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The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore

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Summary

The present study evaluates the interactive effects of protein quality and the ratio of protein to digestible carbohydrate on herbivore performance, various aspects of nutritional homeostasis (feeding and post-ingestive regulation) and food choice. In the first experiment, final-instar caterpillars of generalist-feeding African cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) were confined to one of 20 diets varying in protein:carbohydrate (P:C) ratio (35:7, 28:14, 21:21, 14:28 and 7:35) and in the quality of dietary protein (0%, 20%, 50% and 80% replacement of casein-based protein mix with the low-quality plant protein, zein). Results indicated that the negative impact of low-quality protein on survival, development and growth was amplified as the P:C ratio of the diet fell. Consumption differences were the main underlying cause of this response. The effect of low protein quality was to reduce the efficiency of nitrogen utilization and to increase the conversion rate of ingested nutrient to lipid growth, irrespective of P:C ratio. In the second experiment, caterpillars were allowed to self-compose their preferred diet, selecting between one of four high-protein diets (all 35:7, but containing 0%, 20%, 50% or 80% zein) and one of four equal-P:C-ratio diets (21:21, again containing 0%, 20%, 50% or 80% zein). Caterpillars showed a compensatory increase in their self-selected P:C intake in response to the moderate decline in protein quality of the 35:7 food. No such response was demonstrated for the insects presented with 35:7 food with the lowest protein quality. The significance of these findings is discussed within the context of herbivore food selection.

Key words: carbohydrate, caterpillar, feeding, food selection, foraging, insect herbivore, macronutrient imbalance, nutrient regulation, performance, protein quality.

Introduction

The nutritional value of host plants has profound effects on the ecology, behaviour and physiology of herbivores and is determined by multiple traits, such as the quantity and quality of various nutrients, leaf toughness, water content and secondary chemistry (Bernays and Chapman, 1994; Schoonhoven et al., 1998). Plants vary considerably in the mixture and concentrations of nutrients. Dietary protein and carbohydrate are the two macronutrients that have featured most extensively in the literature on herbivore nutritional ecology, with particular reference to their influences on performance and food selection (Simpson and Simpson, 1990; Waldbauer and Friedman, 1991; Simpson and Raubenheimer, 1993a). Protein is a source of nitrogen for growth and maintenance of tissues, production of enzymes, etc., as well as a source of metabolic energy. Besides being the major source of metabolic energy, carbohydrate is used for structural purposes (cuticle deposition) as well as being converted into body lipids and some non-essential amino acids (O'Brien et al., 2002).

The quantity of dietary protein (or nitrogenous nutrients more generally) has long been recognized as a limiting factor for herbivore survival, growth and fecundity (McNeill and Southwood, 1978; Mattson, 1980; Scriber and Slanksy, 1981; White, 1993). However, there is a growing consensus that the nutritional quality of protein is also of critical importance (Horie and Watanabe, 1983; Broadway and Duffey, 1988; Karowe and Martin, 1989; Felton, 1996). Protein quality is primarily the function of amino acid composition (Lehninger et al., 1993). An imbalanced amino acid composition is associated with reduced growth (Briegel, 1985; Karowe and Martin, 1989). The quality of plant protein is highly variable among different species (Yeoh et al., 1992), changes in response to herbivory (Felton et al., 1992; Bi et al., 1994), and may be subject to both environmental and genetic variation (Felton, 1996). This variability of plant protein quality is likely to impose selective pressure shaping the behavioural and physiological adaptations of herbivores, but few studies have rigorously tested this possibility.

Insect herbivores are well known for their capacity to regulate the intake of both protein and carbohydrate to a certain amount and mixture, where they can meet their optimal nutrient requirement (Chambers et al., 1995; Simpson and Raubenheimer, 2000; Raubenheimer and Simpson, 2004). There is evidence that failure to reach this 'intake target' incurs a significant performance cost as a consequence of eating too much of one nutrient and too little of the other (Simpson et al., 2004). However, the question remains unanswered how the protein–carbohydrate balance interacts with variations in protein quality. Predictions have been made earlier that any negative impact of low-quality protein on herbivore performance will be magnified with the limitation of overall protein availability in the food (Hinks et al., 1993; Felton, 1996; Anderson et al., 2004).

The aim of the present study was first to investigate interactive effects of the ratio of protein to digestible carbohydrate and the quality of protein in the diet on herbivore feeding behaviour, post-ingestive processing and performance (e.g. survival, growth and development). Having established the nature of this potential interaction, the pattern of food selection was studied to seek evidence for compensatory changes in feeding by herbivores in response to low-quality protein. While a number of studies have described how compensation by insect herbivores buffers the effect of nutrient dilution or imbalance (reviewed in Simpson and Simpson, 1990; Slansky, 1993), relatively little is known about food choice with regards to varying protein quality in the diet. It has previously been shown that a greater quantity of low-quality protein is needed by caterpillars to support maximal growth than a high-quality protein source (Bloem and Duffey, 1990). It is therefore likely that an adaptive response from caterpillars would be to increase their protein intake when encountering low-quality protein diet, in an attempt to secure an equivalent performance outcome to that achieved by eating a high-quality protein source. However, eating more of an imbalanced protein source to gain limiting amino acids will be associated with eating more than is required of other amino acids, requiring that these are voided (Zanotto et al., 1994). All experiments were conducted using the generalist-feeding caterpillars of *Spodoptera littoralis* (Boisduval).

Materials and methods

Insects

Caterpillars of *Spodoptera littoralis* (Boisduval) came from a laboratory culture at the NERC Centre for Ecology and Hydrology, Oxford, UK, and were reared on a wheat-germ based semi-artificial diet until they had reached their final stadium. During this pre-experimental period, they were cultured under crowded conditions at a density of 150–200 individuals in a plastic container ($25\times25\times45$ cm^3).

Synthetic foods

Preparation of synthetic foods was based on the method described in Simpson and Abisgold (Simpson and Abisgold, 1985). All 20 diets contained a fixed total amount of protein plus digestible carbohydrate (42% by dry mass), but differed in the ratio of protein to digestible carbohydrate [35% protein with 7% carbohydrate (35:7), 28:14, 21:21, 14:28 or 7:35] and in protein quality. In each P:C, the protein content of the control diets was composed of a 3:1:1 mixture of casein:peptone: albumen [which provides a similar amino acid profile to seedling wheat (Simpson and Abisgold, 1985)]. The gradient of protein quality was generated by replacing 20%, 50% and 80% (dry mass) of this predominantly casein-based protein mixture with equivalent amounts of zein. Zein is a maize-derived storage protein that is considered of low nutritional quality for noctuid caterpillars, attributable to its deficiency of certain essential amino acids [e.g. lysine and tryptophan (Broadway and Duffey, 1988; Karowe and Martin, 1989)]. Preliminary bioassay also showed 100% mortality for *S. littoralis* larvae on all five P:C diets in which the protein content was composed entirely of zein (K.P.L., unpublished data). The substitution of zein for casein-based protein mixture allowed manipulation of the dietary protein quality without changing the total protein quantity in the diets. Sucrose was used as the source of digestible carbohydrate in the diets. Diets all included 4% of essential micronutrients (salts, vitamins, cholesterol and linoleic acid). The remaining portion of the food was 54% of the indigestible bulking agent, cellulose. The dry, granular diets were presented to the insects suspended at a 1:6 ratio in 1% agar solution.

Protocol

Two separate experiments were performed. The first was a no-choice feeding experiment in which the caterpillars were restricted to one of 20 diets. In the second experiment, they were allowed to self-compose their preferred diet from one of 16 food pairings. In this choice experiment, one of four equal-P:C-ratio diets (21:21) that had different protein qualities was paired with one of four protein-biased diets (35:7) that also varied in their protein quality. A total of 10 replicates was used per treatment in both experiments.

Upon moulting to the final stadium (day 0), caterpillars were weighed to the nearest 0.1 mg (obtaining initial fresh mass), and each was placed into its own experimental arena, a 9 cm-diameter Petri dish that had five 1 mm-diameter perforations in the upper lid to allow ventilation. During the experimental period, individual insects received either a single (no-choice) or two food blocks (choice), that were weighed to the nearest 0.1 mg before being presented to the caterpillars. The fresh mass of each block ranged between 1300–2000 mg to ensure that food was never deprived but the surplus was minimal, thus improving the accuracy of intake estimates (Schmidt and Reese, 1986). Once the food and insects were inside, the Petri dishes were sealed with a strip of Parafilm$^{\mathrm{TM}}$ to minimize desiccation of the food block, and maintained at 27°C under a 12:12 light:dark photoregime. After each 24 h period, any remaining food was removed from the arena and replaced with a fresh block. Removed blocks were dried to constant mass at 50°C and subsequently weighed to the nearest 0.1 mg. This procedure was repeated until each caterpillar had ceased to feed prior to pupation. Food consumption was calculated as the difference between the initial dry mass of the food (estimated from the initial fresh mass of the food using regression equations) and the final dry mass of the food. The dry mass of food consumed provided a measure of protein and carbohydrate consumption.

When insects pupated, the duration of their final stadium was recorded to the nearest day. Pupae were killed by freezing and dried to constant mass in a desiccating oven at 50°C. Dried carcasses were weighed to the nearest 0.1 mg and lipid-extracted in three, 24 h changes of chloroform before being re-dried and re-weighed. Lipid content was estimated from their mass change. This chloroform extraction procedure had earlier been shown to be >98% efficient, relative to Soxhlet distillation (Simpson, 1983). The lipid-free carcasses were then analyzed for nitrogen content using the micro-Kjeldahl procedure. The starting lipid and nitrogen content of each caterpillar were estimated from linear regression equations established from a sample of 20 freshly moulted final-instar larvae. The lipid and nitrogen growth were calculated by subtracting these starting content from the pupal content.

Statistical analysis

General linear modeling procedures were used to analyse consumption, pupal dry mass and growth data. These analyses were undertaken using PROC GLM in SAS v. 8.2 for Windows (SAS Institute, Cary, NC, USA). Efficiencies of conversion of ingested nutrient to body growth were investigated using ANCOVA with nutrient consumption data as covariates, to avoid the statistical and interpretive problems associated with ratio-based nutrient indices (Raubenheimer and Simpson, 1992). Prior to these parametric analyses, the data were checked for normality of residuals and homogeneity of variance using Kolmogorov–Smirnov and Bartlett's test, respectively. Any effects due to experiment factors on time data (e.g. stadium duration) were tested using the accelerated failure-time analysis (PROC LIFEREG using Weibull distribution).

Results

Experiment 1: no-choice experiment

Performance consequences

Two insects were excluded from the experiment because they failed to accept the food within the first 2 days. Survival data indicated a strong interaction between dietary P:C ratio and protein quality (Fig. 1A). Mortality rose progressively with the increasing ratio of zein in the protein component when dietary P:C was low, whereas caterpillars all survived in the high P:C ratio treatments, irrespective of the zein content of protein. The analyses of stadium duration and pupal dry mass were undertaken using only the three low-zein diets (0%, 20% and 50%) because of missing data for insects on the 7:35 diet with highest zein content (80%). Development time was significantly prolonged as the dietary P:C ratio fell (accelerated failure-time analysis: $\chi_4^2=197.49$, $P<0.001$) and with declining protein quality ($\chi_2^2=29.06$, $P<0.001$) (Fig. 1B). The latter effect was particularly pronounced when the food was extremely carbohydrate-biased (7:35 diet), as indicated by a significant interaction term between dietary P:C ratio and protein quality ($\chi_8^2=34.01$, $P<0.001$). Pupal dry mass was significantly affected by P:C ratio ($F_{4,128}=11.67$, $P<0.001$), protein quality ($F_{2,128}=4.71$, $P=0.011$), and the interaction between the two factors ($F_{8,128}=4.08$, $P<0.001$). The effect of low protein quality was to reduce pupal mass for insects on the low-P:C diets (14:28 and 7:35) but not for those on the higher ratios (35:7, 28:14 and 21:21). There was only a marginal covariate (initial fresh mass) effect on pupal mass ($F_{1,128}=3.78$, $P=0.054$). Fig. 1B,C shows that caterpillars on the lowest protein quality (80% zein) diets suffered greatest performance costs (i.e. delayed development and reduced growth), irrespective of the dietary P:C ratios.

Nutrient consumption

The data from all experimental animals except two individuals that were excluded at the earlier stage of experiment (see above) were used in the analysis of nutrient consumption. This was done to improve the balance of the data set, and yielded essentially the same results from an analysis using the data from those surviving to reach pupation (using type 4 sums of squares in GLM).

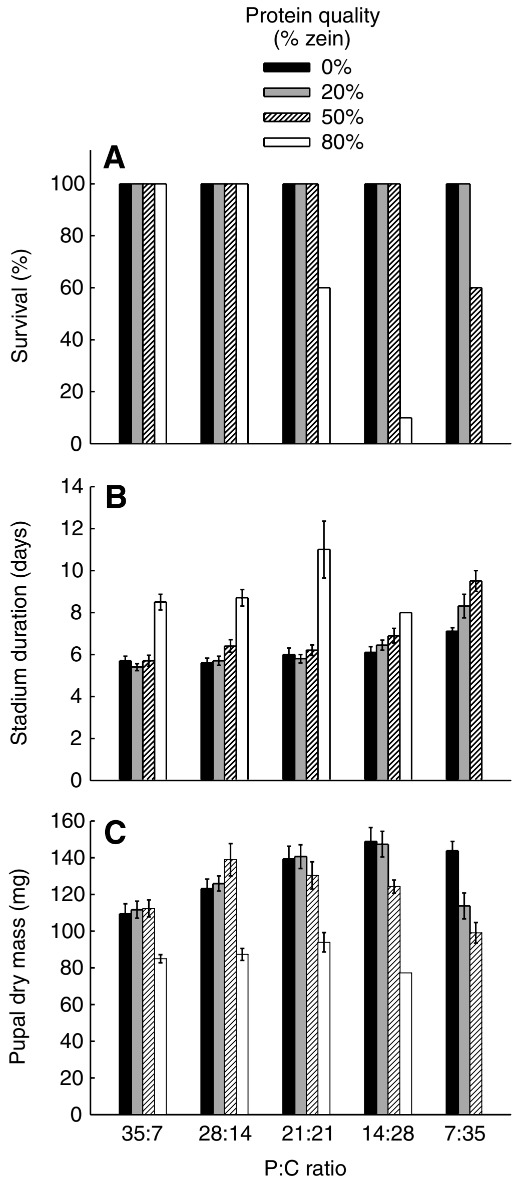

Fig. 1. Patterns of (A) survival, (B) stadium duration and (C) pupal dry mass for final-instar *S. littoralis* caterpillars confined to one of 20 no-choice diets varying in protein:carbohydrate (P:C) ratio (35:7, 28:14, 21:21, 14:28 and 7:35) and in the quality of dietary protein (0%, 20%, 50% and 80% replacement of casein-based protein mix with the low-quality plant protein, zein). Values are means ± 1 s.e.m. (*N*=6–10).

Across development, the cumulative consumption of protein and carbohydrate was significantly affected by dietary P:C ratio, protein quality and the interaction between the two (Table 1). During a fixed period (for instance, days 0–4) (Fig. 2B), the configuration of the intake points connecting the five imbalanced control diets (i.e. intake array) was negative and linear. This straight line later became steeper as the insects on the low P:C diets (7:35 and 14:28) continued to feed beyond day 4. However, most caterpillars on protein-rich diets had completed their feeding by day 4. Caterpillars on the lowest protein quality diets (80% zein) consumed considerably less nutrients (Table 1) and fed for a significantly longer period ($\chi_3^2=154.77$, $P<0.001$) than those given diets with superior protein quality.

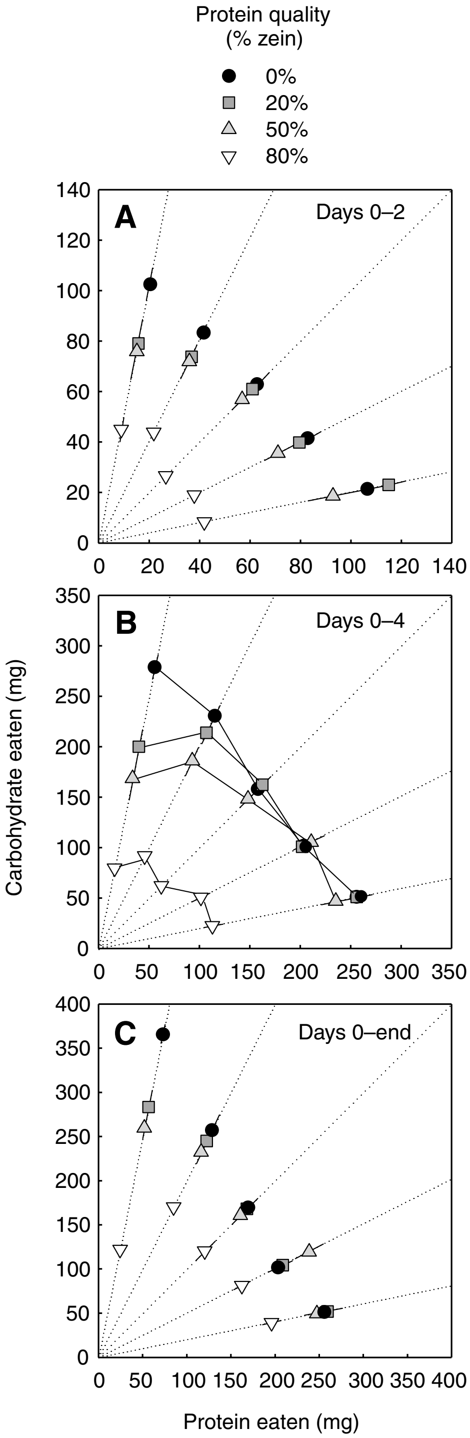

Fig. 2. Bivariate plots for cumulative protein and carbohydrate consumption by *S. littoralis* caterpillars ($N=198$) over the first 2 days (A), 4 days (B) and across the entire feeding period (C) in the no-choice experiment. In each plot, dotted lines radiating from the origin indicate the P:C ratios for 7:35, 14:28, 21:21, 28:14 and 35:7 diets (from left to right) where caterpillars were restricted, respectively. In plot B, the five intake points are connected by solid lines (the intake array) within each level of protein quality to demonstrate the pattern of nutrient balancing. Values are means ± 1 s.e.m. ($N=9$–10).

The degree of the reduction in consumption with deteriorating protein quality was greatest when the food was extremely imbalanced in the direction of low P:C ratio (Table 1). This pattern of interaction was clearly illustrated when the nutrient intake was compared across the three levels of protein quality (0%, 20%, and 50% zein) (Fig. 2B). For example, on the two lowest P:C diets, animals consumed fewer nutrients as the protein quality declined moderately (20% and 50% zein) (ANOVA: 14:28 diet, $F_{2,24}=4.63$, $P=0.020$; 7:35 diet, $F_{2,26}=38.23$, $P<0.001$) while consuming almost identical quantities on the three high P:C diets (35:7 diet, $F_{2,26}=0.66$, $P=0.526$; 28:14 diet, $F_{2,26}=0.16$, $P=0.851$; 21:21 diet, $F_{2,26}=0.70$, $P=0.505$).

Nutrient growth and utilization

Insects gained the least amount of body nitrogen when the quality of dietary protein was the lowest (Fig. 3A). Due to missing data, the analyses of nutrient growth and utilization were performed using only three levels of protein quality (0%, 20%, and 50% zein). Nitrogen growth was reduced for caterpillars on the extremely protein-limiting diet (ANOVA: $F_{4,129}=35.43$, $P<0.001$) but tended to be maintained within the range of 8.0–9.3 mg for those across the other four P:C diets, particularly at the two highest quality levels (0% and 20% zein) (Fig. 3A). The effect of lowered protein quality was to reduce nitrogen growth ($F_{2,129}=24.70$, $P<0.001$). As noted by the significant interaction between the two main effects ($F_{8,129}=2.28$, $P=0.026$), the magnitude of such impact depended on P:C, with the most pronounced effect demonstrated on the extremely protein-deficient diet, where even a slight decrease in protein quality (from 0% to 20% zein) caused substantial reduction in nitrogen growth (Fig. 3A).

The conversion efficiency of ingested nitrogen to growth was analyzed using ANCOVA with nitrogen growth as the response variable and protein eaten as the covariate. The least-square means of nitrogen growth produced following ANCOVA (Fig. 3B) correspond to the nitrogen utilization efficiencies (Raubenheimer and Simpson, 2003). The rate of nitrogen conversion was the lowest in the extremely protein-excess 35:7 diet but rose as the P:C ratio decreased ($F_{4,128}=57.00$, $P<0.001$). An effect of varying protein quality was also apparent ($F_{2,128}=103.40$, $P<0.001$), with the efficiency of nitrogen conversion falling with declining protein quality. There was a significant effect due to an interaction between P:C ratio and protein quality ($F_{8,128}=3.10$, $P=0.003$). A significant covariate effect suggests an increase in body nitrogen with protein consumption ($F_{1,128}=484.80$, $P<0.001$).

As illustrated in Fig. 3C, lipid growth increased in proportion to the carbohydrate content of the diet (ANOVA: $F_{4,129}=59.07$, $P<0.001$). A significant interaction between the main effects was detected ($F_{8,129}=4.86$, $P<0.001$), with the effect of protein quality acting in opposite directions depending on P:C ratio. In the high P:C diets (35:7, 28:14 and 21:21), lipid growth tended to be elevated with a lower quality of protein from 0% to 50% zein. In the lowest P:C diet (7:35), however, lipid growth

Table 1. *ANCOVA results for protein–carbohydrate consumption up to day 2, 4 and the last day of feeding by S. littoralis caterpillars in the no-choice experiment*

		Days 0–2		Days 0–4		Days 0–end	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
P:C	4	10.99	<0.001	5.05	<0.001	6.86	<0.001
PQ	3	155.27	<0.001	153.07	<0.001	56.76	<0.001
P:C×PQ	12	2.88	0.001	2.94	<0.001	6.51	<0.001
Initial mass	1	246.33	<0.001	19.67	<0.001	2.47	0.118
Error	177						

The distance moved along the respective rails in Fig. 2 corresponds to nutrient consumption. Initial fresh mass was used as a covariate to adjust for the effects due to size differences between individuals. P:C, protein:carbohydrate ratio; PQ, protein quality.

dropped markedly when protein quality was low, relative to the control diet (0% zein).

Lipid conversion efficiency was investigated using ANCOVA with lipid growth as the response variable and protein and carbohydrate consumption as joint covariates. Results revealed significant effects of both covariates (protein, $F_{1,127}$=30.20, P<0.001; carbohydrate, $F_{1,127}$=240.17, P<0.001). The conversion rate of nutrients to body lipid was low in the excessively carbohydrate-rich treatment (7:35) ($F_{4,127}$=16.85, P<0.001) (Fig. 3D). Lipid utilization increased in response to lowered protein quality across the five P:C diets ($F_{2,127}$=34.58, P<0.001), and there was no significant effect due to the two-way interaction ($F_{8,127}$=1.04, P=0.408).

Experiment 2: choice experiment

Performance consequences

All insects survived the experiment, except for two individuals provided with a food pairing of the two lowest-quality protein diets (80%). The protein quality of the protein-biased diet (35:7) and of the equal-ratio diet (21:21) significantly affected the duration of the final stadium (accelerated failure-time analysis: χ_3^2=116.42, P<0.001 and χ_3^2=68.96, P<0.001, respectively). A significant two-way interaction (χ_9^2=119.15, P<0.001) was due to pronounced prolongation of development observed for caterpillars given a food pairing of the two lowest-quality protein diets (Fig. 4A). Pupal dry mass was influenced by differing protein qualities of the two choice diets (ANCOVA with initial fresh mass as covariate: 35:7 diet, $F_{3,141}$=11.02, P<0.001; 21:21 diet, $F_{3,141}$=4.41, P=0.005). The observed pattern of pupal mass described in Fig. 4B indicated a significant interaction between the two factors ($F_{9,141}$=3.96, P<0.001), as the mass-reducing effect of low-quality protein in the 21:21 diets became apparent when they were paired with the poorest protein quality 35:7 diet (80% zein). There was a significant covariate effect ($F_{1,141}$=15.47, P<0.001), suggesting that heavier pupae were developed from initially heavier larvae.

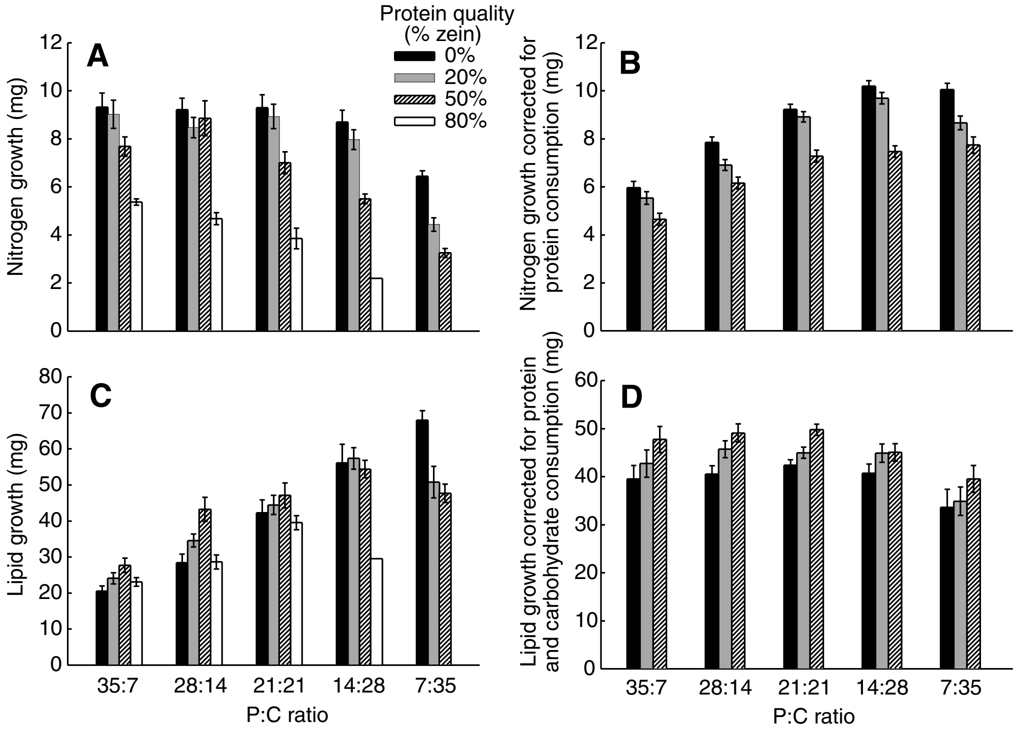

Fig. 3. Patterns of (A) nitrogen growth, (B) nitrogen growth corrected for protein consumption, (C) lipid growth and (D) lipid growth corrected for protein and carbohydrate consumption in final-instar *S. littoralis* caterpillars that had successfully reached their pupal stage in the no-choice experiment Values are means ± 1 s.e.m. (*N*=6–10). The values in B and D are the least-square means calculated from ANCOVA (see text), representing the utilization efficiency of nitrogen and lipid, respectively.

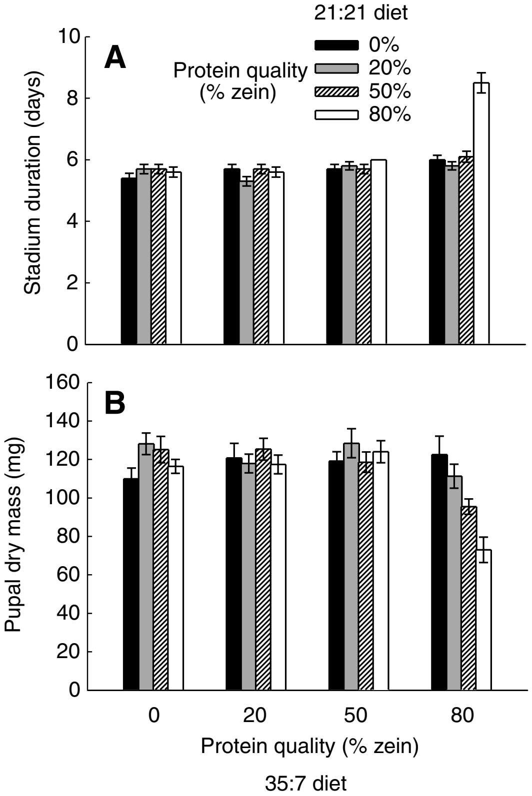

Fig. 4. Patterns of (A) stadium duration and (B) pupal dry mass for final-instar *S. littoralis* caterpillars in the choice experiment. Values are means ± 1 s.e.m. (*N*=8–10).

Nutrient consumption

Two aspects of consumption were investigated across the feeding period, one the radian values of the self-selected P:C ratio and the other the amount of nutrient consumed. Within each measured time period, the most noticeable effect on food selection was found in response to variation in protein quality of the 35:7 diet, such that the self-selected P:C ratio significantly increased for the insects that were given the two moderate 35:7 diets (Fig. 5, Table 2) (1.98:1 and 2.09:1 for 20% and 50% zein, respectively) relative to those provided control (1.74:1) and lowest protein quality 35:7 diet (1.73:1). This pattern was consistent irrespective of the protein quality of the 21:21 diets, as indicated by the non-significant interaction term (Fig. 5, Table 2). The treatment-related difference in the amount of nutrients eaten was analyzed using the distance of each intake point from the origin in the protein–carbohydrate intake space as response variable. During the first 3 days, the nutrient consumption was significantly smaller for caterpillars presented with the food pairing in which the poorest quality 35:7 diet (80% zein) was paired with the poorest quality 21:21 diet (80% zein), indicating the significant interaction between the two factors (Table 2). When the final consumption was examined, the only significant effect was due to the differences in the protein quality of the 35:7 diet, with greater amount of nutrient consumed by the caterpillars on the two moderate quality high-protein food.

Discussion

As generally expected, the results of the no-choice experiment showed that the consequences of feeding on the diets containing low-quality protein were reduced body mass and delayed development, with the most pronounced effect occurring for caterpillars that were fed diets containing the lowest quality protein (80% zein). Such a performance decrease is likely to result from a deficiency of essential amino acids in the food (Broadway and Duffey, 1988; Karowe and Martin, 1989) and may incur ecological costs, such as reduced fecundity (Honek, 1993) and increased vulnerability to parasites and predators (Benrey and Denno, 1997). A moderate decline in protein quality (replacing 20% and 50% casein-based protein mix with zein) did not produce such a marked performance

Table 2. *ANCOVA results for the radian value indicating the self-selected protein:carbohydrate (P:C) ratio and cumulative nutrient consumption up to day 2, 3 and the last day of feeding by S. littoralis caterpillars in the choice experiment*

		Days 0–2		Days 0–3		Days 0–end	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Radian value for self-selected P:C ratio							
PB	3	36.55	<0.001	25.41	<0.001	16.49	<0.001
ER	3	5.00	0.003	3.31	0.022	4.76	0.003
PB×ER	9	0.99	0.452	0.79	0.628	0.82	0.598
Initial mass	1	4.62	0.033	1.79	0.183	1.24	0.268
Error	141						
Nutrient consumption							
PB	3	101.21	<0.001	100.46	<0.001	21.92	<0.001
ER	3	10.78	<0.001	14.59	<0.001	1.07	0.364
PB×ER	9	2.95	0.003	5.23	<0.001	1.49	0.156
Initial mass	1	27.88	<0.001	7.64	0.007	0.24	0.627
Error	141						

The self-selected P:C ratio was converted to the radian value using the function of arctangent (C/P). Nutrient consumption was calculated as the distance between the position of nutrient intake and the origin (see Fig. 5). Initial fresh mass was used as a covariate to adjust for the effects due to size differences between individuals.

PB, protein-biased 35:7 diet; ER, equal-P:C ratio 21:21 diet.

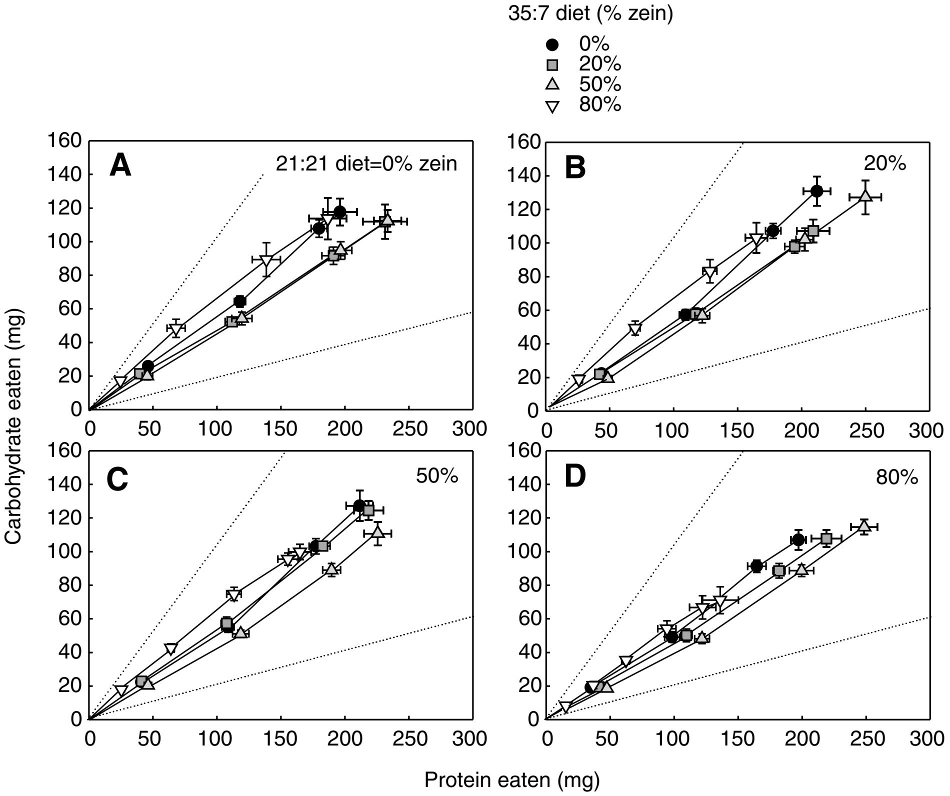

Fig. 5. Bivariate plots for cumulative protein and carbohydrate consumption by *S. littoralis* caterpillars over the successive days during their final larval stadium. (A–D) Nutrient consumption of caterpillars provided with the four equal-ratio 21:21 diets containing 0% (A), 20% (B), 50% (C) and 80% (D) zein in their protein component, respectively. The two dotted lines represent the protein:carbohydrate (P:C) ratio of the two choice foods presented to the insects (21:21 and 35:7 diets). Values are means ± 1 s.e.m. (*N*=8–10).

reduction as for insects on the lowest protein quality (80% zein), indicating that there is a value between 50% and 80% zein, beyond which severe growth limitation starts to occur.

The key finding from the present study is that there was a significant interactive effect of dietary P:C ratio and protein quality, with the negative impact of low protein quality being amplified as the P:C ratio in the diet fell. This finding confirms the prediction that the negative consequences of eating a protein source imbalanced in amino acid composition will be visible only when total protein quantity reached a critically limiting level (Hinks et al., 1993; Felton, 1996; Anderson et al., 2004).

Consistent with previous studies (Lee et al., 2002; Lee et al., 2004), caterpillars of *S. littoralis* displayed an intake array across diets of varying P:C ratio that is typical of generalist-feeders [for the functional explanations in relation to diet breadth, see Raubenheimer and Simpson (Raubenheimer and Simpson, 2003; Raubenheimer and Simpson, 2004)]. Additional to this baseline response, across the five P:C diets, consumption decreased most markedly for caterpillars presented with the lowest quality (80% zein) protein diet relative to those given diets of high protein quality. How poor protein quality reduced herbivore feeding remains to be elucidated but there is the possibility that mechanisms, such as associatively learned aversion in combination with direct nutrient feedback on taste sensitivity, are involved (Simpson and Raubenheimer, 1993b; Simpson and Raubenheimer, 2000). Reduced rate of ingestion of food deficient in essential amino acids has been reported in vertebrates (Gietzen et al., 1986; Chui et al., 1987). It has been proposed that low nutritive quality of plants is one possible measure offering resistance against herbivory (Feeney, 1976; Moran and Hamilton, 1980; Augner, 1995; Berenbaum, 1995), although few studies have tested this possibility experimentally. Adaptive responses of herbivores to low-quality food plants include compensatory increases in food consumption rate and modification of post-ingestive utilization (Slansky and Feeny, 1977; Slansky and Wheeler, 1989; Simpson and Simpson, 1990; Wheeler and Slansky, 1991; Slansky, 1993; Cruz-rivera and Hay, 2000). Theoretically, such herbivore responses will reduce the effectiveness of nutrient-based defenses by plants (Moran and Hamilton, 1980; Berenbaum, 1995). The present data, however, revealed that, at least under a restricted feeding regime, there was no evidence for such compensatory increase in intake for lowered protein quality, indicating that low-quality protein may act as a defensive plant attribute that would hamper insects from compensatory feeding. The failure to increase consumption may result from accumulation of excess amino acids in the haemolymph, resulting in inhibition of further feeding and thus failure to gain more of limiting amino acids (i.e. ‘jamming’ of protein appetite systems) (Simpson and Raubenheimer, 1993b; Zanotto et al., 1994). Interestingly, the present study showed that the reducing effect of low-quality protein on feeding interacted significantly with dietary P:C imbalance, as seen by the fact that the degree of the reduction in consumption with low protein quality was more apparent in the low P:C diets.

Excessive protein consumed by caterpillars on the highest P:C diet (35:7) did not translate into extra nitrogen growth, but was accompanied by a fall in nitrogen conversion efficiency (Lee et al., 2002; Lee et al., 2004). This indicated post-ingestive regulation of nitrogen growth, which is known to include mechanisms such as high rates of nitrogen excretion (Zanotto et al., 1993). It would also seem that the severe carbohydrate deficiency in the 35:7 diet led to caterpillars maintaining their energy balance *via* gluconeogenesis (Thompson and Redak, 2000).

In addition to its effect on feeding, the declining quality of dietary protein resulted in reduced utilization efficiency of ingested nitrogen to body nitrogen. Caterpillars are reported to digest zein less efficiently compared to other protein sources, because of its deficiency of lysine (Broadway and Duffey, 1988). Trypsin is a principal protease in many insects including larval Lepidoptera, and cleaves peptide linkages specifically at the arginine and lysine residues (Chapman, 1998). A limited quantity of lysine is therefore likely to reduce the availability of amino acids or small peptide components to be transported across the gut membrane and assimilated into the body (Felton et al., 1992). Another candidate mechanism that may explain reduced nitrogen utilization is accelerated uric acid production as a consequence of an increased rate of catabolism of all but the deficient amino acids in the nutritionally imbalanced protein source (Horie and Watanabe, 1983; Karowe and Martin, 1989). This will certainly result in reduced level of nitrogen pool incorporated to nitrogen growth.

Despite not being as apparent as for nitrogen growth, there was an indication that lipid growth was subject to post-ingestive regulation, with the lipid utilization efficiency being reduced for caterpillars on the carbohydrate-excess diet (7:35), as reported previously in locusts (Zanotto et al., 1993). A striking aspect of lipid utilization was the elevated conversion rate of ingested nutrient to lipid growth with lowered protein quality irrespective of the P:C ratio. Perhaps this elevated lipid deposition derived from the carbon skeletons from amino acids that were ingested in excess on the imbalanced protein diets and were deaminated to allow excretion of excess nitrogen (Lehninger et al., 1993). The consequence of the increased efficiency of nutrient conversion to lipid was not always related to increased lipid deposition in caterpillars reared on zein-containing diets. The increment in lipid growth occurred for those on protein-rich diets. However, despite the heightened utilization rate, lipid growth fell with declining protein quality on an extremely protein-limiting diet, and this was mainly due to reduced intake.

One hypothesis that has occasionally been called upon to explain the widespread occurrence of diet mixing in herbivores is that mixing results from the need for balancing the requirement for multiple nutrients (Westoby, 1978; Rapport, 1980; Pennings et al., 1993; Simpson et al., 2004). On the basis of the significant two-way interaction between P:C ratio and protein quality, as demonstrated from the no-choice test (see above), caterpillars would be predicted to alter their strategy of nutrient complementation to compensate for the low-quality protein. Results shown from the food choice experiment were largely consistent with this prediction. Once paired with one of the two 35:7 diets with intermediate protein quality (20% and 50% zein), caterpillars selected more protein than carbohydrate compared to the level selected by the insects given the control 35:7 diet, resulting in an increase in the selected P:C ratio in the former. This tendency to ingest more low-quality protein is explicable as caterpillars regulating to compensate for essential amino acids deficient in the food (Greenstone, 1979; Bernays and Woodhead, 1984; Behmer and Joern, 1993). Eating more protein or amino acids than required may come at a cost (Slansky, 1993; Simpson et al., 2004). However, the need for acquiring limiting amino acid (e.g. lysine) is likely to function as a driving force outweighing any metabolic costs associated with processing excess protein intake. When paired with a 35:7 diet with the most inferior quality protein (80% zein), the insects did not increase their self-selected P:C ratio. It would seem that they failed to complement their extra protein requirement by eating this 35:7 diet that would otherwise cause deleterious consequences (see above), but instead preferred 21:21 diets that had a better quality protein (e.g. 0, 20, or 50% zein). Probably as a result of compensatory feeding, both pupal mass and development time were stabilized in caterpillars on all food-pairings except for the one that had the two lowest protein quality choice diets, where caterpillars exhibited reduced body growth and retarded stadium duration because they consumed considerably less nutrients relative to those on the other treatments.

The present study has attempted to identify the complex nature of the interactions between the quantity and quality of nitrogenous nutrients in the diet of an herbivorous insect. It is evident that the extent to which herbivore survival, development and growth were limited by an imbalanced amino acid profile depended on the quantity of protein in relation to other essential macronutrients (in this case, digestible carbohydrate), and differences in nutrient intake seemed to be the main cause of such performance consequences. When presented with varying degrees of protein quality, caterpillars responded by altering their pattern of food selection, which is explicable as compensation for changes in protein quality. This suggests that plant protein quality may play a role in influencing herbivore foraging strategies, in conjunction with other factors such as plant secondary compounds, food frequency and distance (Simpson and Raubenheimer, 2001; Behmer et al., 2001; Behmer et al., 2002; Behmer et al., 2003). Finally, the present study provides evidence that variation in food protein quality has a significant impact on feeding, post-ingestive utilization and chemical composition of animals, with implications for patterns of nutrient transfer across trophic levels in ecosystems (Anderson et al., 2004; Raubenheimer et al., 2005).

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