

# Patterns and Mechanisms of Growth of Fifth-Instar *Manduca sexta* Caterpillars Following Exposure to Low- or High-Protein Food during Early Instars

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## ABSTRACT

For many insect herbivores, variation in protein availability is a pervasive part of the environment. I explore how variable protein availability affects growth rates of fifth-instar *Manduca sexta* caterpillars and how growth is related to behavior and physiology. Groups of larvae were reared on low- or high-protein artificial diets (5.9% and 17.7% casein by dry weight, respectively) and then transferred in the fifth instar to the same or opposite diet. During or after the 24-h period following transfer, I measured growth rate, consumption rate, growth efficiency, midgut proteolytic activity, and masses of midgut contents and tissues. Fifth-instar caterpillars reared in earlier instars on high-protein diet grew about 20% more rapidly over 24 h than did caterpillars reared on low-protein diet. This growth pattern appears to be caused by differences in consumption and growth efficiency: caterpillars reared on high protein consumed more food, and used it more efficiently, than did caterpillars reared on low-protein diet. Over the short term (24 h), in contrast, fifth instars that received low-protein diet grew as rapidly as caterpillars that received high-protein diet. Increased (compensatory) consumption appears to be the primary mechanism by which caterpillars consuming low-protein food maintained growth rates.

## Introduction

For insect herbivores, variation in plant protein concentration is a pervasive part of the environment. Plants—or even leaves within a plant—may contain substantially different concentra-

tions of protein (Schultz et al. 1982; Denno and McClure 1983; Stamp and Bowers 1990; Suomela et al. 1995). Protein concentrations may also change with time, generally decreasing as the growing season progresses (Slansky and Scriber 1985). Individual insects, therefore, depending on the distance and frequency of moves and the duration of the larval period, are likely to encounter variable dietary protein. This variation is important because dietary protein concentration strongly affects growth rates of many insects. In general, low concentrations of protein lead to reduced rates of growth (McNeill and Southwood 1978; Mattson 1980; Scriber 1984; Slansky and Scriber 1985). Factors other than protein concentration—including protein quality, levels of other nutrients, temperature, and predation—also may influence growth rates (Stamp and Casey 1993).

Here I address two questions about insect responses to variable protein, using larval *Manduca sexta* (Lepidoptera: Sphingidae). First, how are caterpillar growth rates affected by current and prior protein availability? Only a handful of authors have examined short- and long-term effects of dietary protein (Taylor 1989; Stockhoff 1992, 1993a, 1993b), and the findings have been mixed (discussed later). Second, how is growth related to the process of protein acquisition? This process involves multiple steps, including consumption, digestion, absorption, and postabsorptive incorporation into tissues. Protein availability may affect these steps directly; also, in response to protein availability, caterpillars may actively adjust their behavior or physiology. I address these questions in the context of four competing hypotheses.

### *The Beneficial Acclimation Hypothesis*

The beneficial acclimation hypothesis (Fig. 1A) predicts that late-instar caterpillars grow more rapidly on a particular diet if they experienced that diet during earlier instars (Stockhoff 1992; Huey and Berrigan 1996). Preexposure should allow caterpillars to make advantageous physiological adjustments—for example, by producing detoxification enzymes (Brattsten 1988; Snyder et al. 1994) or by altering proteolytic activity in the midgut (Ishaaya et al. 1971; Broadway and Duffey 1986) or efficiency of food use (Slansky and Feeny 1977; Slansky and Wheeler 1989, 1991). Several species of caterpillars apparently grow more rapidly on particular host plants after previous exposure to them (Schoonhoven and Meerman 1978; Scriber

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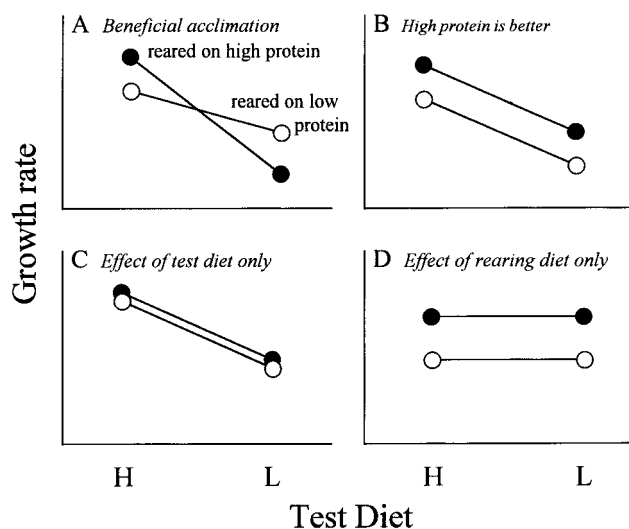


Figure 1. Four hypotheses about how rearing and test diets affect growth rate. See text for description.

1979, 1982; Karowe 1989; but see Stoyenoff et al. 1994), although it is unclear whether patterns of growth were due primarily to host plant nutritional characteristics or secondary chemistry.

#### The High Protein Is Better Hypothesis

The high protein is better hypothesis (Fig. 1B) predicts that caterpillars currently eating high-protein diet grow more rapidly than caterpillars eating low-protein diet and that caterpillars reared on high-protein diet grow more rapidly under a range of conditions than caterpillars reared on low-protein diet. Stockhoff (1992), for example, showed that second-, third-, and fourth-instar larval gypsy moths (*Lymantria dispar*) grew more rapidly when consuming a high-protein diet and also grew more rapidly if they consumed high protein during previous instars. This outcome may reflect that high dietary protein has, in addition to immediate positive effects, long-term positive consequences.

#### The Effect of Test Diet Only Hypothesis

The effect of test diet only hypothesis (Fig. 1C) predicts that caterpillars currently eating high-protein diet grow more rapidly than those eating low-protein diet but that rearing diet has no effect. Stockhoff (1992) also found, for example, that growth rates of fifth-instar *L. dispar* were affected by the protein content of the test diet but not of the rearing diet. This outcome suggests either that the physiology of later instars is not affected by dietary conditions in earlier stages or that later stages are less able to compensate for low-quality diets.

#### The Effect of Rearing Diet Only Hypothesis

The effect of rearing diet only hypothesis (Fig. 1D) predicts that rearing diet, but not test diet, affects growth rate. This outcome may arise if long-term exposure to a particular concentration of protein affects the capacity for growth but not for short-term compensation (Slansky and Scriber 1985; Simpson and Simpson 1990).

I tested these hypotheses using larval *M. sexta*. Larvae were reared in the laboratory on low-protein (5.9% casein, 19.2% total protein) or high-protein (17.7% casein, 30.9% total protein) artificial diets during instars 1–4 and transferred to the same or opposite diet in the fifth instar. I then measured growth rate during a 24-h test period in the middle of the fifth instar. A second goal of this work is to relate growth rate to underlying behavioral and physiological processes. Therefore, during or after the 24-h test period, I also measured consumption rate, efficiency of food use, midgut proteolytic activity, midgut lumen mass, and midgut tissue mass. These traits were chosen because they are associated with the process of protein acquisition (and more generally with nutrient acquisition), and several of them are known from previous work to vary with concentration of dietary protein.

#### Material and Methods

##### Animals

*Manduca sexta* are distributed widely in North America, where they are restricted primarily to Solanaceae (Hodges 1971).

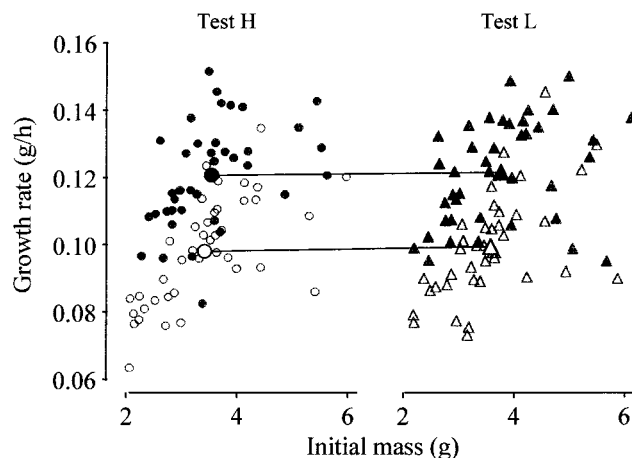


Figure 2. Growth rate of fifth-instar *Manduca sexta* during 24-h trials. The information is arranged as in Figure 1 except that information on initial mass has also been included. The raw data are shown in smaller symbols and the means (both growth rate and initial mass) of the treatment groups are represented by the four larger, boldface symbols. Caterpillars were reared on a high-protein (filled) or low-protein diet (unfilled) and were tested on a high-protein (circles) or low-protein diet (triangles).

Although later instars move readily about their host plants, caterpillars probably move only rarely between disjunct hosts; thus, individuals experience primarily intraplant variation in protein concentration. Leaves of Solanaceae contain highly variable amounts of protein. Tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabacum*), for example, contain 13.8%–37.5% and 12.5%–31.3% protein by dry mass, respectively (Reuter and Robinson 1986). In tobacco, soluble protein concentrations of individual leaves can decrease by two-thirds in less than 2 wk (He et al. 1997).

*Manduca sexta* were obtained as eggs from a laboratory colony at the University of Washington. After hatching, larvae were placed individually on cubes of a wheat germ–based artificial diet (modified from Bell and Joachim 1976; available ad lib.) in 1-oz plastic cups and were reared in an environmental chamber (26°C, 17L:7D). Diet was replaced when the larvae reached the fourth instar. During the molt to the fifth instar, larvae were transferred individually to 9-oz plastic cups and again given fresh cubes of diet.

Two artificial diets were used, a high-protein diet containing 36 g casein/L (17.7% casein by dry weight) and a low-protein diet containing 12 g casein/L (5.9% casein by dry weight). (See Woods and Chamberlin 1999 for a list of the remaining dry ingredients, the same for both diets.) The high-protein diet is the usual food supplied to colony caterpillars. Twenty-four grams of  $\alpha$ -cellulose (indigestible, nonnutritive) was added to the low-protein diet, which held constant the concentrations of all other ingredients. Additional protein was present in two other ingredients, wheat germ and Torula yeast (20 g and 7.2 g/L diet, respectively); thus, total protein in the two diets (dry mass basis) was 30.9% and 19.2%.

### Growth Rate

Larvae were reared through the molt to the fifth instar on either high- or low-protein artificial diet and then were transferred in early to middle fifth instar (at least 1 d following the commencement of feeding) to the same or opposite diet for 24-h trials (2 × 2 factorial design; 38–40 larvae/treatment). Larvae reared on low protein generally molted to the fifth instar 1 d later than larvae reared on high-protein diet and thus were transferred to the test diet 1 or 2 d later. Larval fresh mass was recorded before and after the trial, and growth rate was calculated as final minus initial mass divided by trial length.

### Consumption Rate and Efficiency

For insect herbivores generally, consumption rate increases with decreasing protein concentration, a response that may be compensatory (Slansky and Scriber 1985; Simpson and Simpson 1990). Food consumed and frass produced by larvae in the four experimental groups were determined during the 24-h growth trials. At the beginning of the test period, larvae were placed on preweighed cubes of diet (masses chosen so that larvae would consume more than half of the available food). After 24 h, food remaining was separated from frass, and both were dried at 60°C for 3 d and reweighed. Total fresh consumption rate was calculated as initial cube mass (fresh) minus the final cube mass (dry) divided by fraction dry mass of the food, all divided by trial length. The fraction dry mass of each batch of diet was determined from at least three samples approximately the same size as cubes given to larvae.

Efficiency of food use (dry mass basis) was analyzed with utilization plots (Raubenheimer and Simpson 1994). Larval dry

Table 1: Summary of *F* ratios from two-factor ANCOVA of *Manduca sexta* growth rate, consumption rate, and midgut proteolytic activity, using log initial mass as a covariate and rearing and test diets as factors

Source of Variation	df	Growth Rate	Consumption Rate	Proteolytic Activity
Log initial mass (M) .....	1	81.2***	506***	10.13**
Rearing diet (R) .....	1	97.0***	61.1***	.305
Test diet (T) .....	1	.07	188***	26.98***
M × R .....	1	2.80	4.26*	22.07***
M × T .....	1	.35	1.20	.039
R × T .....	1	.01	1.01	.622
M × R × T .....	1	.22	1.54	.389
Residuals .....	149 <sup>a</sup>			

Note. Numbers are *F* values.

<sup>a</sup> Only 145 residual df for proteolytic activity owing to problems with four reactions.

\* *P* < 0.05.

\*\* *P* < 0.01.

\*\*\* *P* < 0.001.

mass before and after the 24-h trials was calculated using the results of a separate experiment. Larvae (15 per group) were reared and tested as described earlier. Following the test period, larvae were weighed, frozen, dried at 60°C for 3 d, and reweighed, and the fraction dry mass was calculated. Dry masses of larvae from 24-h growth trials were then estimated by multiplying the fresh weight by the average fraction dry mass obtained in the separate experiment. Dry consumption was calculated by subtracting the dry mass of remaining food from the wet mass of the initial cube multiplied by the fraction dry mass of the diet. The mass of  $\alpha$ -cellulose was subtracted from the masses of food and frass of individuals receiving the low-protein diet during the test period.

#### Proteolytic Activity

Midgut proteolytic activity determines the rate at which proteins are broken into fragments. In several Lepidoptera, soluble proteolytic activity appears to be stimulated by high protein (Ishaaya et al. 1971; Broadway and Duffey 1986). The proteolytic activity of soluble midgut enzymes was estimated for individual caterpillars in the four treatment groups by measuring in vitro breakdown of azocasein by crude gut extracts (Johnston et al. 1995; Kingsolver and Woods 1997). Specifically, the assay measures (spectrophotometrically) the conversion of azocasein to trichloroacetic acid-soluble fragments (smaller than about 10 amino acids).

At the completion of the growth trials, caterpillars were placed on fresh cubes of diet for 2–4 h, then on ice for 15–30 min. Midguts were dissected free, rinsed with ice-cold saline, and separated from Malpighian tubules, and the midgut contents including peritrophic membranes were placed individually in test tubes, which were centrifuged at 4°C for 15 min at 16,000 g. Next 300  $\mu$ L of the resulting supernatant was removed and frozen at –20°C.

All reactions were carried out at room temperature (about 24°C). Reactions were started by mixing 10- $\mu$ L thawed gut fluid with 500- $\mu$ L reaction solution consisting of 2% azocasein in 50 mM dibasic sodium phosphate, pH 11. This pH was chosen because it is within the range of pHs measured in *M. sexta* midgut (Dow 1984), and it gives high proteolytic reaction rates (H. A. Woods, unpublished data). After 30 min, reactions were stopped by the addition of 500- $\mu$ L 10% trichloroacetic acid. Vials were placed on ice for 30 min, then spun at 4°C for 15 min at 16,000 g. Next 300  $\mu$ L of the supernatant was neutralized with 300  $\mu$ L 1N NaOH and diluted with 600  $\mu$ L reaction buffer, and its optical density (OD) at 450 nm was determined on a spectrophotometer (Beckman DU 640). For each caterpillar, a control tube (accounting for pre- and auto-degraded azocasein and for absorbance by gut extract) was prepared by adding 500  $\mu$ L 10% trichloroacetic acid to 500  $\mu$ L 2% azocasein solution, followed by 10  $\mu$ L gut supernatant. Control ODs (on average 17% of the total OD) were subtracted from experimental ODs.

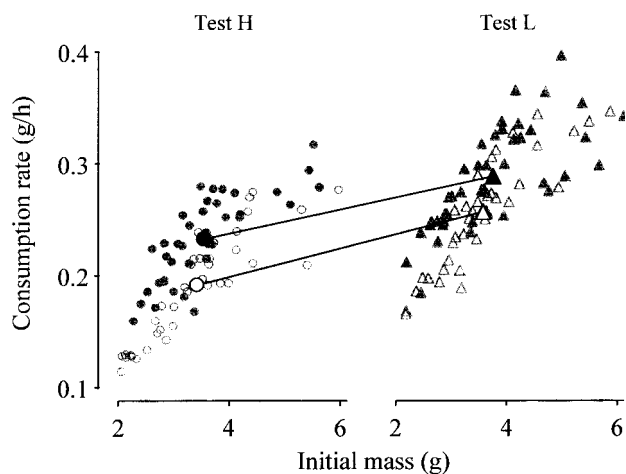


Figure 3. Consumption rate (fresh) by fifth-instar *Manduca sexta*, as a function of initial caterpillar mass. See Figure 2 for a description of the symbols.

#### Mass of Midgut Lumen and Tissue

The midgut lumen and epithelium are the principal sites of protein breakdown and absorption, and compensating for low protein may involve increases in the masses of lumen contents and midgut tissue (Sibly 1981; Yang and Joern 1994). The masses of lumen contents and midgut tissues were determined following dissection of fifth-instar caterpillars. In a separate experiment, caterpillars were reared as described above (11–13 per treatment). After the 24-h test period, caterpillars were chilled (4°C) and dissected into lumen contents (including peritrophic membrane), midgut tissue, and carcass (all remaining tissue). Lumen contents, tissues, and carcasses were dried at 60°C for 3 d and weighed.

#### Statistics

The data were analyzed by ANCOVA (Raubenheimer and Simpson 1992), with rearing diet and test diet as main factors and initial fresh mass (log transformed) or carcass mass (dry) as covariate.

## Results

#### Growth Rate

Fifth-instar *Manduca sexta* reared on high-protein diet grew significantly more rapidly (by about 20%) during the 24-h test period than caterpillars reared on low-protein diet (Fig. 2; Table 1). Growth rate, however, was not significantly affected by the concentration of protein in the diet received during the 24-h test period.

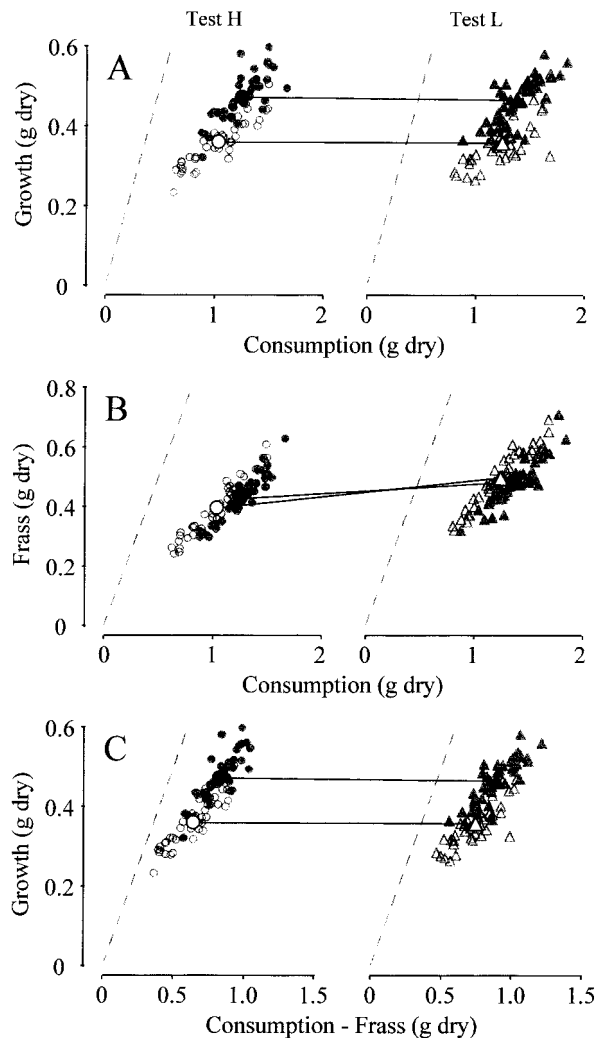


Figure 4. Efficiency of growth (A), nutrient extraction (B), and nutrient conversion (C) by fifth-instar *Manduca sexta* during 24-h trials. See Figure 2 for a description of the symbols.

#### Consumption Rate

Consumption rate during the 24-h test period was significantly affected by both rearing and test diets (Fig. 3; Table 1). Caterpillars reared on low-protein diet consumed food less rapidly than did caterpillars reared on high-protein diet. In contrast, caterpillars tested on low-protein diet consumed food more rapidly than did caterpillars tested on high-protein diet. In addition, there was a significant interaction between initial mass (log transformed) and rearing diet, which reflects the more pronounced separation between rearing groups at lower initial masses.

#### Food Use

The growth obtained (dry) per food consumed (growth efficiency) was significantly affected by both rearing and test diets (Fig. 4A; Table 2). Caterpillars reared on low protein were less efficient than those reared on high protein (apparent as a vertical separation between *filled* and *unfilled symbols*). In addition, caterpillars tested on low protein were less efficient than those tested on high protein (Fig. 4A; apparent as a right-shifting of the cloud of *triangles*). Thus, caterpillars tested on low protein reached roughly the same final mass (*triangles* and *circles* are about the same height) but consumed more food in doing so.

Growth efficiency is separable into two components (Fig. 4B, C). Figure 4B shows the efficiency with which nutrients were extracted from the food (amount of frass produced per food consumed). Caterpillars reared on low protein were significantly less efficient (Fig. 4B; *unfilled symbols* are left shifted; Table 2). In addition, caterpillars tested on low protein were slightly (but significantly) less efficient at extracting nutrients (Fig. 4B; *triangles* are higher than *circles*; Table 2). Figure 4C shows the efficiency with which extracted nutrients were used for growth. Caterpillars were significantly less efficient after having been reared on low-protein diet or when receiving the low-protein diet during the test period, with the rearing effect being larger.

#### Proteolytic Activity

Proteolytic activity of soluble midgut enzymes was affected by test diet but not rearing diet (Fig. 5; Table 1): caterpillars tested on high protein had significantly higher proteolytic activity (mean absorbance, 0.33) than caterpillars tested on low protein (mean absorbance, 0.28; Table 3). Absorbance is approximately isometrically related to amount of azocasein breakdown (H. A. Woods, unpublished data); therefore, caterpillars eating the high-protein test diet broke down approximately 18% more azocasein per unit time than caterpillars eating the low-protein test diet. Four observations were excluded from the analysis because of problems with the reactions. The data also show a significant interaction between initial mass (log transformed) and rearing diet (Table 1). Inspection of Figure 5 suggests that soluble proteolytic activity is not related to initial mass in caterpillars reared on high protein but is positively related to initial mass in caterpillars reared on low-protein diet.

#### Masses of Midgut Contents and Tissue

After accounting for differences in carcass mass between the groups, caterpillars tested on a low-protein diet maintained significantly more food in their midguts than caterpillars tested on high protein (Fig. 6; Table 3). Caterpillars reared on low protein showed the opposite effect, although it was only marginally significant (Table 3;  $P = 0.09$ ). Most of the apparent

vertical separation between the rearing groups (Fig. 6A) is due to the smaller (i.e., left-shifted) carcass masses of caterpillars reared on low protein.

Allocation to midgut tissue was significantly affected by both rearing and test diets. After accounting for carcass mass, caterpillars reared on low-protein diet had heavier midgut tissues. This difference does not appear as a vertical separation of symbols in Figure 6B because the main difference between groups was in the covariate (carcass mass). Similarly, caterpillars tested on low protein had relatively heavier midguts, an effect due to their having slightly higher mean midgut masses and slightly lower carcass masses.

**Discussion**

The experiments support the effect of rearing diet only hypothesis (Fig. 1D), which predicted that rearing diet, but not test diet, would affect growth rate. Caterpillars reared on a high-protein diet grew about 20% more rapidly, regardless of test diet, than caterpillars reared on a low-protein diet (Fig. 2). These data are only partially consistent with studies of other Lepidoptera. Stockhoff (1992) showed that growth rates of second through fourth instar *Lymantria dispar* were lower when larvae were reared and tested on low protein (as in Fig. 1B); fifth instars, while growing slower on a low-protein test diet, were unaffected by the rearing diet (pattern similar to Fig. 1C). Taylor (1989) demonstrated yet another pattern using larval *Samea multiplicalis* reared and tested on high- or low-nitrogen leaves of *Salvinia molesta*. Test diet had a significant effect; larvae grew considerably more slowly on low-nitrogen leaves. The effect of rearing diet, however, depended on the test diet: larvae tested on high-nitrogen leaves grew slower if they had been reared on low nitrogen, but larvae tested on low-nitrogen leaves grew equally rapidly regardless of rearing diet. In the

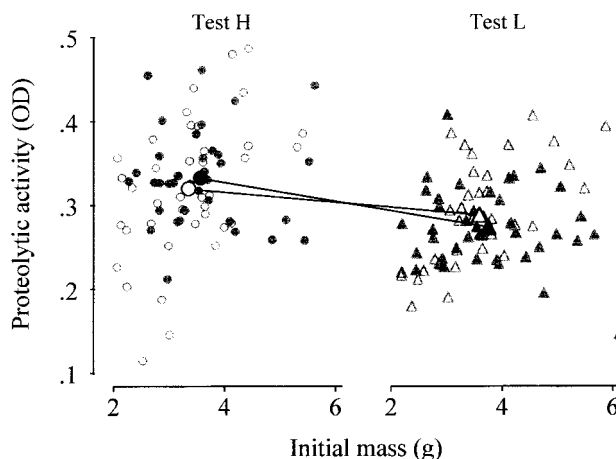


Figure 5. Proteolytic activity in the midgut fluid of fifth-instar *Manduca sexta* following 24-h trials, as a function of initial caterpillar mass. See Figure 2 for a description of the symbols.

absence of data on more species, therefore, generalizations would be premature.

The pattern of growth obtained in this study (Fig. 2) suggests two questions: why did caterpillars reared on low-protein diet grow relatively slowly in the fifth instar? In addition, why did caterpillars tested on low-protein diet grow as rapidly as those tested on high protein? I analyze these questions by comparing the observed pattern of growth rates among treatment groups with the patterns of various steps in protein (and nutrient) acquisition.

*Effects of Rearing Diet*

Caterpillars reared on low-protein diet grew less rapidly in the fifth instar, regardless of diet consumed, than caterpillars reared

Table 2: Summary of *F* ratios from two-factor ANCOVA of *Manduca sexta* efficiency of food use, using consumption or consumption minus frass as a covariate and rearing and test diets as factors

Source of variation	df	Growth (Dry) <sup>a</sup>	Frass (Dry)	Growth (Dry) <sup>b</sup>
Consumption or consumption minus frass (C) .....	1	458 <sup>***</sup>	1,358 <sup>***</sup>	743 <sup>***</sup>
Rearing diet (R) .....	1	168 <sup>***</sup>	127 <sup>***</sup>	104 <sup>***</sup>
Test diet (T) .....	1	41.1 <sup>***</sup>	15.3 <sup>***</sup>	39.9 <sup>***</sup>
C × R .....	1	1.12	1.53	.98
C × T .....	1	.77	1.84	1.24
R × T .....	1	1.02	.72	1.06
C × R × T .....	1	.04	1.27	.73
Residuals .....	149			

Note. Numbers are *F* ratios.

<sup>a</sup> Analyzed with consumption as the covariate.

<sup>b</sup> Analyzed with consumption minus frass as the covariate.

\*\*\* *P* < 0.001.

Table 3: Summary of *F* ratios from two-factor ANCOVA of *Manduca sexta* mass of midgut contents and midgut tissue, using dry carcass mass as a covariate and rearing and test diets as factors

Source of Variation	df	Midgut Contents Mass	Midgut Tissue Mass
Carcass (C) .....	1	60.5 <sup>***</sup>	159 <sup>***</sup>
Rearing diet (R) .....	1	3.05	11.8 <sup>**</sup>
Test diet (T) .....	1	26.4 <sup>***</sup>	7.38 <sup>**</sup>
C × R .....	1	.01	1.45
C × T .....	1	.06	.27
R × T .....	1	.11	.32
C × R × T .....	1	.27	.35
Residuals .....	145		

Note. Numbers are *F* ratios.  
<sup>\*\*</sup> *P* < 0.01.  
<sup>\*\*\*</sup> *P* < 0.001.

on high-protein diet. Mechanisms accounting for this pattern are likely to be those showing reduced capacity in response to low protein. Of the traits measured in this study, consumption rate (Fig. 3) and efficiency (Fig. 4) show the appropriate pattern: individuals reared on a low-protein diet consumed less food, and used it less efficiently, than did individuals reared on a high-protein diet. In addition, the magnitude of decrease in both traits (about 20%) matches the magnitude of decrease in growth.

The patterns of other measured traits do not appear closely related to the pattern of growth rate. Caterpillars reared on low protein maintained less food in the midgut (Fig. 6A), but most of the decrease was accounted for by the smaller body (carcass) sizes. Caterpillars also allocated relatively more tissue to the midgut, a shift that cannot explain the decrease in growth rate. In addition, caterpillars reared on low protein did not maintain consistently lower soluble proteolytic activity in the midgut than did caterpillars reared on high protein (Fig. 5); thus, limitation of protein digestion in caterpillars reared on low protein, a mechanism suggested by Taylor (1989), does not appear to have contributed to lower growth rates.

*Effects of Test Diet*

Caterpillars grew equally rapidly during the 24-h test period, regardless of diet. A different set of mechanisms appears to have contributed to this short-term response. Consumption rate again shows the appropriate pattern: individuals tested on low protein consumed food more rapidly during the 24-h test period, possibly preventing a decline in growth rate. In addition, caterpillars maintained higher (possibly compensatory [Sibly

1981]) masses of food in the midgut (Fig. 6A). Caterpillars also allocated relatively more tissue to the midgut (Fig. 6B), although the increase is of questionable biological significance. Both growth efficiency (Fig. 4) and proteolytic activity (Fig. 5) were lower in caterpillars tested on low protein, physiological responses unlikely to account for the absence of a decline in growth.

Another factor contributing to the short-term independence of growth rate and dietary protein may be that protein acquisition is less critical to fifth instars than to earlier instars. Larval physiology, for example, may be redirected in later instars toward accumulating compounds, such as glycogen (Siegert 1987), that are used during and after metamorphosis.

*Effects of Rearing and Test Diets Considered Together*

The steps of protein acquisition and nutrient use measured in this study are not exhaustive. Two others in particular—digestion of peptide fragments by peptidases associated with the midgut epithelium (Horie and Watanabe 1982; Santos et al. 1983) and absorption of digestion products by the epithelium—are important and may depend on dietary history.

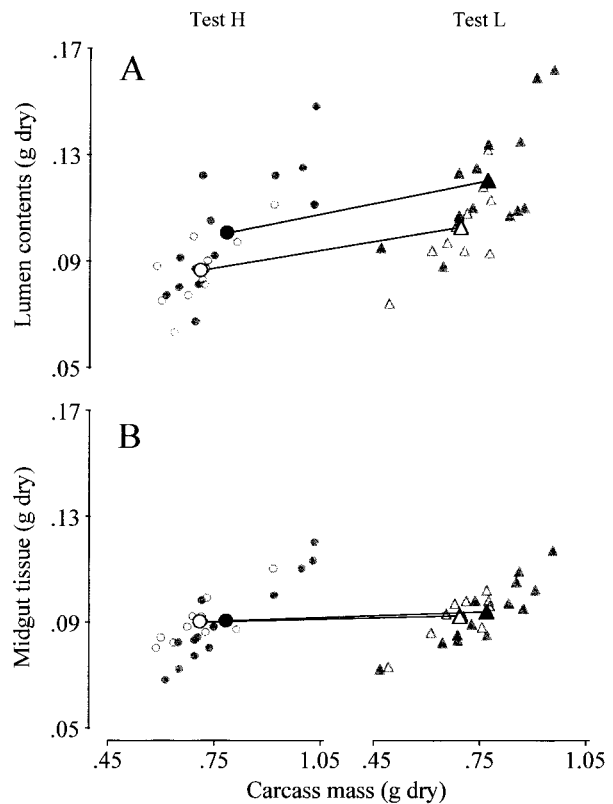


Figure 6. Mass (dry) of midgut contents and midgut tissue from fifth-instar *Manduca sexta* following 24-h trials, as a function of carcass mass. See Figure 2 for a description of the symbols.

No data are available to evaluate the former, and only scant data bear on the latter. Woods and Chamberlin (1999) have shown, using an *in vitro* preparation of posterior midgut from *M. sexta*, that dietary protein does not affect rate of transport of L-proline. Whether transport of other protein breakdown products is similarly unaffected is unknown.

Of the measured mechanisms, consumption emerges as most closely related to growth. During the 24-h test period, caterpillars that received low-protein food consumed greater amounts of it. This result is consistent with compensatory feeding observed in a variety of Lepidoptera and other insects (Slansky and Scriber 1985; Simpson and Simpson 1990), in which individuals appear to "make up" for poor-quality food by eating more of it. Why, then, did caterpillars reared on low protein not also consume food more rapidly in the fifth instar and thereby grow as rapidly as caterpillars reared on high protein?

One possibility is that caterpillars reared on a low-protein diet were unable to consume diet at a high rate, which might have occurred if the feeding musculature or midgut function were physiologically compromised by insufficient protein in early instars. Caterpillars reared and tested on low protein, however, consumed more rapidly than those reared and tested on high protein (Fig. 3). If consumption were limiting, it seems that caterpillars switched from the low-protein rearing diet to the high-protein test diet would have continued to eat at the same (presumably maximal) rate. Instead, those switched to high protein consumed food less rapidly.

Two other explanations appear more likely. First, low protein during early instars may have reduced the capacity of post-absorptive processes to transform nutrients into tissue. If so, fifth-instar caterpillars may have consumed low-protein food at lower rates to avoid nutrient (e.g., amino acid) excess (Harper 1970; Martin and Van't Hof 1988). A second, related explanation is that low protein during early instars altered a feedback loop between hemolymph nutritional status and feeding behavior (Simpson and Simpson 1992; Timmins and Reynolds 1992; Simpson and Raubenheimer 1993a), such that dietary protein at any concentration was less likely to stimulate and maintain feeding by larvae reared on low protein. Alteration of a feedback loop could provide a proximate behavioral mechanism for avoiding nutrient excess.

A fundamentally different hypothesis about the effects of protein concentration on growth concerns nutrient balance. In addition to containing different concentrations of protein, the two diets also contained different ratios of protein to other nutrients. Growth depends on nutrient ratios, and insects may use behavioral and postingestive mechanisms to compensate for nutrient imbalance (Waldbauer et al. 1984; Raubenheimer and Simpson 1993, 1997). Caterpillars appear to perform best on diets containing protein and carbohydrate in roughly equal amounts or, in some cases, more protein-rich diets (Simpson and Raubenheimer 1993b; Waldbauer et al. 1984). In the present study, the high-protein diet (53 : 47, casein : sucrose)

probably was reasonably balanced, whereas the low-protein diet (27 : 73, casein : sucrose) probably provided relatively too much sucrose. Thus, the low growth rates of fifth instars that had been reared on low-protein diet may have been a consequence of nutrient imbalance experienced during earlier stages.

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