Journal of Insect Physiology 81 (2015) 21–27

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Revisiting macronutrient regulation in the polyphagous herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): New insights via nutritional geometry

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ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form 27 June 2015
Accepted 29 June 2015
Available online 30 June 2015

Keywords:
Nutrition
Protein
Carbohydrates
Physiology
Geometric framework

ABSTRACT

Insect herbivores that ingest protein and carbohydrates in physiologically-optimal proportions and concentrations show superior performance and fitness. The first-ever study of protein–carbohydrate regulation in an insect herbivore was performed using the polyphagous agricultural pest *Helicoverpa zea*. In that study, experimental final instar caterpillars were presented two diets – one containing protein but no carbohydrates, the other containing carbohydrates but no protein – and allowed to self-select their protein–carbohydrate intake. The results showed that *H. zea* selected a diet with a protein-to-carbohydrate (p:c) ratio of 4:1. At about this same time, the geometric framework (GF) for the study of nutrition was introduced. The GF is now established as the most rigorous means to study nutrient regulation (in any animal). It has been used to study protein–carbohydrate regulation in several lepidopteran species, which exhibit a range of self-selected p:c ratios between 0.8 and 1.5. Given the economic importance of *H. zea*, and it is extremely protein-biased p:c ratio of 4:1 relative to those reported for other lepidopterans, we decided to revisit its protein–carbohydrate regulation. Our results, using the experimental approach of the GF, show that *H. zea* larvae self-select a p:c ratio of 1.6:1. This p:c ratio strongly matches that of its close relative, *Heliothis virescens*, and is more consistent with self-selected p:c ratios reported for other lepidopterans. Having accurate protein and carbohydrate regulation information for an insect herbivore pest such as *H. zea* is valuable for two reasons. First, it can be used to better understand feeding patterns in the field, which might lead to enhanced management. Second, it will allow researchers to develop rearing diets that more accurately reflect larval nutritional needs, which has important implications for resistance bioassays and other measures of physiological stress.

1. Introduction

The ability of insect herbivores to acquire an optimal mixture of dietary nutrients has profound effects on their performance and fitness (Bernays and Bright, 1993; Bernays and Minkenberg, 1997; Raubenheimer and Jones, 2006; Unsicker et al., 2008; Behmer, 2009; Simpson et al., 2015). In general, plant nutrient content is highly variable, both spatially and temporally (Elser et al., 2000; McGroddy et al., 2003; Deans, 2014), indicating that the majority of herbivores forage in a highly heterogeneous nutritional landscape. To deal with this variability, insect herbivores assess the nutrients present in different plant tissues and regulate the intake of specific nutrients to meet their physiological demands (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 1999; Behmer, 2009; Simpson et al., 2015). The process of acquiring the optimal balance of key nutrients to fuel growth and reproduction strongly impacts insect performance, with consequences for the evolution of plant–insect interactions and host–plant associations (Bernays and Chapman, 1994; Bernays and Bright, 2005), dispersal and movement patterns (Simpson et al., 2006, 2010; Bazazi et al., 2008; Srygley et al., 2009; Hansen et al., 2011) and even the evolution of higher order social interactions (Guttal et al., 2012; Lihoreau et al., 2014, 2015). For these reasons, delineating the nutritional requirements of an insect species is integral to understanding its feeding ecology, life history strategies, and physiological capabilities.

The cotton bollworm, *Helicoverpa zea*, is a highly polyphagous agricultural crop pest that feeds on over 100 different host plants in North America (Fitt, 1989). *H. zea* was also the first species to

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http://dx.doi.org/10.1016/j.jinsphys.2015.06.015
0022-1910/

be used for exploring nutrient regulation in herbivorous insects (Waldbauer et al., 1984). In this study, a choice test was performed to determine the extent to which *H. zea* larvae regulated their protein (p) and carbohydrate (c) intake. To do this, larvae were offered one of two artificial diet pairings over the course of their final instar, either a diet with a protein-to-carbohydrate ratio (p:c) of 100:0 (all protein) and one with a p:c of 0:100 (all carbohydrates), or two diets both with a 50:50 ratio. The consumption results indicated that, when allowed to self-select, larvae ingested a diet with an average p:c ratio of 80:20, or 4:1; in the experimental approach of the geometric framework geometric framework (GF), this self-selected p:c ratio is referred to as an intake target (IT) (Simpson and Raubenheimer, 1995).

Since Waldbauer et al. (1984), protein and carbohydrate regulation has been tested in several lepidopteran species using the GF, including *Heliothis virescens* (Lee et al., 2006; Telang et al., 2001; Roeder and Behmer, 2014), *Heliothis subflexa* (Lee et al., 2006), *Manduca sexta* (Thompson and Redak, 2005), *Malacasoma disseria* (Despland and Noseworthy, 2006), *Plutella xyostella* (Warbrick-Smith et al., 2009), *Spodoptera exigua* (Merkx-Jacques et al., 2008), *Spodoptera exempta* (Lee et al., 2004b), *Spodoptera littoralis* (Lee et al., 2004a), and *Spodoptera litura* (Lee, 2010). Across these species, the ITs range from slightly carbohydrate-biased ratio for *S. exempta* (0.8:1) to slightly protein-biased ratio for *H. virescens* (1.5:1) and *S. littoralis* (1.3:1), with several species selecting for a balanced 1:1 p:c ratio. Comparatively, the 4:1 IT for *H. zea* as determined in Waldbauer et al. (1984) stands apart from these other caterpillar species because it is extremely protein-biased. It is also much more protein-biased than the 1.5:1 IT reported for *H. virescens* (Lee et al., 2006), which is a close relative to *H. zea*, and which shows similar feeding biology to *H. zea* (both are extreme generalists) (Mitter et al., 1993).

Given the economic significance of *H. zea* and the major discrepancy between Waldbauer et al. (1984) and other lepidopteran studies employing the GF, we wanted to reassess protein–carbohydrate regulation in *H. zea*. We had two objectives. The first was to determine the IT for *H. zea* using the experimental approach of the GF. To do this, a choice-experiment was performed in which individuals were offered pairings of two diets that differed in their p:c ratios; for each treatment newly-molted final instar caterpillars were maintained individually and consumption of each food was measured over the final instar. The total amount of protein and carbohydrates consumed over the study was then used to calculate the IT. The second objective was to understand how diet p:c impacts performance when larvae cannot choose. This was done with a no-choice experiment by rearing larvae from neonate to pupation on diets with a specific p:c ratio and then measuring growth rate, developmental time, and pupal mass. Given the IT results for other caterpillar species, especially the IT reported for the closely related *H. virescens* (Lee et al., 2006), we expected the IT for *H. zea* to be only slightly protein-biased, approximating the upper range found in these other studies. We also hypothesized that performance in the no-choice study would be best on the diet treatment that most closely matched the IT calculated from the choice experiments, given that ITs have been shown to be functionally optimal (Behmer and Joern, 2008; Roeder and Behmer, 2014).

2. Methods

2.1. Insects

H. zea eggs were purchased from Benzon Research (Carlisle, PA, USA). Upon hatching, neonates were individually placed, using a fine-tipped paint brush, into 1 oz. clear condiment cups with paper lids. Each cup also contained one or two blocks of experimental food that differed in soluble protein and digestible carbohydrate content (see below). All individuals were kept in a growth chamber (Model I-66VL; Percival Scientific, Perry, IA, USA) set at 25 °C with a 14:10 L:D cycle for the duration of each experiment.

2.2. Artificial diet

The synthetic diet used in this study was originally developed by Ritter and Nes (1981), and then later modified as described by Jing et al. (2013). The key ingredients were vitamin-free casein, sucrose, cellulose, Wesson's salt mix, Torula yeast, lipids (cholesterol, linoleic and linolenic acid) and vitamins. In total, 11 different diets were made that had unique protein and digestible carbohydrate profiles by altering the proportion of casein and sucrose in the diets. All other ingredients, except for cellulose, were held constant between the different diets; the amount of cellulose in a diet varied inversely with total macronutrient content. Although the nutritional components of this artificial diet may differ from plant tissues in important ways, larvae readily feed on this diet and show high survival and successful development to adulthood. Despite being an animal-based protein, casein (the primary protein source in this diet) is considered a high quality protein source for lepidopterans (Lee et al., 2008) and is commonly used in insect artificial diets (Cohen, 2003). The original diet from Ritter and Nes (1981) contained 34% protein (p) and 12% sucrose (digestible carbohydrate (c)). This diet (p34:c12), plus three others (p12:c34, p17:c29 and p23:c23) had the same total macronutrient content (p + c) of 46%, but varied in p:c ratio from 0.35 to 2.8 (see Fig. 1a). Collectively, these four diets were used in various combinations in a choice experiment (described below).

The remaining seven diets (see Fig. 1b) were used in a no-choice experiment (see below). To maintain ecological relevance to natural conditions, these ratios and concentrations were selected to mimic the empirically-determined range of macronutrient content found in different cotton tissues under different growing conditions (Deans, 2014). Cotton is a common resource for *H. zea*, and as a result, larvae are likely to encounter resources of this quality in a natural setting. Table 1 shows the relationship between our experimental diets and the nutrient values for different cotton tissues. Three of these diets had total macronutrient content of 21%, but varied in p:c ratio from 0.4 to 2.5 (p6:c15, p12:c9 and p15:c6). The next three had a higher total macronutrient content of 42% with the same p:c ratios (p12:c30, p24:c18 and p30:c12), and the final diet had total macronutrient content of 68% and a p:c ratio of 1.6 (p42:c26). This resulted in the same three ratios being tested at two different total macronutrient concentrations: 0.4 (p15:c6 and p12:c30), 1.3 (p12:c9 and p24:c18), and 2.5 (p15:c6 and p30:c12) (see Fig. 1b).

All of the experimental diets were mixed as dry ingredients with a slightly warm 1% agar solution. After cooling, the diets were cut into blocks and presented to the experimental caterpillars. In this way caterpillars received both nutrients and water.

2.3. Experimental protocol

2.3.1. Choice experiment

All caterpillars were reared on the original Ritter and Nes (1981) diet (p34:c12, 46% total macronutrients) from hatching through to the start of the final instar. Upon molting to the final instar, larvae were weighed, then transferred to a petri-dish (with holes in the lid for ventilation) and offered two foods that differed in p:c ratio. There were three unique treatments: (1) p12:c34 paired with p34:c12; $n = 15$, (2) p17:c29 paired with p34:c12; $n = 15$, and (3) p23:c23 paired with p34:c12; $n = 14$. Diet cubes were individually weighed and placed at opposites ends of the petri-dish (100 mm diameter). Both diet cubes were replaced every 1–2 days so that

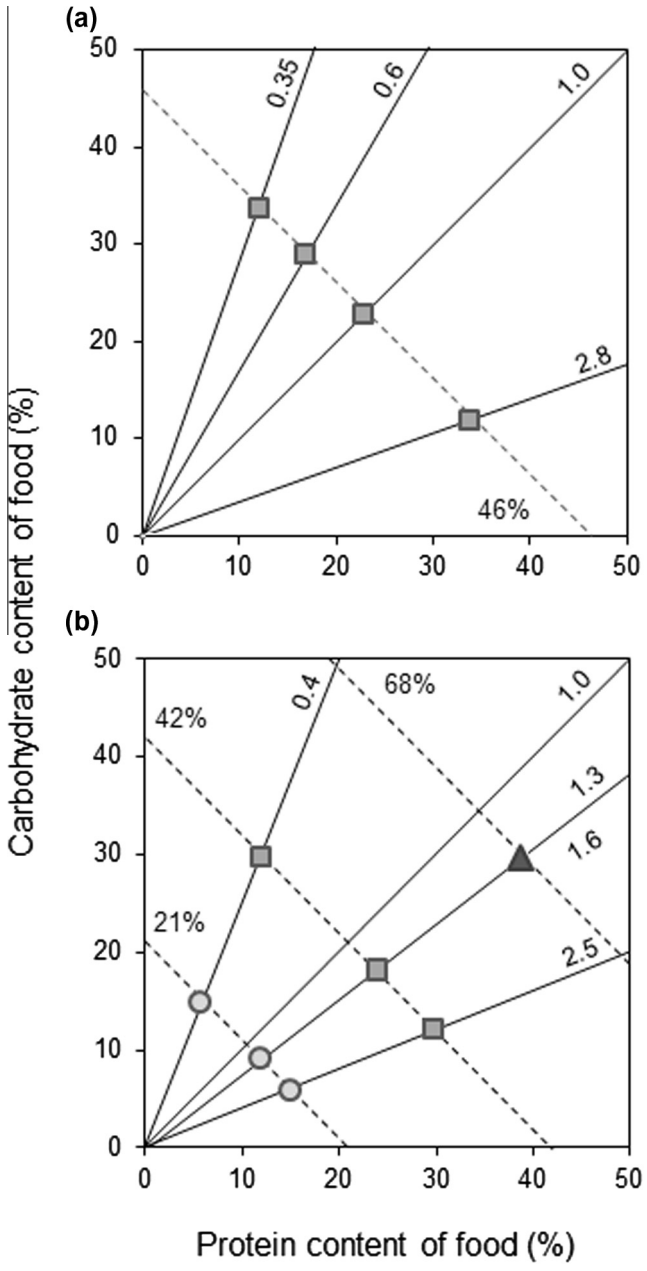

Fig. 1. The protein–carbohydrate content of the experimental foods for the (a) choice and (b) no-choice experiments. For each panel, individual points indicate the protein (*x*-axis) and carbohydrate (*y*-axis) content of the test food, expressed as percent dry mass of the food. Each panel also has 4 rails, that represent the different food p:c ratios (indicated at the end of each rail). Dashed lines that intersect the rails show total macronutrient concentration of individual diets.

Table 1
The empirically-determined range of total macronutrient content (%) and p:c ratios for different cotton tissues across both field and greenhouse conditions (only greenhouse values are reported for seed) (Deans, 2014).

Tissue	Concentration (%)	P:C ratio
Terminal growth (immature leaves)	34.0–41.8	1.18–1.19
True leaves (mature leaves)	26.9–41.8	1.16–1.68
Squares (pre-flowers)	17.7–30.6	1.18–14.80
Bolls (fruits)	29.5–41.3	0.41–1.17
Seed (found in bolls)	68.0	1.60

both diets were always available to the larvae. Consumption of each diet was measured by obtaining the wet and dry mass of each diet portion, using a wet–dry mass regression calculated separately to determine differences in initial and final dry mass. The total amount of protein and carbohydrates consumed was calculated using the total amount eaten from each block of food. Survival to pupation, developmental time until pupation, and pupal mass were measured for each experimental insect. There were 15 replicates per treatment and the sex ratio for each treatment, identified at the pupal stage, was a 1:1 ratio.

2.3.2. No-choice experiment

Larvae were reared on one of the seven experimental diets from neonate through to pupation. Individual food blocks were replenished as needed, but changed a minimum of every 2–3 days to prevent the food from drying out. Developmental time until pupation, pupal mass, and growth rate were measured for each experimental insect. Each treatment was replicated (p6:c15, $n = 14$; p12:c9, $n = 11$; p15:c6, $n = 15$; p12:c30, $n = 13$; p24:c18, $n = 13$; p30:c12, $n = 14$; p42:c26, $n = 13$), and the sex ratio for each treatment, identified at the pupal stage, was approximately 1:1. Survival across all treatments was high, with only one death occurring on the p42:c26 treatment.

2.4. Data analysis

For the choice experiment, protein–carbohydrate consumption was analyzed using MANCOVA. The MANCOVA model included P and C consumption as the dependent variables, diet and sex as fixed factors, and initial larval mass as a covariate. For both experiments an ANCOVA was used to determine differences in pupal mass and growth rate, with diet and sex as fixed factors, and initial larval mass as a covariate. A Kaplan–Meier survival analysis (specifically the Mantel Cox test) was used to determine differences in developmental time between diet treatments and sex. The Tukey-*b* test was used for all ANCOVA post hoc tests. All data sets met the assumptions of normality and homogeneity of variance. All analyses were done using SPSS version 21 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Choice experiment

Strong regulation for P and C was apparent in the choice experiment. The MANCOVA results showed that P and C consumption ($F_{4,80} = 1.44$, $P = 0.229$) were statistically similar across all treatments, indicating that larvae standardized their consumption of these two macronutrients across all diet pairings (Fig. 2). Univariate tests also showed that protein consumption ($F_2 = 2.60$, $P = 0.087$) and carbohydrate consumption ($F_2 = 0.57$, $P = 0.570$) were similar across all three treatments (Table 2). Average protein consumption throughout the experiment was 103.0 mg (SEM ± 6.7), and average carbohydrate consumption was 76.3 mg (SEM ± 4.4). This resulted in a protein-biased intake target of 1.6 (SEM ± 0.14). There was no significant effect of sex on consumption ($F_{2,36} = 0.11$, $P = 0.895$).

There was no mortality during this experiment and as Fig. 3 shows that there was also no effect of diet treatment on developmental time (Mantel–Cox, $X^2 = 1.50$, df = 2, $P = 0.472$) or pupal mass ($F_{6,38} = 0.34$, $P = 0.712$). There were also no differences in developmental time (Mantel–Cox, $X^2 = 1.27$, df = 1, $P = 0.260$) or pupal mass ($F_{2,42} = 3.65$, $P = 0.064$) between males and females.

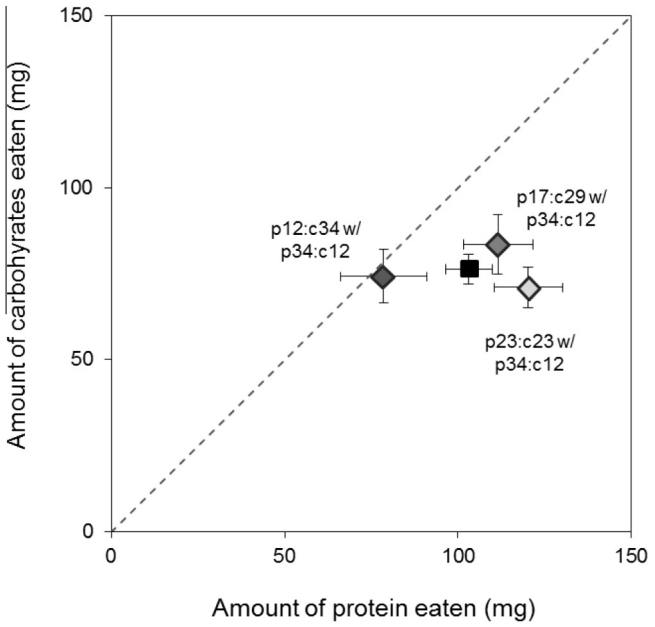

Fig. 2. Self-selected protein–carbohydrate intake from the choice experiment. Each diamond shows the mean (±SEM) amount of protein and carbohydrate eaten for each of the three choice treatments; they were not statistically different from one another. The black square shows the mean (±SEM) amount of protein and carbohydrate eaten when calculated across all treatments.

3.2. No-choice experiment

3.2.1. Developmental time

There was a significant effect of diet on developmental time (Mantel–Cox, $X^2 = 37.64$, df = 6, $P < 0.001$), and an influence of both total macronutrient concentration and p:c ratio was apparent. Larvae on the p6:c15 diet, the most carbohydrate-biased of the 21% total nutrient diets, exhibited the longest developmental time, while those on the same ratio (p12:c30) at 42% total nutrients took significantly less time to pupate ($X^2 = 10.86$, $P = 0.001$). Also, across both the 21% and 42% diets, larvae on the protein-biased ratios (p12:c9, p15:c6, p24:c18, p30:c12) had consistently shorter developmental times (Fig. 4a). There was a significant difference in the developmental time curves between males and females across diets (Mantel–Cox, $X^2 = 6.41$, df = 1, $P = 0.011$); however, a significant difference between sexes effect was only evident for the p30:c12 diet ($X^2 = 7.11$, $P = 0.008$), with females showing faster development than males.

3.2.2. Pupal mass

There was a significant diet effect on pupal mass ($F_{6,86} = 6.85$, $P < 0.001$). Among the 21% diets, p:c ratio had no impact; however, at 42% total nutrients, the most carbohydrate-biased diet (p12:c30) showed significantly higher pupal mass (Fig. 4b). In general, pupal mass was lower on the high-protein diets, while mass was relatively stable over carbohydrate concentrations. There was no significant effect of sex on pupal mass ($F_{1,90} = 0.37$, $P = 0.545$).

Table 2
Results of the MANCOVA (with initial mass as a covariate) for consumption across treatments in the choice experiment.

Variable	Factor	df	*F*-ratio	*P*-value
P & C consumption	Treatment	4	1.338	0.264
	Sex	2	0.111	0.895
	Initial mass	2	2.895	0.068

3.2.3. Growth rate

There was a significant effect of diet treatment on growth rate ($F_{6,86} = 5.84$, $P < 0.001$). Fig. 4c shows that larvae on the high 68% nutrient diet (p42:c26) had the lowest growth rate overall, and for those on the dilute diets (21% total nutrients), the moderate p:c ratio (p12:c9) showed the highest growth rate. There was no significant difference in growth rate across the different p:c ratios for the 42% total nutrient diets. There was also a significant sex effect on growth rate ($F_{1,90} = 4.09$, $P = 0.047$), with females showing a significantly faster growth rate than males overall.

4. Discussion

In sharp contrast to the earlier work of Waldbauer et al. (1984), we found that *H. zea* larva select dietary protein and carbohydrates in a 1.6:1 ratio, rather than a 4:1 ratio. As Fig. 5 shows, this result is much more consistent with the ITs of other lepidopterans that have been measured in GF studies and most closely matches that found for *H. virescens* (Lee et al., 2006; Roeder and Behmer, 2014), a generalist New World species that is closely related to *H. zea*. These results support our initial hypothesis that the IT for *H. zea* would be slightly protein-biased and be similar to that of *H. virescens*.

So why did we find such a different IT compared to Waldbauer et al. (1984)? Important methodological differences likely provide the best explanation. In our study, we had three unique diet pairings (Fig. 2), and the p:c ratios of the four foods we used (Fig. 1) mimicked concentrations found in cotton (Table 1; Deans, 2014), a natural host plant on which *H. zea* commonly feeds. In contrast, Waldbauer et al. (1984) only used two diet pairings, generated from three different diets. The first pairing was a control treatment

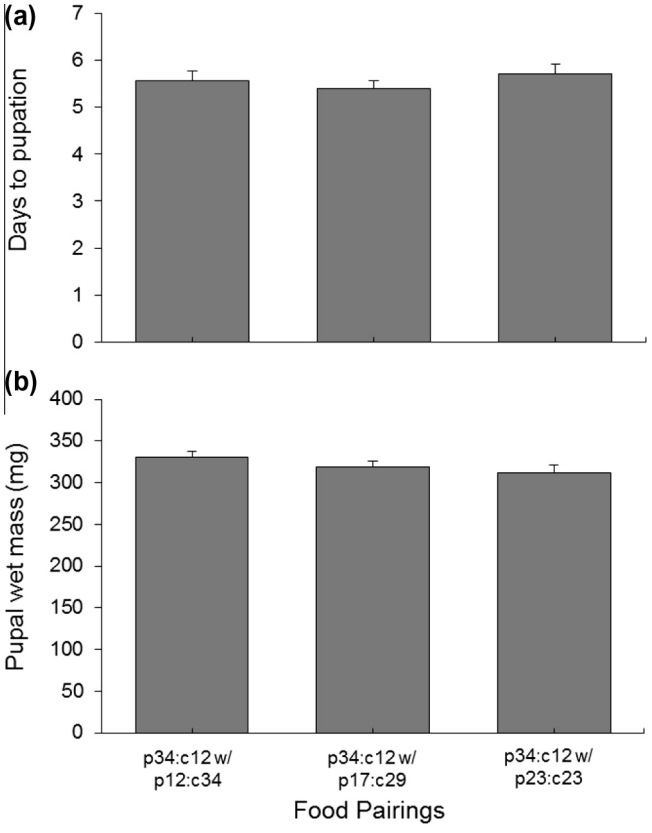

Fig. 3. Performance for caterpillars from the choice experiment. Panel (a) shows mean (±SEM) days to pupation, and panel (b) shows mean (±SEM) pupal wet mass. The *x*-axis indicates the diet pairings for each treatment.

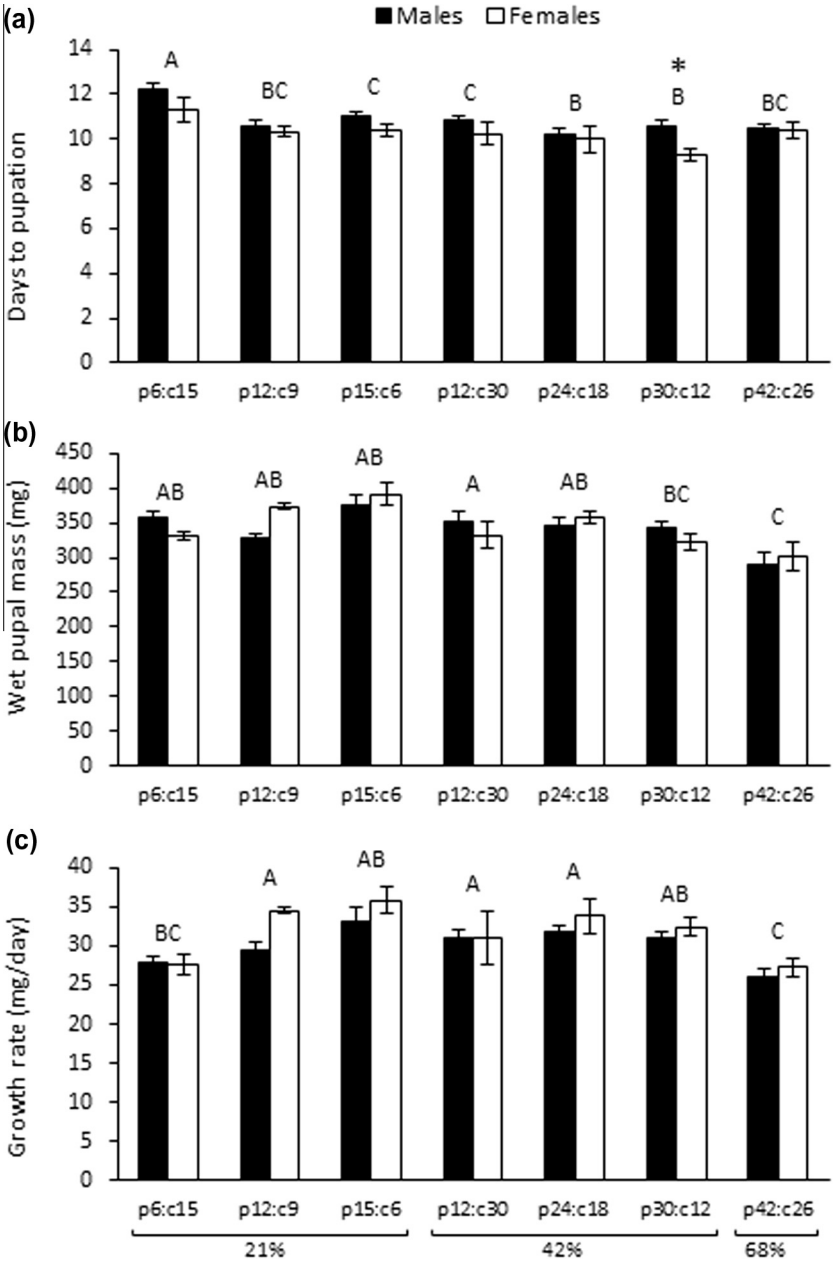

Fig. 4. Performance in the no-choice experiment for males and females. Panel (a) shows mean (±SEM) days to pupation, panel (b) shows mean (±SEM) pupal wet mass, and panel (c) shows mean (±SEM) growth rate (mg/day). Foods are grouped by their total macronutrient content, and then by increasing p:c ratio. Bars of similar color have similar p:c ratios. Different letters above each bar indicate significant differences between diets ($P < 0.05$), while an asterisk indicates significant differences between sexes.

were both diet cubes were identical – each had a 50:50 p:c ratio. The second treatment paired a p100:c0 diet with a p0:c100 diet. However, the use of diets that contain only protein or carbohydrates does not accurately represent a resource that an herbivore would encounter in nature, and the use of such extreme diets may have resulted in aberrant feeding behavior. The strong preference caterpillars showed for the protein containing diets (Waldbauer et al., 1984) may simply reflect the possibility that the carbohydrate-only diet was barely recognizable as food. Interestingly, Mormon crickets in the field show very little interest in carbohydrate-only artificial diets (Simpson et al., 2006). However, when they encounter a food dish containing protein-only artificial diet, they stop and feed for extended periods of time. This suggests that for chewing insect herbivores dietary protein might be needed to sustain feeding.

Several GF studies have documented strong differences in macronutrient regulation in herbivores fed diets that were either extremely protein- or carbohydrate-biased and/or extremely low in total macronutrient content. For example, Lee et al. (2002) explored the effects of diet p:c ratio and total macronutrient concentration on protein and carbohydrate regulation in a caterpillar species (*S. littoralis*). This paper showed that macronutrient regulation was significantly affected by food total macronutrient concentration and that larvae increased protein consumption when offered dilute diets (25.2% total macronutrients versus 42%). This trend has also been observed in *M. sexta* (Thompson and Redak, 2005) and *Drosophila melanogaster* (Lee et al., 2008). More recently Le Gall and Behmer (2014) performed a choice experiment using a grasshopper species (*Melanoplus differentialis*) and showed that individuals prioritized dietary protein when the total

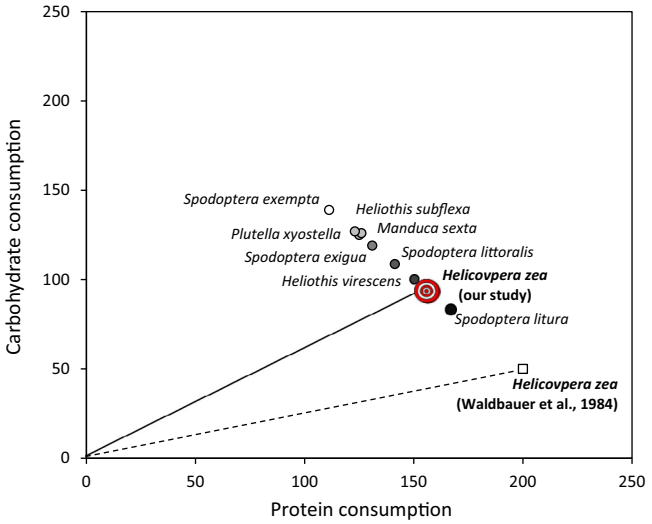

Fig. 5. Published intake targets (ITs) for different lepidopteran species, including *S. exempta* (Lee et al., 2004b), *H. subflexa* (Lee et al., 2006), *Plutella xyostella* (Warbrick-Smith et al., 2009), *M. sexta* (Thompson and Redak, 2005), *S. exigua* (Merkx-Jacques et al., 2008), *S. littoralis* (Lee et al., 2004a), *H. virescens* (Lee et al., 2006; Telang et al., 2001; Roeder and Behmer, 2014), *S. litura* (Lee, 2010), as well as the ITs for *H. zea* reported by Waldbauer et al. (1984) and this study. The amounts of protein and carbohydrate eaten for each species have been standardized relative to the amounts eaten by *H. zea* in the current study.

macronutrient content of the diets was low. Taken together, these results suggest that decision rules for nutrient regulation on extreme diets may not be indicative of regulation across more typical diets. The fact that protein is often prioritized in sub-optimal nutritional situations may indicate its importance in signaling the presence of an acceptable food source. This is particularly true given that the balance of multiple nutrients, rather than simply the concentration of total nutrients, can greatly impact herbivore feeding via chemosensory stimuli (Simpson and Raubenheimer, 1993) and has also been shown to impact the regulation of digestive enzymes in the gut, particularly in *H. zea*'s sister species *H. armigera* (Kotkar et al., 2009; Clissold et al., 2010; Sarate et al., 2012).

Despite detecting regulation for a specific protein–carbohydrate IT, when we reared neonates on diets with a range of different p:c ratios and total macronutrient concentrations we observed few differences in performance (Fig. 1b), and those that did occur were relatively small. Waldbauer et al. (1984) also ran a no-choice experiment from neonate to pupation, but only tested a p1:c1 and p4:c1 diet. They reported higher survival and shorter developmental time for larvae on the protein-biased diet; however, they did not see a difference in pupal wet mass. Unlike the Waldbauer et al. (1984) study, we saw no differences in survival across all diets (we had very low mortality overall), and on the diets that contained 42% macronutrient content (the Waldbauer et al. (1984) diets had 43% total macronutrient content), no difference in developmental time was observed. Our results are also very similar to what has been found in other GF studies. For example, in caterpillars (Lee et al., 2002, 2003, 2004a,b, 2006, 2012; Despland and Noseworthy, 2006; Lee, 2007, 2010; Roeder and Behmer, 2014) and nymphal grasshoppers (Raubenheimer and Simpson, 2003; Behmer and Joern, 2008; Le Gall and Behmer, 2014), developmental time increases on very carbohydrate-biased diets. In addition, GF studies often report the lowest pupal mass for caterpillars and adult mass for grasshoppers reared on protein-biased diets, largely due to the fact that insects on protein-biased diets are lean, and contain very low lipid levels, due to low dietary carbohydrate concentration (Lee et al., 2002, 2003, 2004a,b, 2012; Lee, 2010; Roeder and Behmer, 2014).

Roeder and Behmer (2014) measured larval, pupal, and reproductive performance of *H. virescens* (a close relative of *H. zea*) on artificial diets with different p:c ratios, and they too found larval performance results similar to those reported in the current study. However, they did show that diet p:c ratio had a significant negative effect on eclosion success and time to eclosion, especially when the p:c ratio of the test diets diverged strongly away from the IT. Additionally, they showed that when larval, pupal and reproductive performance was integrated, and extrapolated to the population level, insects reared on diets that most closely matched the IT generated the largest populations, and had the shortest generation times. Collectively, the Roeder and Behmer (2014) results suggest that larval performance is, at best, a weak indicator of fitness for lepidopterans. Exploring the effects of food protein–carbohydrate ratio and total macronutrient concentration on *H. zea* over its entire lifetime would more fully characterize latent nutritional effects.

Nutrition impacts a herbivore's ability to deal with a range of stressors, including plant secondary compounds (Simpson and Raubenheimer, 2001; Behmer et al., 2002), pathogens (Lee et al., 2006, 2008; Ponton et al., 2011), pesticides (Gordon, 1961), and even their ability to withstand periods of starvation (Lee and Jang, 2014). Insects are also capable of modifying their feeding behavior and resulting macronutrient intake to mitigate the effects of these stresses (Simpson and Raubenheimer, 2009; Behmer, 2009). Having accurate nutrient regulation data for important economic pests such as *H. zea* is valuable because it provides a reference point for understanding their feeding behavior in the field. This in turn enables predictions to be made related to movement and distributions, and can help us anticipate how this species will respond to different control methods including, but not limited to, pesticides, transgenic plants (e.g., *Bt*), and biological control agents such as the fungal entomopathogen *Beauveria bassiana*. Up-dating and providing a more accurate account of how *H. zea* responds to different nutritional environments also provides a stronger foundation for further exploring its physiology and nutritional ecology. For example, the use of more realistic artificial diets in laboratory studies can be used to standardize nutritional environments across different physiological experiments, or enhance the ecological relevance of applied studies such as diet-based resistance monitoring programs (e.g., Ali et al., 2006; Luttrell and Jackson, 2012).

Acknowledgements

We would like to thank all those who have contributed to this project by providing methodological, analytical, or editorial feedback. In particular, we would like to thank Xiangfeng Jing, Marion Le Gall, Paul Lenhart, Cesar Valencia, and Rebecca Clark. We would also like to acknowledge our funding sources for this project, including the C. Everette Salyer Fellowship (Texas A&M Entomology Department), the Charles R. Parencia Endowment (Texas A&M Entomology Department), and this research was partially supported by an NRI-CSREES-USDA Grant (#2007-35302-18185) to Spencer Behmer.

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