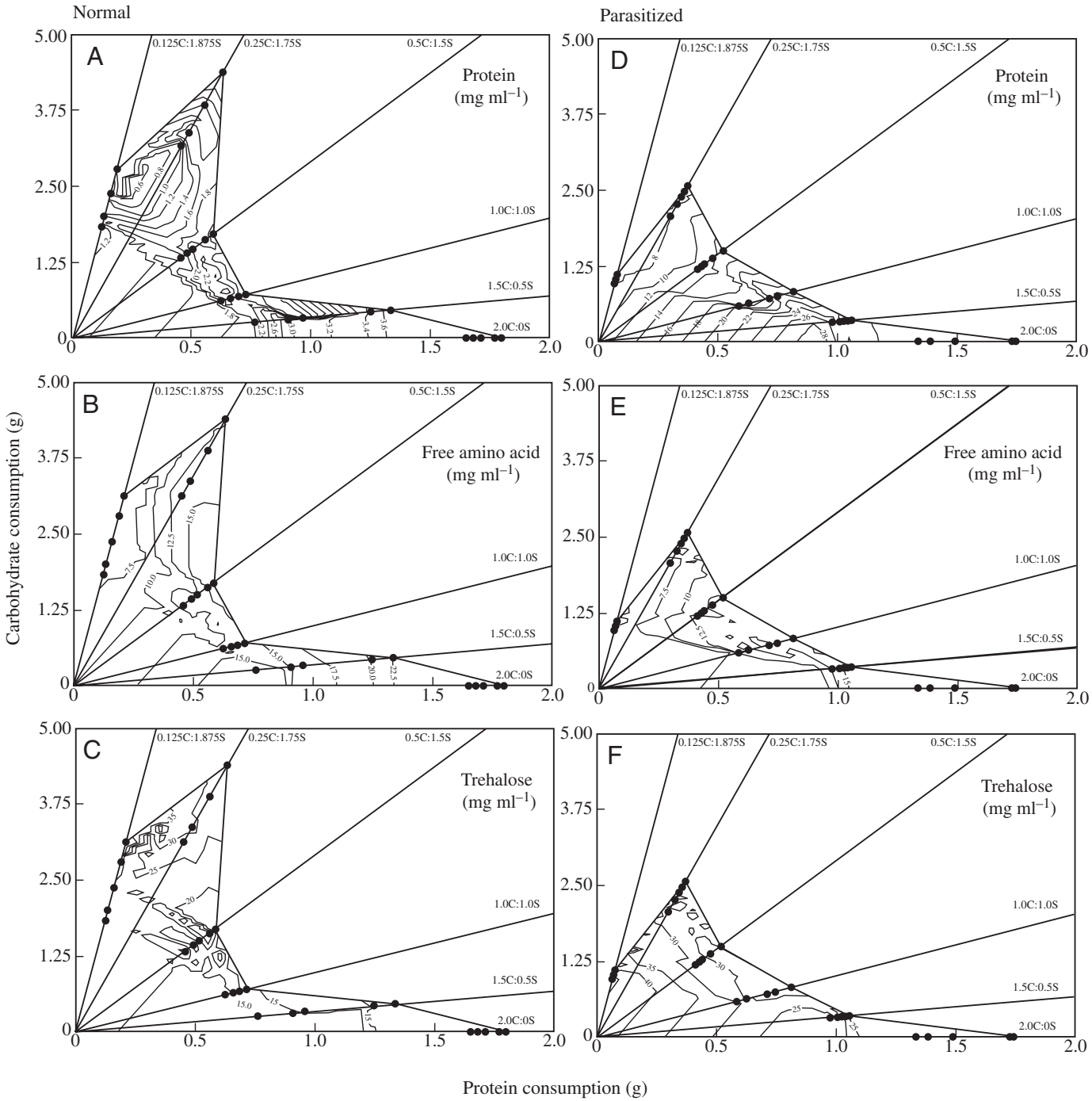


Fig. 6. Contour maps illustrating the effects of nutrient consumption on blood protein, free amino acids and trehalose concentrations (mg ml^{-1}) during the fifth stadium of normal unparasitized fifth instar *M. sexta* larvae and larvae parasitized by *C. congregata*, maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. (A,D) Protein, (B,E) free amino acid and (C,F) trehalose concentrations of (A–C) normal unparasitized larvae and (D–F) parasitized larvae.

Table 3. *ANOVA summary demonstrating the effects of dietary nutrient ratio and parasitism by* Cotesia congregata *on blood metabolite concentrations (mg ml^{-1}) in fifth instar* Manduca sexta *larvae maintained over the fourth and fifth stadia on a chemically-defined artificial diet*

Dependent variable	d.f.	Mean square	*F* value	Probability
Trehalose				
Dietary nutrient ratio	5	1156.3481	48.55	<0.0001
Parasitism	1	303.1372	12.73	=0.0009
Interaction	5	78.9895	3.32	=0.0130
Error	42	23.8169		
Free amino acids				
Dietary nutrient ratio	5	1488.8322	45.56	<0.0001
Parasitism	1	65.8926	10.08	=0.0028
Interaction	5	87.0714	2.66	=0.0349
Error	43	6.5362		
Protein				
Dietary nutrient ratio	5	402.1148	23.93	<0.0001
Parasitism	1	515.4811	30.67	<0.0001
Interaction	5	36.3667	2.16	=0.0759
Error	43	16.8047		

Table 4. *ANCOVA summary demonstrating the effects of dietary nutrient ratio and parasitism by* Cotesia congregata *on blood metabolite concentrations (mg ml^{-1}) in fifth instar* Manduca sexta *larvae after accounting for differences in nutrient consumption between diets*

Dependent variable	d.f.	Mean square	*F* value	Probability
Trehalose				
Dietary nutrient ratio	5	97.8387	4.12	=0.0041
Parasitism	1	257.0710	10.83	=0.0021
Interaction	5	74.2238	3.13	=0.0179
Covariate (protein consumption)	1	0.3873	0.02	=0.8990
Covariate (carbohydrate consumption)	1	46.3462	1.95	=0.1701
Error	40	23.7456		
Free amino acids				
Dietary nutrient ratio	5	23.0511	4.58	=0.0021
Parasitism	1	28.2098	5.60	=0.0228
Interaction	5	17.0593	3.39	=0.0119
Covariate (protein consumption)	1	74.5340	14.80	=0.0004
Covariate (carbohydrate consumption)	1	8.9627	1.78	=0.1896
Error	41	5.0371		
Protein				
Dietary nutrient ratio	5	120.1045	9.25	<0.0001
Parasitism	1	127.6456	9.25	=0.0032
Interaction	5	17.2800	1.33	=0.2706
Covariate (protein consumption)	1	170.3922	13.12	=0.0008
Covariate (carbohydrate consumption)	1	0.3553	0.03	=0.8694
Error	41	12.9890		

Dietary nutrient ration and parasitism were main treatment effects and protein and carbohydrate consumption were joint covariates.

differences in nutrient consumption are taken into account, parasitized larvae generally display lower mass gain and, therefore, lower utilization efficiency, than normal larvae on the same diet. For both normal and parasitized larvae, utilization efficiency was greatest on the 0.5C:1.5S diet. Based on Waldbauer's (1968) ECI (efficiency of conversion of ingested food to body substance) nutrient ratio, the results of studies by others imply increased utilization efficiency (ECI) in lepidopteran larvae parasitized by *Cotesia* spp. (Slansky, 1978; Benz and Barbosa, 1990; Alleyne, 1995). None of those studies, however, considered the influence of differences in diet or nutrient consumption between normal and parasitized insects. We did not calculate the above ratio, or other commonly employed nutritional ratios because of difficulties inherent with their application and interpretation, first outlined by Schmidt and Reese (1986) and elaborated by Packard and Boardman (1988). ANCOVA analyses offer distinct experimental as well statistical advantages based on less restrictive assumptions that apply to ratio based parameters (Raubenheimer and Simpson, 1992).

Numerous investigators have reported suppression of host growth and food consumption by parasitized lepidopteran larvae, including *M. sexta* (Vinson and Iwantsch, 1980; Alleyne and Beckage, 1997; Benz and Barbosa, 1990; Quickie, 1997; Nakamatsu et al., 2001). The present study, however, is the first to demonstrate that host nutrition mediates parasitism effects on host growth and consumption; whether parasitism reduces growth depends on diet. Parasitized larvae on diets having nutrient ratios displaced from the 1.0C:1.0S nutrient ratio generally display reduced growth and nutrient consumption. To achieve growth equivalent to normal larvae, parasitized larvae must consume a diet having a nutrient ratio close to that of the intake target, but also consume more nutrients than normal larvae.

There have been a few reports of hosts with large numbers or burdens of *Cotesia* spp. attaining greater total final mass than normal unparasitized larvae at the same developmental stage (Slansky, 1978; Beckage and Riddiford, 1983; Tanaka et al., 1992). We failed to observe larger parasitized larvae under any of the nutritional conditions investigated during this study. Parasite biomass contributes significantly to total host gain but the relative contribution varies with dietary nutrient ratio. Clearly, parasitism compromises host gain, but parasite biomass alone does not account for the difference in response of normal and parasitized insects to dietary nutrient ratio. Increased metabolic expenditure required to support and sustain parasite development may play a role. Final host mass excluding the parasite contribution is perhaps the more relevant measure when considering potential effects of host size on the parasite, as this is the biomass providing nourishment for

parasite growth and development. This issue, however, is the subject of a separate investigation.

Analysis of the host blood demonstrated the effects of dietary nutrient ratio and parasitism on some blood metabolites. Generally, the blood concentrations of trehalose, free amino acids and proteins conformed to dietary nutrient ratio. As the dietary nutrient ratio shifted toward higher protein and lower carbohydrate, the blood protein and amino acid concentrations increased while trehalose concentration decreased. The effect of dietary nutrient ratio, however, differed between normal and parasitized insects. Two explanations are generally offered for the effects of parasitism on host tissue metabolite levels. These are that parasitism alters the metabolic capacity of host larvae and changes in metabolite levels reflect this alteration or that parasite absorption and utilization of host metabolites brings about these changes (Vinson and Iwantsch, 1980; Thompson, 1993; Nakamatsu and Tanaka, 2004). Clearly, the explanations are not mutually exclusive. The finding that host fat body, the tissue regulating much of the insect's intermediary metabolism, is often diminished in parasitized larvae (Dahlman and Green, 1981; Nakamatsu and Tanaka, 2002), including those of *M. sexta*, strongly suggests that the metabolic capacity of the host is compromised. Protein synthesis, for example, occurs principally in the fat body and the overall reduction of blood protein levels observed here in parasitized *M. sexta* is consistent with decreased fat body content. However, trehalose synthesis also occurs in the fat body but overall trehalose concentrations were increased in parasitized *M. sexta* larvae. Despite any decrease in fat body, this results from induction of gluconeogenesis and occurs in parasitized larvae even under nutritional conditions that fail to induce trehalose formation in normal larvae (Thompson, 2001; Thompson et al., 2002). It may be, therefore, that redirection of host metabolic capacity in addition to, or rather than, reduction of capacity plays an important role in supporting parasite growth and development. In any case, the concentrations of blood metabolites in parasitized larvae when related to host size reflect the nutrients available to the developing parasite. How these factors, host blood metabolite levels and host size, influence parasite growth and development is the subject of another study.

The results on blood metabolite concentrations are equivocal regarding their potential to influence short-term feeding preferences. In the case of trehalose, only on the carbohydrate-free diet, 2.0C:0.5S, were blood sugar levels low enough to suggest a feeding preference for carbohydrate if these larvae were offered a dietary choice of a high carbohydrate and a high protein diet. Trehalose concentration, however, was not different between normal and parasitized larvae on that diet. Effects of variations in blood free amino acids and protein concentrations on feeding behavior are not known for *M. sexta*. It is not possible at this time to predict how the variations of these metabolites may affect short-term feeding preferences. Investigations with other insects, however, demonstrate a relationship between diet, blood amino acid level and food choice (Abisgold and Simpson, 1988; Simpson and Raubenheimer, 1993b; Zanotto et al., 1996).

The influence of diet and nutrient consumption on growth of *M. sexta* larvae determined here suggests that normal and parasitized insects exhibit differences in dietary breadth that may be reflected in different long-term feeding patterns. The intake arrays indicate that overall, parasitized larvae exhibit less nutritional flexibility, and to attain maximal host size require greater nutrient levels and a more specific nutrient balance than normal larvae. How this might influence the plant feeding habits of parasitized larvae is unknown, but parasitized larvae may prefer to feed at sites within plants that differ in nutrient content from those preferred by normal larvae. Alternately, the feeding by parasitized larvae may be more restrictive than normal larvae. The results with parasitized larvae clearly suggest narrower nutritional preferences than normal larvae, both, however, within the specialist category.

References

Abisgold, J. D. and Simpson, S. J. (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. M., Waldbauer, G. P. and Friedman, S. (1989). A defined artificial diet for the larvae of *Manduca sexta*. *Entomol. Exp. Appl.* **53**, 189-192.

Alleyne, M. (1995). Alterations in host growth and metabolism of *Manduca sexta* larvae parasitized by *Cotesia congregata*. MS thesis, University of California, Riverside, CA, USA.

Alleyne, M. and Beckage, N. E. (1997). Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by *Cotesia congregata*. *J. Insect Physiol.* **43**, 407-424.

Baker, F. C., Tsai, L. W., Reuter, C. C. and Schooley, D. A. (1987). *In vivo* fluctuations of JH, JH acid, and ecdysteroid titer, and JH esterase activity, during development of fifth stadium *Manduca sexta*. *Insect Biochem.* **17**, 989-996.

Beckage, N. E. and Gelman, D. B. (2001). Parasitism of *Manduca sexta* by *Cotesia congregata*: a multitude of disruptive endocrine effects. In *Endocrine Interactions of Insect Parasites and Pathogens* (ed. J. P. Edwards and R. J Weaver), pp. 59-81. Oxford: BIOS Scientific.

Beckage, N. E. and Riddiford, L. M. (1983). Growth and development of the endoparasitic wasp *Apanteles congregatus*: dependence on host nutritional status and parasite load. *Physiol. Entomol.* **8**, 231-241.

Bell, R. A. and Joachim, R. G. (1976). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. Entomol. Soc. Amer.* **69**, 365-373.

Benz, J.-A. and Barbosa, P. (1990). Effects of dietary nicotine (0.1%) and parasitism by *Cotesia congregata* on the growth and food consumption and utilization of the tobacco hornworm, *Manduca sexta*. *Entomol. Exp. Appl.* **57**, 1-8.

Dahlman, D. L. and Green, J. R. (1981). Larval hemolymph protein patterns in tobacco hornworms parasitized by *Apanteles congregatus*. *Ann. Entomol. Soc. Amer.* **74**, 130-133.

Friedman, S., Waldbauer, G. P., Eertomoed, J. E., Naeem, M. and Ghent, A. W. (1991). Blood trehalose levels have a role in the control of dietary self-selection by *Heliothis zea* larvae. *J. Insect Physiol.* **37**, 919-928.

Horton, D. R. and Redak, R. A. (1993). Further comments on analysis of covariance in insect dietary studies. *Entomol. Exp. Appl.* **69**, 263-275.

House, H. L. (1977). Nutrition of natural enemies. In *Biological Control by Augmentation of Natural Enemies* (ed. R. L. Ridgway and S. B. Vinson), pp. 151-182. New York: Plenum.

Lee, K. P., Behmer, S. T., Simpson, S. J. and Raubenheimer, D. (2002). A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *J. Insect Physiol.* **48**, 655-665.

Nakamatsu, Y. and Tanaka, T. (2002). Larvae of an endoparasitoid, *Cotesia*

kariyai (Braconidae: Hymenoptera), feed on the host fat body directly in the second stadium with the help of teratocytes. *J. Insect Physiol.* **48**, 1041-1052.

Nakamatsu, Y. and Tanaka, T. (2004). Correlation between concentration of hemolymph nutrients and amount of fat body consumed in lightly and heavily parasitized hosts (*Pseudaletia separata*). *J. Insect Physiol.* **50**, 135-141.

Nakamatsu, Y., Gyotoku, Y. and Tanaka, T. (2001). The endoparasitoid *Cotesia kariyai* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* larvae by venom and Ck polydnavirus. *J. Insect Physiol.* **47**, 573-584.

Packard, G. C. and Boardman, T. J. (1988). The misuse of ratios, indices, and percentages in ecophysiological research. *Physiol. Zool.* **61**, 1-9.

Quickie, D. L. J. (1997). *Parasitic Wasps*, pp. 221-255. New York: Chapman and Hall.

Raubenheimer, D. and Simpson, S. J. (1992). Analysis of covariance: an alternative to nutritional indices. *Entomol. Exp. Appl.* **62**, 221-231.

Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl*. **91**, 67-82.

Raubenheimer, D. and Simpson, S. J. (2003). Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *J. Exp. Biol.* **206**, 1669-1681.

Schiff, N. M., Waldbauer, G. P. and Friedman, S. (1989). Dietary self-selection by *Heliothis zea* larvae: roles of metabolic feedback and chemosensory stimuli. *Entomol. Exp. Appl*. **52**, 261-270.

Schmidt, D. J. and Reese, J. C. (1986). Sources of error in nutritional index studies of insects on artificial diet. *J. Insect. Physiol.* **32**, 193-198.

Simpson, S. J. and Raubenheimer, D. (1993a). A multi-level analysis of feeding behavior: the geometry of nutritional decisions. *Phil. Trans. R. Soc.* **342**, 381-402.

Simpson, S. J. and Raubenheimer, D. (1993b). The central role of haemolymph in the regulation of nutrient intake in insects. *Physiol. Entomol.* **18**, 395-403.

Simpson, S. J. and Raubenheimer, D. (1996). Feeding behavior, sensory physiology and nutrient feedback: a unifying model. *Entomol. Exp. Appl.* **80**, 55-64.

Simpson, S. J., Simmonds, M. S. J. and Blaney, W. M. (1988). A comparison of dietary selection behavior in larval *Locusta migratoria* and *Spodoptera littoralis*. *Physiol. Entomol.* **13**, 225-238.

Slansky, F. (1978). Utilization of energy and nitrogen by larvae of the imported cabbageworm, *Pieris rapae*, as affected by parasitism by *Apanteles glomeratus*. *Environ. Entomol*. **7**, 179-185.

Slansky, F. (1982). Insect nutrition: an adaptationist's perspective. *Flor. Entomol.* **65**, 45-71.

Tanaka, T., Yagi, S. and Nakamatu, Y. (1992). Regulation of parasitioid sex allocation and host growth by *Cotesia (Apanteles) kariyae* (Hymenoptera: Bracondidae). *Ann. Entomol. Soc. Amer.* **85**, 310-316.

Thompson, S. N. (1993). Redirection of host metabolism and effects on parasite nutrition. In *Parasites and Pathogens of Insects*, Vol. 1 (ed. N. E. Beckage, S. N. Thompson and B. A. Federici), pp. 125-144. New York: Academic Press.

Thompson, S. N. (2001). Parasitism enhances the induction of glucogenesis by the insect *Manduca sexta* L. *Int. J. Biochem. Cell Biol.* **33**, 163-173.

Thompson, S. N. and Redak, R. A. (2000). Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. *Biochim. Biophys. Acta* **1523**, 91-102.

Thompson, S. N., Redak, R. A. and Wang, L.-W. (2001). Altered dietary nutrient intake maintains metabolic homeostasis in parasitized larvae of the insect *Manduca sexta* L. *J. Exp. Biol.* **204**, 4065-4080.

Thompson, S. N., Redak, R. A and Borchardt, D. B. (2002). The glucogenic response of a parasitized insect *Manduca sexta* L. is partially mediated by differential nutrient intake. *Biochim. Biophys. Acta* **1571**, 138-150.

van Loon, J. J. A. (1991). Measuring food utilization in plant feeding insects – towards a metabolic and dynamic approach. In *Insect–Plant Interactions*, Vol. III (ed. E. A. Bernays), pp. 79-124. Boca Raton: CRC Press.

Vinson, S. B. and Iwantsch, G. F. (1980). Host regulation by insect parasitoids. *Q. Rev. Biol*. **55**, 143-165.

Waldbauer, G. P. (1968). The consumption and utilization of food by insects. *Adv. Insect Physiol*. **5**, 229-288.

Waldbauer, G. P. and Friedman, S. (1991). Self-selection of optimal diets by insects. *Ann. Rev. Entomol.* **36**, 43-63.

Zanotto, F. P., Raubenheimer, D. and Simpson, S. J. (1996). Haemolymph amino acid and sugar levels in locusts fed nutritionally unbalanced diets. *J. Comp. Physiol. B* **166**, 223-229.