FORUM

Diet-Induced Thermogenesis in Insects: A Developing Concept in Nutritional Ecology

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ABSTRACT Diet-induced thermogenesis (DIT) is a concept that has been well known in one form or another for more than a century in vertebrate nutrition and physiological ecology. Yet, it is practically unknown in the physiology and nutritional ecology of insects. We suggest that DIT is a ubiquitous mechanism occurring in most if not all organisms and functions to maintain nutritional homeostasis by metabolically oxidizing excess energy intake to maintain a metabolic pool of nutrients that is balanced in both energy and nutrients. There is sufficient evidence to suggest the phenomenon exits in insects and should be considered as a viable hypothesis to enrich the paradigms of insect nutritional ecology and biological stoichiometry. We demonstrate evidence for DIT in the phytophagous spruce budworm (*Choristoneura fumiferana* [Clemens]). Budworm larvae with the highest dietary metabolizable energy/protein ratio and highest assimilated food are the least metabolically efficient and are apparently able to oxidize excess metabolizable energy intake (i.e., they exhibit diet-induced thermogenesis). Metabolic adaptations such as DIT would allow organisms to use foodstuffs that are high in energy but critically low or unbalanced in essential nutrients to maintain normal growth, survival, and reproduction. Understanding the role DIT plays in nutritional and elemental stoichiometric homeostasis of insects may be an important element in interpreting their nutritional ecology.

KEY WORDS diet-induced thermogenesis, unbalanced diets, biological stoichiometry, spruce budworm, *Choristoneura*, insects

DIET-INDUCED THERMOGENESIS (DIT), an exponential rise in body heat production associated with feeding, is a complex set of energy dissipating physiological processes that have been well recognized in mammals and birds since at least the beginning of the 20th century (Rubner 1902, Kleiber 1975). In fact, various animal physiologists have proposed numerous terms to describe this phenomenon, such as specific dynamic action, heat increment of feeding, thermic effect of food, and postprandrial thermogenesis (Webster 1981, James 1992). DIT has been subdivided into obligatory DIT_o and regulatory DIT_r (Girardier and Stock 1983) to distinguish the obligatory costs associated with food consumption (i.e., chewing, processing, and biosynthesis) from nonobligatory or regulatory costs. Only recently, special uncoupling genes (UCP1, UCP2, and UCP3) have been discovered that partially regulate DIT in both mice and humans by manufacturing special uncoupling proteins that affect how mitochondria use energy (Flier and Lowell 1997, Ricquier and Bouillaud 2000). Although mostly studied for its influence on weight regulation and thermoregulation in homeotherms (Fleury et al. 1997), DIT may have evolved as part of a fundamental suite of mechanisms for enhancing the uptake of critically limiting nutrients such as nitrogen and phosphorus (Rothwell and Stock 1981). Supporting evidence for the nutrient sequestration enhancement hypothesis comes from numerous energy balance studies of vertebrates indicating that DIT occurs when animals consume low-protein, "unbalanced diets" (Tulp et al. 1979, Donald et al. 1981, Rothwell et al. 1982, 1983, Kevonian et al. 1984, Rothwell and Stock 1987, Trier 1996). Natural foodstuffs are rarely nutritionally balanced (Mattson and Scriber 1987, Raubenheimer and Simpson 1997) and thus, feeding often leads to surfeits of energy and nutrients above metabolic needs. Based on a study of DIT in the herbivorous prairie vole, *Microtus ochrogaster*, Trier (1996) argued for the general relevance of DIT to the nutritional ecology and bioenergetics of animals. Every heterotroph has its own elemental stoichiometric configuration (e.g., C:N:P) which in turn determines its unique dietary sequestration needs and problems (Elser et al. 1996, 2000a, b). Being able to self-select diets (sensu Waldbauer and Friedman 1991) and then to partition inevitable dietary excesses by various mechanisms such as oxidation (DIT), storage, hyperactivity, and excretion by means of special filtration

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systems (as in some Homoptera) may facilitate the acquisition and balancing of limiting nutrients (Kleiber 1945, Rothwell et al. 1982, Zanotto et al. 1997).

Surprisingly, DIT is largely unknown in invertebrates (except as Specific Dynamic Action, e.g., Acosta et al. 1993, Chapelle et al. 1994). Is it because poikilotherms are vastly different physiologically from homeotherms and thus cannot or do not exhibit similar whole organism and cellular level processes associated with food intake and food processing? Instead, we hypothesize that DIT is a universal physiological process and should therefore be demonstrable especially in insects that, like the prairie vole, are confronted with nutritionally mercurial diets. Supporting this general supposition are the following: firstly, we now know that the makeup of the human genome and that of all other species are remarkably similar and demonstrate a convincing unity of life, so that at a very fundamental genetic and biochemical level, even widely disparate species such as humans and *Drosophila* are remarkably alike (Ackerman 2001, Venter et al. 2001). Secondly, and more to the point, at least three earlier studies have already provided tempting evidence that the wax moth (*Galleria melonella* L.) (Jindra and Sehnal 1989), cinnabar moth (*Jacobea tyrae* L.) (McEvoy 1984), and the migratory locust (*Locusta migratoria* L.) (Zanotto et al. 1997) exhibit some of the key features suggestive of DIT (though this term has not been applied). Our data on the spruce budworm in this paper also lend support to the hypothesis that DIT occurs in insects. While substantive verification of this hypothesis is yet to be offered, we propose that there are sufficient shards of evidence to justify further testing for the presence of DIT as a ubiquitous mechanism for coping with unpredictable fluxes in the kinds and amounts of nutrients entering the metabolic pool. If this is substantiated, then physiological and ecological studies addressing growth and energy balance in insects may benefit from considering DIT as one of many fundamental mechanisms for coping with unbalanced diets to maintain nutritional homeostasis (Raubenheimer and Simpson 1997). Furthermore, earlier studies on insect nutritional and physiological ecology may need to be carefully reinterpreted with these fundamental processes in mind.

To test for DIT in a phytophagous insect, we chose the spruce budworm (*Choristoneura fumiferana* (Clemens), Lepidoptera: Tortricidae), a vernal, outbreak species adapted to conifer foliage known for its highly dynamic nutrient content (Lawrence et al. 1997). We measured the physiological responses of sixth instar larvae to artificial diets varying in the proportion of metabolizable energy (ME) to protein per unit of food. We predicted that large nutrient imbalances would substantially alter budworm feeding and thus the amount of energy entering its metabolic pool, thereby affording the chance to test if (1) growth per unit assimilated food (AF) declines with increasing increments of AF, and (2), net metabolic efficiency (growth per unit AF) varies inversely with estimated assimilated energy, that is, ME intake, (3) respiration increases and net efficiency declines as the ratio of ME to protein increases in the diet, and (4) heat production per unit AF increases with increasing increments of AF. Data confirming any of these four specific experimental hypotheses would provide support for the general hypothesis that–just like birds and mammals–DIT also occurs in insects and is driven by nutrient imbalance and the flow of surplus energy into the metabolic pool.

Materials and Methods

Diapausing second instar spruce budworm larvae were obtained from the Insect Rearing Facility, Forest Pest Management Institute in Slt. Ste. Marie, Ontario. We reared the larvae on a modified (reduced N) McMorran diet (i.e., casein ≈54%, and wheat germ ≈92% of the standard McMorran diet) (McMorran 1965) in an incubator at 22 to 23°C, 50% RH, and a 16:8 L:D photoperiod. At the start of the sixth instar, 15 males and 15 females were weighed and randomly allocated to each treatment diet, housed singly in one oz plastic creamer cups, and maintained as before. To minimize experimental error often associated with nutritional indices studies, only enough food was supplied so that the majority of it (at least 75%) was consumed (Schmidt and Reese 1986). After 72 h, frass, silk, larvae, and uneaten food were separated, frozen, and later oven-dried at 55°C to constant weight, and immediately transferred to a special dry-box containing an analytical balance and drying agent, wherein they were weighed after 24 h of final equilibration to an accuracy of 0.01 mg. Though laborious and awkward, the dry-box protocol is crucial because it guarantees that oven-dry weights for very small masses, which can be notoriously labile once out of the oven, are not altered by atmospheric humidity before and during weighing, and therefore ensures accurate, stable dry masses. When materials are handled in this way, we have found that dry weights are remarkably consistent when reweighed even months later.

There were five treatment diets, each with different amounts of cellulose (alphacel) substituted for sucrose on a g/g basis, while keeping N fixed at 2.8% dwt (Table 1). This created five diets differing in % alphacel and estimated ME, ranging from 8.7 kJ/g ME for the lowest fiber diet (22.4% alphacel) and decreasing incrementally to 4.8 kJ/g ME for the highest fiber diet (46.4% alphacel) (Table 1).

Whole-organism growth and traditional nutritional indices were calculated gravimetrically following Waldbauer (1968). Weight-specific relative rates were calculated using the mean exponential larval weight, W_e, where $W_e = G/\ln(W_f/W_i)$. W_f and W_i refer to larvae final and initial dry weights, respectively, and G = growth, in mg (Gordon 1968). Initial dry weights of food and insects were estimated by linear regression of dry weights on wet weights of food and insects matched to those used at the start of all treatments. As stated before, all dry weights were fastidiously measured to 0.01 mg at near zero humidity in a sealed, humidity-controlled drybox.

Table 1. Composition of artificial diets

Ingredients (g)	Diet (% alphacel)				
	22.4	30.5	38.7	44.8	46.4
Sucrose	30.0	20.0	10.0	2.5	0.5
Alphacel	27.5	37.5	47.5	55.0	57.0
Casein	15.0	15.0	15.0	15.0	15.0
Wheat germ	22.0	22.0	22.0	22.0	22.0
Other[a]	28.3	28.3	28.3	28.3	28.3
Total	122.8	122.8	122.8	122.8	122.8
% Nitrogen[b]	2.8	2.8	2.8	2.8	2.8
Metabolizable energy[c] (kJ/g)	8.7	7.4	6.1	5.1	4.8
Metabolizable [energy]/[Protein][d] (kJ/g)	49.7	42.3	34.8	29.1	27.4

[a] See McMorran (1965).
[b] Calculated as: [casein N (g casein × 0.14) + wheat germ N (g wheat germ × 0.06)]/g total (McLaughlin 1986). The N content of alphacel (0.10 % dwt), nor that of agar (0.18 % dwt) was included because neither is probably metabolizable. Likewise the N content of choline chloride (10.0 % N based on dwt of choline chloride) was excluded because it was only 0.8 g out of 122.84 g total dry ingredients.
[c] Calculated as: sucrose at 16.1 kJ/g (Gebhardt and Matthews 1981) + casein at 90.6% protein × 16.7 kJ/g (Rothwell et al. 1982) + wheat germ at 15.1 kJ/g (Netzer 1994); assumes alphacel is nonmetabolizable (Friend 1958).
[d] [Protein] = N/g × 6.25.

Abbreviations for Nutritional Indices Are as Follows. RGR = relative growth rate; TFC = total food consumption; RCR = relative consumption rate; AD = approximate digestibility; AF = assimilated food; ECD = efficiency of conversion of digested or assimilated food; RMR = relative metabolic rate. AD and ECD are expressed as % dry weight. MEI is an estimate of metabolizable energy intake in kJ calculated by multiplying TFC by ME conversion factors calculated from estimates based on nutritional studies of vertebrates (Table 1). ME intake provides an estimate of assimilated energy that is analogous to AF. As a check on our gravimetric efficiency data, we directly measured the energy density (kJ/g) of treated larvae in the highest and lowest fiber diets by bomb calorimetry.

All ratio data generated from nutritional index calculations were first tested for isometry (Packard and Boardman 1988), and homogeneity of variance before applying analyses of variance. Data were subjected to analysis of covariance (ANCOVA) when the covariates were colinear among all treatment groups. If the colinearity assumption was violated, bivariate plots and linear regressions were employed to interpret relationships and compare with the standard ANOVAs of nutritonal indices. If not isometric, adjusted nutritional indices were calculated by regressing the numerator variable of the ratio against its denominator variable, and using the derived slope as an unbiased estimate of the ratio (Sokal and Rohlf 1995).

Results

Sex. Because there were no significant sex effects on key nutritional indices (ECD, AD) and there were no sex by treatment interactions, further discussion is omitted in this report.

Growth Per Unit Assimilated Food Versus Assimilated Food. As expected, the amount of assimilated food per diet decreased sharply with increasing fiber, ranging from ≈42% AD at the lowest to ≈17% AD at the highest dietary fiber loading (Table 2), which resulted in an AF of 30.61 to 15.59 mg, respectively (Table 2). However, efficiencies of conversion of assimilated food into growth (ECDs) increased ($P < 0.0001$) with dietary fiber loading, ranging from 58% at the lowest, to 75 to 84% at the two highest fiber loadings (Table 2). Because ECDs were not isometric for

Table 2. Group mean comparisons of nutritional indices. Treatments were compared using a 2 × 5 factorial model: $\mu+T_i + S_j + TS_{ij} + e_{ijk}$, where S is sex effect (i.e., male or female effect) and TS_{ij} is the interaction term. RMRs were compared using AF as a covariate (test of parallelism: $P = 0.28$) All effects were fixed

Variable	Diet (% alphacel)				
	22.4 ($n = 30$)	30.5 ($n = 29$)	38.7 ($n = 29$)	44.8 ($n = 29$)	46.4 ($n = 30$)
AD (%)	41.82a ± 0.96	32.37b ± 0.55	24.24c ± 0.59	16.91d ± 0.39	17.60d ± 0.36
ECD (%)	57.91a ± 2.0	65.01b ± 0.9	68.42bc ± 1.0	83.60d ± 1.7	75.46e ± 1.3
Growth (mg)	17.2a ± 0.8	15.9ab ± 1.1	14.8abc ± 1.2	11.7c ± 0.89	11.6c ± 0.89
RGR (mg/mg/d)	0.58a ± 0.018	0.55ab ± 0.024	0.50bc ± 0.020	0.45c ± 0.025	0.44c ± 0.016
TFC (mg)	72.87a ± 3.6	75.65a ± 5.0	89.91a ± 7.2	81.44a ± 5.7	86.70a ± 6.0
RCR (mg/mg/d)	2.46a ± 0.07	2.58a ± 0.10	3.00b ± 0.10	3.14b ± 0.15	3.37b ± 0.11
AF (mg)	30.61a ± 1.8	24.80ab ± 1.8	22.24b ± 1.9	13.97c ± 1.1	15.59c ± 1.2
TFC_{ME} (kJ)	0.63a ± 0.03	0.56a ± 0.04	0.54a ± 0.04	0.41b ± 0.03	0.42b ± 0.03
RCR_{ME} (kJ/mg/d)	$0.0213a \pm 0.62 \times 10^{-3}$	$0.0190ab \pm 0.72 \times 10^{-3}$	$0.0182bc \pm 0.55 \times 10^{-3}$	$0.0159c \pm 0.75 \times 10^{-3}$	$0.0162c \pm 0.53 \times 10^{-3}$
RMR (mg/mg/d)	0.251a ± 0.020	0.174b ± 0.007	0.149bd ± 0.006	0.067c ± 0.009	0.106cd ± 0.007

Means are presented ± SEM. Significant differences within rows (among diets) are denoted by different letters (ANOVA and Tukey's HSD test, $P < 0.05$).

Table 3. Tests of isometry. Nutritional indices were tested for isometry in each of five fiber treatment diets by regressing the numerator of each ratio on their respective denominators

Variable	Diet (% alphacel)				
	22% $b_i \pm SE$, a_i[a] r^2	31% $b_i \pm SE$, a_i r^2	39% $b_i \pm SE$, a_i r^2	45% $b_i \pm SE$, a_i r^2	46% $b_i \pm SE_i$, a_i r^2
ECI_{reg}	23.5 ± 0.4 0.99	21.1 ± 0.3 0.99	16.5 ± 0.3 0.99	14.4 ± 0.3 0.99	13.5 ± 0.3 0.99
ECD_{reg}	39.2 ± 4.3, 5.2 0.74	59.2 ± 2.0, 1.3 0.97	61.6 ± 1.5, 1.1 0.99	82.6 ± 1.5 0.99	73.5 ± 1.1 0.99
AD_{reg}	48.6 ± 3.0, −4.8 0.96	33.0 ± 0.4 0.99	25.0 ± 0.3 0.99	17.3 ± 0.4 0.99	20.6 ± 0.8, −2.2 0.96
RCR_{reg}	1.79 ± 0.18, −1.8 0.77	2.60 ± 0.08 0.97	3.67 ± 0.22, −1.7 0.91	3.14 ± 0.11 0.96	3.29 ± 0.09 0.98
RGR_{reg}	0.40 ± 0.05, −4.9 0.73	0.55 ± 0.02 0.96	0.52 ± 0.02 0.98	0.45 ± 0.02 0.95	0.45 ± 0.01 0.97
RMR_{reg}	0.44 ± 0.03 0.88	0.31 ± 0.02 0.93	0.38 ± 0.03, −3.7 0.91	.092 ± 0.01 0.75	0.21 ± 0.02, 1.4 0.75

[a] b_i denotes the slope (± SE) and a_i the intercept in the linear regression model $Y_i = b_i (X_i) \pm a_i$. Intercepts (a_i) are shown only if significantly different from zero ($P < 0.05$). If the intercept is significantly different from zero, then the original ratio is not isometric. All regression slopes are significantly different from zero ($P < 0.05$). r^2 is listed for each analysis.

the three lowest fiber treatment groups (Table 3), which raises questions about the validity of using only simple ratios to interpret the treatment effects on the process of conversion efficiency, we therefore also plotted and regressed growth (the numerator) on assimilated food (the denominator) for each diet (Fig. 1). The pattern, though not identical, was the same. Analysis of variance (ANOVA) indicated a significant difference in slopes among the five diet-specific regression lines ($P < 0.001$), with slopes that were even more disparate than the simple ECD ratios, increasing from 0.39 for the lowest fiber diet to 0.73 to 0.83 for the two highest fiber diets (Fig. 1; Table 3). The slopes represent the mean change in growth per unit change in assimilated food extracted from each diet. In other words, they are unbiased estimates of ECD within the range of the experimental data. Additionally, a contrast of slopes for the highest and lowest fiber groups was also significantly different ($P < 0.001$), indicating a trend commensurate with expectations for DIT.

To explore more generally how budworm growth response per unit of AF responded to changes in the amount of assimilated food extracted across all diets, if at all, we plotted the five regression slopes ($S_{ei} = dG_{ei}/dAF_{ei}$) against their respective grand mean assimilated food per diet ($\overline{AF}$). The result was a significant, declining linear function: $S = 1.14 - 0.02\ \overline{AF}$, ($r^2 = 0.97$) (Fig. 2). There is no reason, a priori, to presume that the slope of a linear relationship between x (AF in this case) and y (dG_{ei}/dAF_{ei}) is going to vary with the mean of x values studied. Therefore, the null hypothesis (slope = 0) is rejected, favoring the interpretation that the slope does in fact change. In other words, the anabolic process that the slope represents is not invariant with respect to substantive changes in mean AF. This further substantiates the hypothesis that growth increment per unit increment in AF declines with increasing AF intake, and that it may decline in a linear fashion. And, conversely, it supports the hypothesis that DIT increases with AF intake.

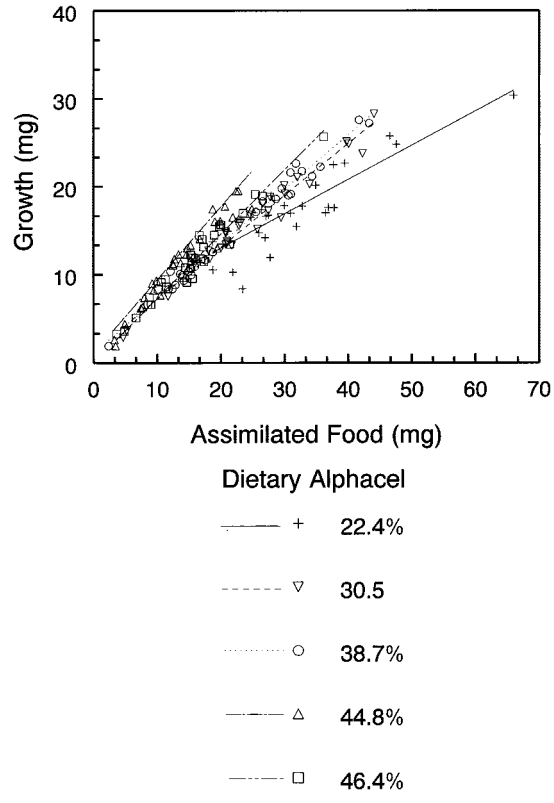

Fig. 1. Relationship between budworm growth and assimilated food. Individual regression equations for mg growth (y) on mg assimilated food (x) are as follows: 22.4%: $y = 5.24+0.39x$, $r^2 = 0.75$, $P < 0.0001$, $n = 30$; 30.5%: $y = 1.28+0.59x$, $r^2 = 0.97$, $P < 0.0001$, $n = 29$; 38.7%: $y = 1.18+0.62x$, $r^2 = 0.98$, $P < 0.0001$, $n = 29$; 44.8%: $y = 0.83x$, $r^2 = 0.99$, $P = 0.0001$, $n = 29$; 46.4%: $y = 0.73x$, $r^2 = 0.99$, $P < 0.0001$, $n = 30$. A regression ANOVA of slopes indicated significant differences among treatments ($P < 0.001$) and a contrast of the lowest fiber group and highest fiber group revealed that slopes differed significantly ($P < 0.001$).

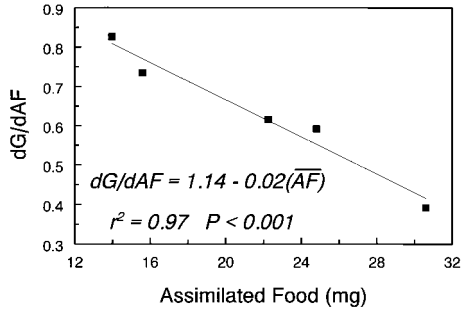

Fig. 2. Relationship between mean budworm growth increment per unit of assimilated food ($S_{ei} = dG_{ei}/dAF_{ei}$) and respective mean assimilated food per insect on five different diets.

The evidence, however, is not unequivocal because declining growth per unit of AF intake could be counterbalanced by more lipids being laid down under conditions of high AF intake. However, measurements of mean larval energy gain per gram of assimilated food (kJ/g_{AF}) lend support to the DIT hypothesis. Net energy conversion efficiencies (calculated from the ratio of micro-bomb measurements of body energy of 16–20 insects) per unit of assimilated food (g) in the two extreme diets (22.4% versus 46.4%) were significantly different (Student's *t*-test, $P = 0.0001$): 13.7kJ/g_{AF} and 16.8kJ/g_{AF}, respectively.

Growth Per Unit Assimilated Food and Metabolizable Energy Intake. Next, regressing ECD_{reg} (i.e., the slopes from the regressions in Table 3) on estimated metabolizable energy intake (MEI) provided another estimate of the relationship between ECD and energy assimilation: ECD_{reg} consistently and linearly dropped with increasing MEI ($ECD_{reg} = 1.49–1.67MEI$, $r^2 = 0.92$). In short, budworm ECDs appear tightly and inversely coupled to the energy flow across the gut into the metabolic pool. Moreover, declining ECDs cannot simply be attributed to rising obligatory costs of food handling, that is, gathering, mastication, digestion, and defecation, and so forth, because contrary to expectation, ECDs showed a positive logarithmic relationship—not negative—with rising TFC ($ECD_{reg} = -4.2 + 1.1 \log TFC$, $r^2 = 0.36$). This implies that the obligatory costs of feeding must be small relative to regulatory costs, and hence are greatly overshadowed by them.

Growth Per Unit Assimilated Food and Respiration Rate Vary with Metabolizable Energy to Protein Ratio of Diets. Changes in ECD and estimated respiration rate (RMR) were also linked—but in opposite ways—to the balance of nutrients in the diet. ECD_{reg} declined ($r^2 = 0.88$) and relative metabolic rates (RMR_{reg}, mg/mg/d) increased ($r^2 = 0.64$) as the ratio between the concentrations of ME to protein in the diet increased (N was held constant in the diets) (Fig. 3). Furthermore, covariance analysis of RMRs (covariate = AF) substantiated that, given equivalent intakes, larvae on low fiber, high ME diets had higher RMRs per unit of AF than those on high fiber, low ME diets (Table 2).

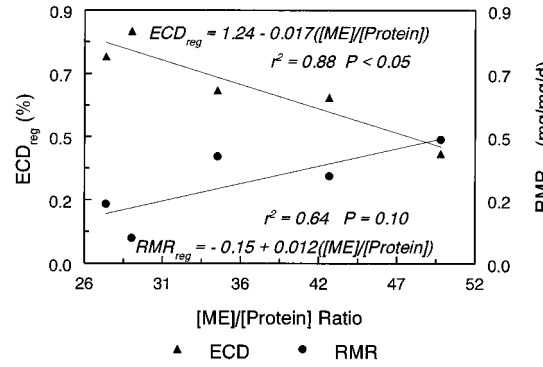

Fig. 3. ECDs decline and RMRs increase as the ratio of metabolizable energy to dietary protein increases, suggesting that DIT occurs as a homeostatic response to dietary imbalance.

Heat Production Per Unit Assimilated Food and AF Intake. We also found that heat production (HP) per unit AF increased as AF increased. We estimated HP gravimetrically as HP = AF-G, and then regressed HP on AF for each of the five treatments, the slope of each line being an estimate of the extent of DIT (Fig. 4) (Gabarrou et al. 1997). ANOVA substantiated that slopes differed among treatments ($P < 0.001$) and a contrast of the lowest fiber group (22.4% alphacel, $HP = -5.2 + 0.6AF$) and highest fiber group (46.4% alphacel, $HP = -0.8 + 0.3AF$) revealed significantly different slopes ($P < 0.001$), thus demonstrating different DIT responses occurring in directions commensurate with predictions, that is, the low fiber group had the higher DIT response.

Discussion

A basic tenet of vertebrate nutrition is that as ME intake increases, metabolic heat production increases exponentially, that is, increments of ME intake lead to a declining efficiency of energy retention (Blaxter and Boyne 1978, Webster 1983). This commonly has been termed diet-induced thermogenesis (Trayhurn and James 1981). We propose that a similar phenomenon, but here-to-fore largely unrecognized as such, may be the well-known negative correlation between ECD and AD which has been reported for hundreds of species of insects (Scriber and Slansky 1981, Slansky and Scriber 1985). Of course, there could be other plausible explanations as well, such as pure measurement error (Schmidt and Reese 1986). Because ADs are often—but not always—positively linked to ME intake, stronger evidence for DIT would be a negative correlation between ECDs and ME intake. This is exemplified in the means from Table 2, when ECD is regressed on TFC_{ME} and similarly, AF: $ECD = 121.4–100.3TFC_{ME}$, $r^2 = 0.93$, $P < 0.01$, and $ECD = 100.3–1.4AF$, $r^2 = 0.95$, $P < 0.01$. Similarly, the southern army worm, *Spodoptera eridania,* adjusted its consumption in relationship to the varying AD's of diets diluted with different amounts of fiber such that its ME intake

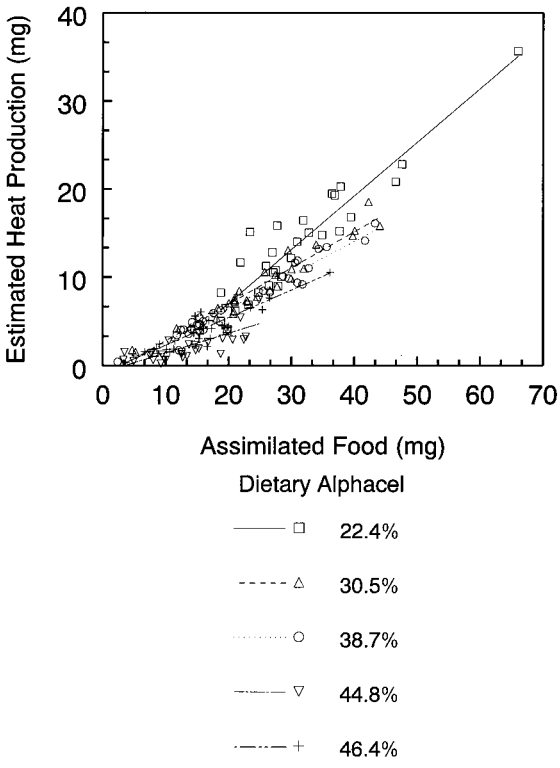

Fig. 4. Individual regression equations for estimated heat production (HP) (y) in mg regressed on mg assimilated food (AF) (x) are as follows: 22.4%: $y = -5.2 + 0.61x$, $r^2 = 0.87$, $P < 0.0001$, $n = 30$; 30.5%: $y = -1.28 + 0.41x$, $r^2 = 0.94$, $P < 0.0001$, $n = 29$; 38.7%: $y = -1.18 + 0.38x$, $r^2 = 0.96$, $P < 0.0001$, $n = 29$; 44.8%: $y = 0.17x$, $r^2 = 0.83$, $P = 0.0001$, $n = 29$; 46.4%: $y = 0.26x$, $r^2 = 0.95$, $P < 0.0001$, $n = 30$.

remained invariant and so did its ECD (Peterson et al. 1988). But Gordon (1968) found that when *Blatella germanica* fed on a diets diluted with fiber, ME, intake plummeted and ECD increased. Gordon argued that when ME is reduced, insects compensate by shunting larger proportions of ME into "higher-priority" growth processes. He further hypothesized that low ECDs can result when "one or more essential nutrients is deficient," leading to "excess-nutrient removal-catabolism" (Gordon 1968). The Mitchell hypothesis (Mitchell 1934) proposed the same idea for vertebrates, that is, "balanced" diets are more efficiently used, having the lowest DIT, and DIT may be a mechanism for "removal of excess food–in the interests of physiological efficiency." More recently, Zanotto et al. (1993, 1997) demonstrated that locusts show enhanced respiration rates in response to excess ingestion of carbohydrate, suggesting a homeostatic "wastage respiration" occurs in these herbivorous insects.

Slansky and Feeny (1977) argued that for phytophagous insects, natural selection is likely to favor a "power" instead of an "efficiency" strategy, that is, consuming high volumes of food but processing it at low efficiency. Such a strategy begs for mechanisms for distilling the limiting nutrients from the plethora of chemicals. That insects are known to use low-efficiency metabolic pathways such as futile cycles (Newsholme et al. 1972) and oxidative phosphorylation uncoupling (Jindra and Sehnal 1990) suggests that they, like mammals, possess the metabolic machinery to facilitate DIT, although we now know that one type of cycle, the triglyceride/fatty-acid substrate cycle, apparently does not mediate DIT in migratory locusts (see Zanotto et al. 1997). We propose as an important hypothesis that DIT is probably an underpinning mechanism in all organisms, operating across levels of organization from cells to whole organisms. In fact, the recently discovered vertebrate genes UCP1 (in brown fat), UCP2 (in most tissues), and UCP3 (primarily in skeletal muscles) that give rise to special uncoupling proteins that mediate how cells use energy and apparently influence an organism's propensity for obesity, may turn out to be widespread (Fleury et al. 1997, Flier and Lowell 1997, Ricquier and Bouillaud 2000).

We stress the apparent universality of the Mitchell hypothesis (Mitchell 1934), that unbalanced diets result in lowered efficiencies of diet utilization. Hamilton clearly demonstrated this in rats (Hamilton 1939), and House (1969) declared it a "basic law" of insect nutrition. Not surprisingly, optimal growth and survivorship for many herbivorous insects occurs at midrange dietary N concentrations (Brewer et al. 1985, Broadway and Duffey 1986), and highly digestible foodstuffs often (but not always) result in low ECDs in no-choice studies (Bauce et al. 1994). To correctly interpret performance indices in dietary studies, it is critical that ME intake as well as nutrient concentrations be considered, especially where variations in dietary diluents can influence AD and hence the flow of energy and nutrients into the metabolic pool. Thus, although the consensus is that insect performance is often limited by the availability of N, it may be that the role of energy has been underappreciated, thereby preventing the accurate assessment of the potential food quality of host plants. Truly poor quality foodstuffs will be low not only in essential nutrients, but also in metabolizable energy (owing to dilution by recalictrant, refractory substances such as cellulose, hemicellulose, lignin, silica, calcium oxalate, or tannins) to hinder the herbivore strategy of increasing food consumption and then 'wasting' excess energy to gain essential nutrients (Mattson and Scriber 1987, Peterson et al. 1988).

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References Cited

Ackerman, J. G. 2001. Chance in the house of fate: a natural history of heredity. Houghton Mifflin Co., New York.

Acosta, F.A.M., C. M. Correa, and G. J. Rodriguez. 1993. Effect of zinc on the specific dynamic action of the freshwater shrimp *Macrobrachium olfersii* (Wiegmann, 1836). Acta Cient. Venez. 44: 292–296.

Bauce, E., M. Crepin, and N. Carisey. 1994. Spruce budworm growth, development and food utilization on young and old balsam fir trees. Oecologia 97: 499–507.

Blaxter, K. L., and A. W. Boyne. 1978. The estimation of the nutritive value of feeds as energy sources for ruminants and the derivation of feeding systems. J. Agric. Sci., Camb. 90: 47–68.

Brewer, J. W., J. L. Capinera, R.E.J. Deshon, and M. L. Walmsley. 1985. Influence of foliar nitrogen levels on survival, development, and reproduction of western spruce budworm, *Choristoneura occidentalis* (Lepidoptera: Tortricidae). Can. Entomol. 117: 23–32.

Broadway, R. M., and S. S. Duffey. 1986. The effect of dietary protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua.* J. Insect Physiol. 32: 673–680.

Chapelle, G., L. S. Peck, and A. Clarke. 1994. Effects of feeding and starvation on the metabolic rate of the necrophagous Antarctic amphipod *Waldeckia obesa* (Chevreux, 1950). J. Exp. Marine Biol. Ecol. 183: 63–76.

Donald, P., G. C. Pitts, and S. L. Pohl. 1981. Body weight and composition in laboratory rats: effects of diets with high or low protein concentrations. Science 211: 185–186.

Elser, J. J., D. R. Dobberfuhl, N. A. MacKay, and J. H. Schampel. 1996. Organism size, life history and N:P stoichiometry. BioScience 46: 674–684.

Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Killham, E. McCauley, K. L. Schultz, E. H. Siemann, and R. W. Sterner. 2000a. Nutritional constraints in terrestrial and freshwater food webs. Nature (Lond.) 408: 578–580.

Elser, J. J., R. W. Sterner, E. Gorokhova, W. F. Fagan, T. A. Markow, J. B. Cotner, J. F. Harrison, S. E. Hobbie, G. M. Odell, and L. J. Weider 2000b. Biological stoichiometry from genes to ecosystems. Ecol. Lett. 3: 540–550.

Fleury, C., M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M. F. Seldin, R. S. Surwit, D. Ricquier, and C. H. Warden. 1997. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat. Genet. 15: 269–272.

Flier, J. S., and B. B. Lowell. 1997. Obesity research springs a proton leak. Nat. Genet. 15: 223–224.

Friend, W. G. 1958. Nutritional requirements of phytophagous insects. Annu. Rev. Entomol. 3: 57–74.

Gabarrou, J. F., P. A. Geraert, M. Picard, and A. Bordas. 1997. Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. J. Nutr. 127: 2371–2376.

Gebhardt, S. E., and R. H. Matthews. 1981. Nutritive Value of Foods. USDA, Washington, DC.

Girardier, L., and M. J. Stock. 1983. Mammalian thermogenesis: an introduction, pp. 1–7. *In* L. Girardier, and M. J. Stock [eds.], Mammalian Thermogenesis, Chapman & Hall Ltd., London.

Gordon, H. T. 1968. Quantitative aspects of insect nutrition. Am. Zool. 8: 131–138.

Hamilton, T. S. 1939. The heat increments of diets balanced and unbalanced with respect to protein. J. Nutr. 17: 583–599.

House, H. L. 1969. Effects of different proportions of nutrients on insects. Entomol. Exp. Appl. 12: 651–669.

James, W.P.T. 1992. From SDA to DIT to TEF, pp. 163–186. *In* J. M. Kinney, and H. N. Tucker [eds.], Energy Metabolism: Tissue Determinants and Cellular Corollaries, Raven Press, Ltd., New York.

Jindra, M., and F. Sehnal. 1989. Larval growth, food consumption, and utilization of dietary protein and energy in *Galleria mellonella.* J. Insect Physiol. 35: 719–724.

Jindra, M., and F. Sehnal. 1990. Linkage between diet humidity, metabolic water production and heat dissipation in the larvae of *Galleria mellonella.* Insect Biochem. 20: 389–395.

Kevonian, A. V., J. G. Vander Tuig, and D. R. Romsos. 1984. Consumption of low protein diet increases norepinephrine turnover in brown adipose of adult rats. J. Nutr. 114: 543–549.

Kleiber, M. 1945. Dietary deficiencies and energy metabolism. Nutr. Abst. Rev. 15: 207–222.

Kleiber, M. 1975. The Fire of Life: an introduction to animal energetics. Robert E. Krieger, Huntington.

Lawrence, R. K., W. J. Mattson, and R. A. Haack. 1997. White spruce and the spruce budworm: defining the phenological window of susceptibility. Can. Entomol. 129: 291–318.

Mattson, W. J., and J. M. Scriber. 1987. Nutritional ecology of insect folivores of woody plants: nitrogen, water, fiber, and mineral considerations, pp. 105–146. *In* F.J. Slansky, J.G. Rodriguez (eds.), Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates, Wiley, Inc., New York.

McEvoy, P. B. 1984. Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae.* Physiol. Entomol. 9: 191–195.

McLaughlin, B. M. 1986. Performance of the spruce budworm, *Choristoneura fumiferana,* in relation to dietary and foliar levels of sugar and nitrogen. Michigan State University Masters Thesis, East Lansing.

McMorran, A. 1965. A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Entomol. 97: 58–62.

Mitchell, H. H. 1934. Balanced diets, net energy values and specific dynamic effects. Science 80: 558–561.

Netzer, C. T. 1994. Complete Book of Food Counts. Doubleday, New York.

Newsholme, E. A., B. Crabtree, S. J. Higgins, S. D. Thornton, and C. Start. 1972. The activities of fructose diphosphatase in flight muscles from bumble-bee and the role of this enzyme in heat generation. Biochem. J. 128: 89–97.

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

Peterson, S. S., J. M. Scriber, and J. G. Coors. 1988. Silica, cellulose, and their interactive effects on the feeding performance of the southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). J. Kansas Entomol. Soc. 61: 169–177.

Raubenheimer, D., and S. J. Simpson. 1997. Integrative models of nutrient balancing: application to insects and vertebrates. Proc. Nutr. Soc. 10: 151–179.

Ricquier, D., and F. Bouillaud. 2000. . Mitochondrial uncoupling proteins: From mitochondria to the regulation of energy balance. J. Physiol. (Lond.) 529: 3–10.

Rothwell, N. J., M. E. Saville, and M. J. Stock. 1982. Effects of feeding a "cafeteria" diet on energy balance and diet-induced thermogenesis in four strains of rat. J. Nutr. 112: 1515–1524.

Rothwell, N. J., and M. J. Stock. 1981. Thermogenesis: comparative and evolutionary considerations, pp. 335–343. *In* L. A. Cioffi, W.P.T. James, and T. B. Van Itallie [eds.], The Body Weight Regulatory System: Normal and Disturbed Mechanisms, Raven Press, New York.

Rothwell, N. J., and M. J. Stock. 1987. Influence of carbohydrate and fat intake on diet-induced thermogenesis and brown fat activity in rats fed low protein diets. J. Nutr. 117: 1721–1726.

Rothwell, N. J., M. J. Stock, and R. S. Tyzbir. 1982. Energy balance and mitochondrial function in liver and brown fat of rats fed "cafeteria" diets of varying protein content. J. Nutr. 112: 1663–1672.

Rothwell, N. J., M. J. Stock, and R. S. Tyzbir. 1983. Mechanisms of thermogenesis induced by low protein diets. Metabolism 32: 257–261.

Rubner, M. 1902. Die Gesetze des Energieverbrauchs bei der Ernahrung. Deuticke, Leipzig.

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

Scriber, J. M., and F. J. Slansky. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183–211.

Slansky, F. J., and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47: 209–228.

Slansky, F. J., and J. M. Scriber. 1985. Food consumption and utilization, pp. 87–163. *In* F. J. Slansky, and J. M. Scriber [eds.], Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon, New York.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W.H. Freeman and Co., New York.

Trayhurn, P., and P. T. James. 1981. Thermogenesis: dietary and non-shivering aspects, pp. 97–105. *In* L. A. Cioffi, W.P.T. James, and T. B. Van Itallie [eds.], The Body Weight Regulatory System: Normal and Disturbed Mechanisms, Raven Press, New York.

Trier, T. M. 1996. Diet-induced thermogenesis in the prairie vole, *Microtus ochrogaster.* Physiol. Zool. 69: 1456–1468.

Tulp, O. L., P. P. Krupp, E. J. Danforth, and E. S. Horton. 1979. Characteristics of thyroid function in experimental protein malnutrition. J. Nutr. 109: 1321–1332.

Venter, J. C., M. D. Adams, E. W. Myers, and P. W. Li. 2001. . The sequence of the human genome. Science 291: 1304–1351.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects, pp. 229–288. *In* J.W.L. Beament, J. E. Treherne, and V. B. Wigglesworth [eds.], Advances in Insect Physiology, Academic Press, London.

Walbauer, G. P., and S. Friedman. 1991. Self-selection of optimal diets by insects. Annu. Rev. Entomol. 36: 43–64.

Webster, A.J.F. 1981. The energetic efficiency of metabolism. Proc. Nutr. Soc. 40: 121–128.

Webster, A.J.F. 1983. Energetics of maintenance and growth, pp. 178–207. *In* L. Girardier, and M. J. Stock [eds.], Mammalian Thermogenesis. Chapman & Hall Ltd., London.

Zanotto, F. P., S. M. Gouveia, S. J. Simpson, D. Raubenheimer, and P. C. Calder. 1997. Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? J. Exp. Biol 200: 2437–2448.

Zanotto, F. P., S. J. Simpson and D. Raubenheimer. 1993. The regulation of growth by locusts through postingestive compensation for variation in the levels of dietary protein and carbohydrate. Physiol. Entomol. 18: 425–434.

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