OXFORD

Effect of Dietary Protein and Carbohydrates on Survival and Growth in Larvae of the *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae)

Zai-Ling Wang, Xiang-Ping Wang, Chuan-Ren Li,[1] Zhen-Zhou Xia, and Shi-Xiang Li

Department of Entomology. Yangtze University, Jingzhou, Hubei 434025, P.R. China and [1]Corresponding author, e-mail: 13986706558@163.com

Abstract

The ratio of protein and carbohydrate in an insect's nutritional regime can significantly influence its survival, growth, and fecundity. The effects of 11 different artificial diets containing protein (p): carbohydrate (c) ratios were determined in larvae of the phytophagus ladybug, *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae). We recorded the developmental times and survival rates of the larvae and weighed their pupae. When the concentration of carbohydrates was kept constant while the concentration of proteins was increased (p29:c20, p31:c20, p33:c20, and p35:c20), *H. vigintioctopunctata* could successfully complete the larval and pupal stages. The highest survival rate and greatest pupal mass of *H. vigintioctopunctata* were 72% and 19.5 mg, respectively, when reared on the p33:c20 diet. *H. vigintioctopunctata* larvae, however, were unable to develop into adults when the concentration of protein remained constant while the level of carbohydrates was increased (p20:c23, p20:c25, p20:c27, and p20:c29), or when the total amount (p + c) was kept at 48% (p22:c26, p 24:c24, p26:c22). Evidently, changing the availability of quality diet, especially the total protein levels, can significantly affect the performance to *H. vigintioctopunctata.* Our results indicated that the maximum development and survival of *H. vigintioctopunctata* larvae occurred within a narrow range—when the p:c ratio was (33:20).

Key words: *Henosepilachna vigintioctopunctata*, protein, carbohydrate, developmental time, survival

Insects consume specific diets to obtain the necessary mixture of nutrients that are essential to fuel their survival, growth, and fecundity (Behmer 2009). Proteins (p) and carbohydrates (c) are two significant macronutrients that provide the essential amino acids and energy, respectively, that influence their development, growth, and fecundity (Karasov and del Rio 2007; Simpson and Raubenheimer 2012). Most animals need to ingest the optimal nutritional blend of protein and carbohydrate to reduce fitness costs. Recent literature has documented that some insects such as grasshoppers, crickets, ants, and various caterpillars, among others, are capable of regulating their intake of protein and carbohydrate independently when provided the opportunity (Simpson and Raubenheimer 1993, Simpson et al. 2004, Lee et al. 2008, Dussutour and Simpson 2009, Altaye et al. 2010, Cease et al. 2012). The phytophagus ladybug beetle, *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae), typically feeds on Solanaceae plants such as black nightshade in the wild, but has adapted to many cultivated crops including tomato, potato, eggplant, etc. *H. vigintioctopunctata* is widespread throughout much of China (Zhou et al. 2015, Wang et al. 2017, Huang et al. 2018) and Japan (Nakamura 1976, Kawazu 2014, Sharma et al. 2012). The nutritional value of food plants varies considerably

depending on the plant type, age, and growing conditions (Bernays and Chapman 1994, Awmack and Leather 2002). Because of this variability, it may be difficult for *H. vigintioctopunctata* beetles to continuously find and consume equally nutritious hosts. The consequences of such variation in host plant quality may be considerable to a beetle's growth, survival, and reproductive capability.

That insects may actively regulate their intake of nutrients was only confirmed when researchers began to use defined artificial diets (Behmer 2009). Altering the nutrient composition of an artificial diet, either qualitatively and/or quantitatively, can often lead to a diet that is satisfactory to the target species, but understanding the precise interactions of the required ingredients remains a necessary to researchers. (Raubenheimer and Simpson 1997). Presently, lab populations of *H. vigintioctopunctata* are raised almost exclusively on natural host plants (Wang et al. 2017). This system of continually supplying host plant leaves as the diet for lab populations of *H. vigintioctopunctata* can be very cumbersome, both time- and labor-intensive, especially during the winter season when obtaining host plants may be difficult (Han et al. 2012). To date, however, attempts to develop artificial diets for *H. vigintioctopunctata* have not been successful. In 1979, Kono (1979) tried unsuccessfully to

substitute sliced potato tubers as an alternative food for rearing _H. vigintioctopunctata._ The developmental time of the larvae was twice that of individuals reared on tomato plant leaves, and only half-sized adults were obtained. Murata et al. (1994) and Kawazu (2014) used different artificial diets to rear _H. vigintioctopunctata._ Both diets were successful in rearing third and fourth instar _H. vigintioctopunctata_ larvae; the earlier instars, however, required tomato leaves to develop.

The aim at the present study was to elucidate the role of protein and carbohydrate ratios play in the development and growth of _H. vigintioctopunctata._ Using this information, we hope to develop a successful artificial diet for _H. vigintioctopunctata_ larvae by altering the content and ratio of protein and carbohydrate. We used the two-sex life table method to evaluate the consequences of using different diets containing variable types of dietary protein and carbohydrates, we then assessed which protein: carbohydrate ratios were nutritionally favorable for optimal larval growth and development. Identifying the optimal p: c ratio would maximize _H. vigintioctopunctata_ larval performance, producing a major impact on breeding _H. vigintioctopunctata_ populations in the laboratory, and provide a basis for understanding _H. vigintioctopunctata_ nutritional regulation.

Materials and Methods

Insects and Diets
Ten pairs of _H. vigintioctopunctata_ adults were collected from potato plants in Jingzhou city (Hubei, China, 30.05 N, 112.14 E) and were

successively reared on their wild host plant, _Solanum nigrum_, in the laboratory at 25 ± 1°C, 75% ± 10% RH, 12L: 12D for one generation. The eggs used in this study were laid within a 24-h period and obtained from 20 pairs of randomly chosen _H. vigintioctopunctata_ adults. They were then placed in an environmental chamber (GZX-400BS-III, Shanghai Xin-Miao Medical Equipment Manufacturing Co., Ltd., China) at 25 ± 1°C and 75% ± 10% RH, with a photoperiod of 16L: 8D and allowed to develop.

The mixed diet consisted of commercial, chemically defined food products, including casein, cholesterol, ascorbic acid, choline, chloride, myo-insoitol, locust bean gum, Wesson's salt, vitamin mix, cellulose, sucrose (Beijing Solarbio Science and Technology Co., Ltd., China), and yeast (Guangdong Huan Kai Microbial Technology Co., Ltd., China). The ingredients of artificial diet were weighed using an Ohaus balance (Model AX2242H/E). Table 1 gives the amount and composition of protein and carbohydrate in each ingredient. Cellulose as indigestible carbohydrate cannot be digested by _H. vigintioctopunctata._ Both casein and yeast provided protein, yeast and sucrose provide carbohydrate for _H. vigintioctopunctata._ Each of the 11 treatment combinations contained equal amounts of all ingredients, other than the three variables: Casein, sucrose, and cellulose, so that the concentration of micronutrients was constant across all diets (Raubenheimer and Simpson 1993, Yang 1994, Lee 2006).

Experimental Design
We formulated 11 treatment combinations containing different ratios of dietary protein (p) and carbohydrate (c) (Table 1). We

Table 1. The ratios and compositions of protein and carbohydrate in the ingredients used in the artificial diet

Ratio	Total quality (g)	Yeast (g)	Casein (protein) (g)	Sucrose (carbohydrate) (g)	Cellulose (g)	Other (g)
p35:c20	12.6725	2.0000	3.7050 (3.3345)	2.5673 (2.5160)	0.7276	3.6700
p33:c20	12.6725	2.0000	3.4234 (3.0811)	2.5673 (2.5160)	1.0093	3.6700
p29:c20	12.6725	2.0000	3.1419 (2.8277)	2.5673 (2.5160)	1.2908	3.6700
p27:c20	12.6725	2.0000	2.8603 (2.5743)	2.5673 (2.5160)	1.5724	3.6700
p20:c23	12.6725	2.0000	1.5933 (1.4340)	2.9552 (2.8961)	2.4515	3.6700
p20:c25	12.6725	2.0000	1.5933 (1.4340)	3.2138 (3.1495)	2.1929	3.6700
p20:c27	12.6725	2.0000	1.5933 (1.4340)	3.4723 (3.4029)	1.9344	3.6700
p20:c29	12.6725	2.0000	1.5933 (1.4340)	3.7309 (3.6563)	1.6758	3.6700
p22:c26	12.6725	2.0000	1.8749 (1.6874)	3.3430 (3.2762)	1.7821	3.6700
p24:c24	12.6725	2.0000	2.1564 (1.9408)	3.0845 (3.0228)	1.7591	3.6700
p26:c22	12.6725	2.0000	2.4380 (2.1942)	2.8259 (2.7694)	1.7361	3.6700

The total dry mass of artificial diet is 12.67 g. The total mass of yeast, casein, sucrose, and cellulose is 9 g. The values for dietary protein and carbohydrate are based on yeast containing 55% protein and 0.9% carbohydrate, and casein containing 90% protein, and sucrose containing 98% carbohydrate.

Table 2. The developmental time of _H. vigintioctopunctata_ larvae and pupa reared on different diet by maintaining the concentration of carbohydrates, and then increasing the concentration of protein (p29:c20, p31:c20, p33:c20, and p35:c20)

Stage	The ratio of protein: carbohydrate			
	29:20	31:20	33:20	35:20
L1 (d)	4.84 ± 0.10[a,b]	4.74 ± 0.09[b]	5.02 ± 0.10[a]	4.96 ± 0.08[a,b]
L2 (d)	3.58 ± 0.08[b]	3.82 ± 0.12[a,b]	3.80 ± 0.06[a]	3.55 ± 0.07[b]
L3 (d)	5.21 ± 0.14[a]	4.68 ± 0.15[b]	5.06 ± 0.09[a]	5.00 ± 0.13[a,b]
L4 (d)	10.56 ± 0.41[a]	10.07 ± 0.37[a]	9.11 ± 0.11[b]	9.10 ± 0.14[b]
Pupa (d)	7.12 ± 0.16[a]	6.28 ± 0.10[b]	6.08 ± 0.10[b]	5.47 ± 0.12[c]
Total larval stage (d)	23.75 ± 0.67[a]	23.19 ± 0.46[a]	22.95 ± 0.22[a]	22.61 ± 0.27[a]

Values are means ± standard errors. Standard errors were evaluated using the bootstrap procedure with 100,000 resampling and different letters in the same row indicated significantly different ($P < 0.05$) between the ratio of p:c using the paired bootstrap test. (L1, the first instar; L2, the second instar, L3, the third instar, L4, the fourth instar.)
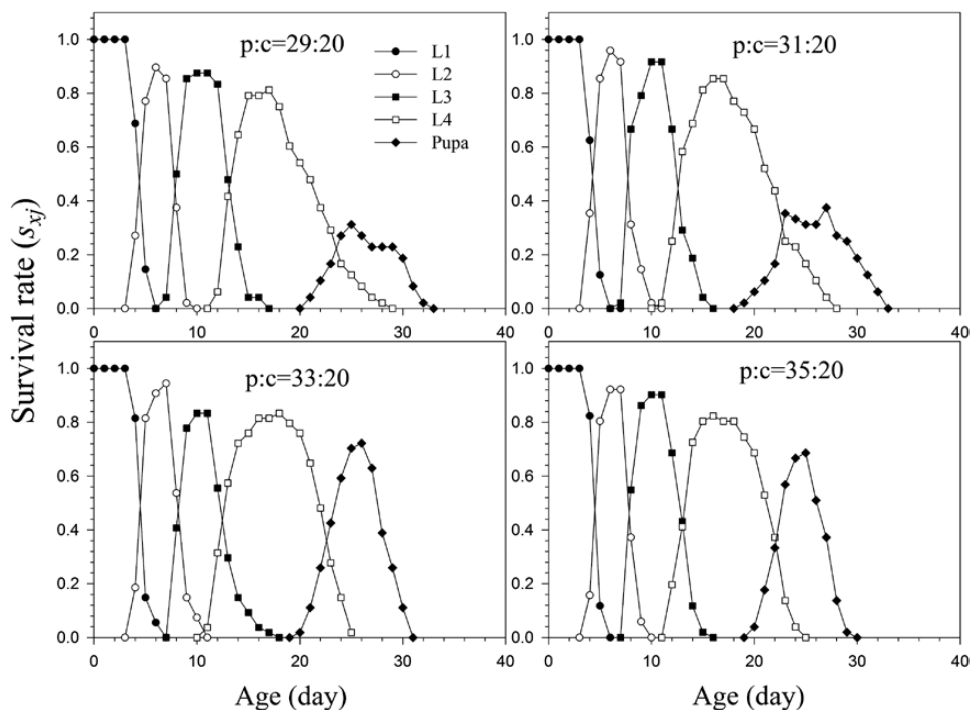
Fig. 1. Age-stage-specific survival rate (s_{xj}) of *H. vigintioctopunctata* larvae reared on diet with different protein: carbohydrate ratios by maintaining the concentration of carbohydrates, and then increasing the concentration of protein (p29:c20, p31:c20, p33:c20 and p35:c20).
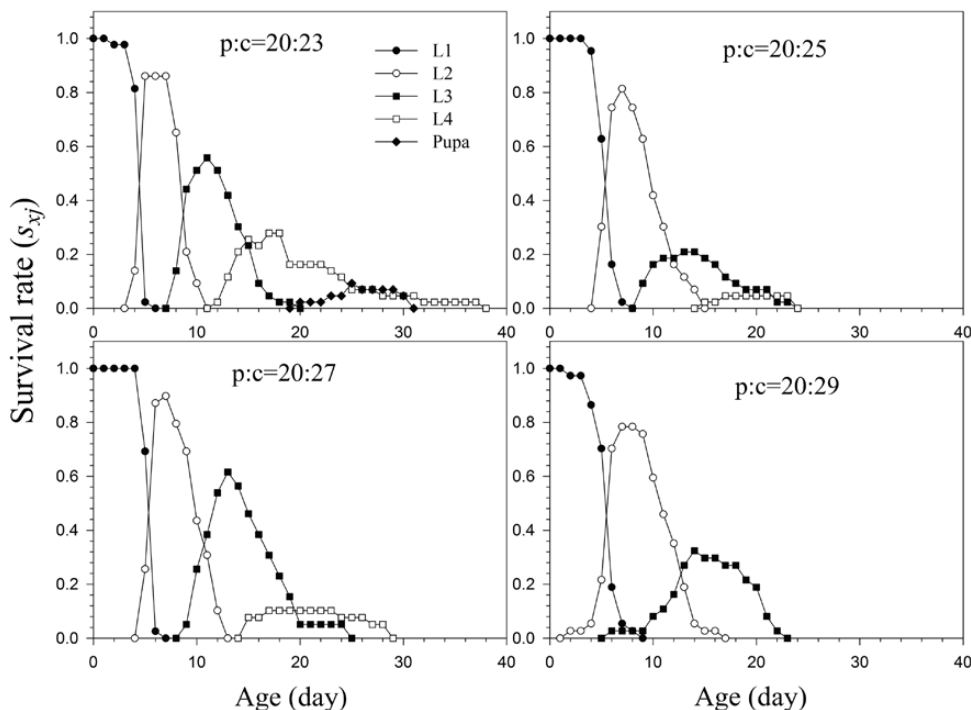

Fig. 2. Age-stage-specific survival rate (s_{xj}) of *H. vigintioctopunctata* reared on diet with different protein: carbohydrate ratios by maintaining the concentration of protein and then increasing the level of carbohydrates (p20:c23, p20:c25, p20:c27 and p20:c29).

began by maintaining the concentration of carbohydrates, and then increasing the concentration of protein (p29:c20, p31:c20, p33:c20, and p35:c20). Next, we maintained the concentration of protein, and increased the level of carbohydrates (p20:c23, p20:c25, p20:c27, and p20:c29). Finally, we kept the total protein (p) and carbohydrate

(c) amount (48% by dry mass) but changed the p:c ratios (p22:c26, p 24:c24, p26:c22). Identical amounts of artificial diet were added to plastic containers (27 cm length, 15 cm width) containing 32 litter box which was disinfected prior to usage, using 75% alcohol, and then exposed to UV light for 1 h to reduce microbial contamination.
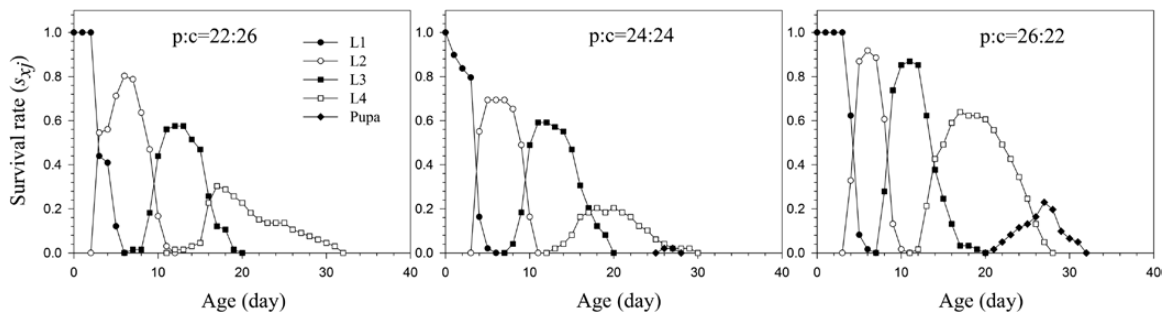
Fig. 3. Age-stage-specific survival rate (s_{xj}) of *H. vigintioctopunctata* reared on diet with different ratio of protein and carbohydrate by keeping the total protein (p) and carbohydrate (c) amount (48% by dry mass) but changed the p:c ratios (p22:c26, p24:c24 and p26:c22).

The newly hatched larvae were individually transferred to containers with one of the 11 food treatments. Each diet was replaced every 3 d, and the developmental data, survival rate, and pupal weight for all individuals were recorded daily until the emergence of adults.

Statistical Analysis

The raw life table data for *H. vigintioctopunctata* larvae reared on different ratios of protein and carbohydrate were analyzed using the age-stage, two-sex life table theory developed by Chi and Liu (1985) and Chi (1988). The bootstrap technique (Efron and Tibshirani 1993, Huang and Chi 2012) with 100,000 resampling was used to calculate the standard errors of the larval and pupal developmental times and pupal masses. Data were analyzed using the computer program, TWOSEX-MSChart (Chi 2016) (available at: http://140.120.197.173/Ecology/Download/TWOSEX-MSChart.rar). The relationship between the age-specific survival rate (*lx*) which is the probability that a newly laid egg will survive to age *x* and the age-stage specific survival rate curves (s_{xj}) which is the probability that a newly laid egg will survive to age *x* and stage *j* were calculated as:

$$l_x = \sum_{j=1}^{k} s_{xj} \qquad (1)$$

Where *k* is the number of stages.

Results

We recorded the developmental time and survival rate of *H. vigintioctopunctata* larvae reared on different protein: carbohydrate ratios when the carbohydrate level remained constant (Table 2 and Fig. 1). Although the lengths of the total larval stage (d) did not significantly differ in the four different protein levels, the pupal period reared on p35:c20 (5.47 d) was significantly shorter than that of the three other dietary ratios (Table 2). The pupal wet mass was the heaviest (19.5 mg) when larvae were fed on the p33:c20 diet, and the lightest (17.1 mg) when fed on the p31:c20 diet. The pupal mass when fed on diets p33:c20 (19.5 mg) and p29:c20 (18.5 mg) were both significantly higher than on diet p31:c20 (Fig. 2). The age-stage-specific survival rate curves (s_{xj}) (Figs. 1,3,4) clearly show the stage differentiation and overlap of *H. vigintioctopunctata* larvae reared on the eleven diets with different p: c ratios. The highest s_{xj} of the L1, L2, and L3 instars were not significant difference among the four different protein levels. However, the mortality of L4 larvae fed on p29:c20 and p 31:c20 was significantly higher than on the p33:c20 and p35:c20 diets (Fig. 1). The survival rates for larvae fed on diets p33:c20 (72%) and p35:c20 (68%) were significantly higher than those on the p29:c20 (31%) and p31:c20 (37%) diets (Fig. 1).
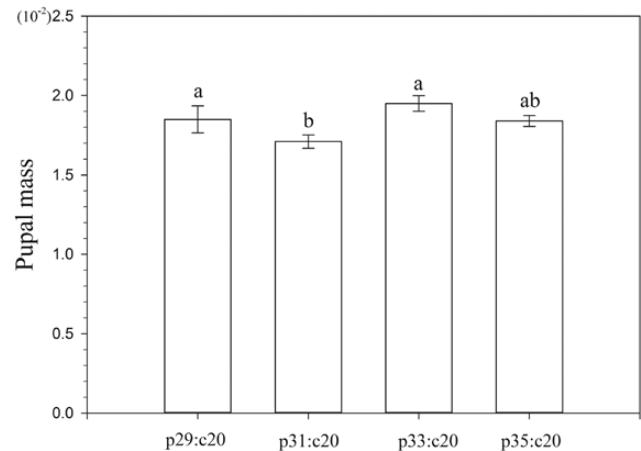

Fig. 4. The means of pupal mass of *H. vigintioctopunctata* reared on different diet by maintaining the concentration of carbohydrates, and then increasing the concentration of protein (p29:c20, p31:c20, p33:c20 and p35:c20).

The number of *H. vigintioctopunctata* larvae surviving to the fourth instar was significantly decreased with increases in carbohydrate concentration (Fig. 3). The developmental times of L1 and L2 reared on p20:c23 were significantly faster than those on p20:c25, p20:c27, and p20:c29 (Table 3). *H. vigintioctopunctata* larvae could successfully develop to the pupal stage when fed on the p20:c23 diet, but the highest pupal s_{xj} was 9.3%. The highest s_{xj} values for L4 reared on the p20:c25 and p20:c27 diets were 4.6% and 10.3%, respectively. The larvae fed on the p20:c29 diet only survived to the third instar.

When the total protein (p) and carbohydrate (c) amounts (48% by dry mass) were kept the same but differed in their p:c ratios (22:c26, p 24:c24, p26:c22), the *H. vigintioctopunctata* larvae reared on the p26:c22 and p24: p24 diets were able to complete the entire larval stage; those fed on diet p22: c26, however, were only able to develop to L4 (Fig. 4). The developmental periods of L2 and L3 reared on the p26:c20 diet were both significantly shorter than on diet p24:c24 (Table 4).

Discussion

In this study, we used the life table approach to analyze the effects that two key macronutrients have on larval performance, and to obtain the optimal p:c ratio for *H. vigintioctopunctata*. Life tables, as one of the basic research tools for population ecology studies, are often used to determine the survival, development and reproductive capabilities of a population cohort when exposed to different treatment variables such as cultivars, temperature, humidity, etc. (Morris

Table 3. The developmental time of *H. vigintioctopunctata* larva reared on different diet by maintaining the concentration of protein and then increasing the level of carbohydrates

Stage	The ratio of protein: carbohydrate			
	20:23	20:25	20:27	20:29
L1 (d)	4.83 ± 0.06^b	6.11 ± 0.12^a	5.83 ± 0.08^a	5.90 ± 0.20^a
L2 (d)	4.38 ± 0.14^a	5.58 ± 0.43^b	5.67 ± 0.28^b	7.17 ± 0.42^c
L3 (d)	5.42 ± 0.27^b	7.00 ± 0.71^a	$5.60 \pm 0.35^{a,b}$	-
L4 (d)	9.8 ± 0.52	-	-	-

Values are means ± standard errors. Standard errors were evaluated using the bootstrap procedure with 100,000 resampling and different letters in the same row indicated significantly different ($P < 0.05$) between the ratio of p:c using the paired bootstrap test. ' - ' means the *H. vigintioctopunctata* cannot finish this stage. (L1, the first instar; L2, the second instar, L3, the third instar, L4, the fourth instar.).

Table 4. The developmental time of *H. vigintioctopunctata* larva reared on different ratio of protein: carbohydrate levels by keeping the total protein (p) and carbohydrate (c) amount (48% by dry mass) but changed the p:c ratios

Stage	The ratio of protein: carbohydrate		
	26:20	24:24	22:26
L1 (d)	4.70 ± 0.07^b	3.10 ± 0.07^c	5.20 ± 0.07^a
L2 (d)	4.12 ± 0.07^c	6.00 ± 0.18^a	4.39 ± 0.11^b
L3 (d)	5.65 ± 0.19^b	6.65 ± 0.25^a	$6.00 \pm 0.30^{a,b}$
L4 (d)	11.94 ± 0.41	-	-

Values are means ± standard errors. Standard errors were evaluated using the bootstrap procedure with 100,000 resampling, and different letters in the same row indicated significantly different ($P < 0.05$) between the ratio of p:c using the paired bootstrap test. ' - ' means the *H. vigintioctopunctata* cannot finish this stage.

and Miller 1954, Jahn et al. 2005, Hu et al. 2010, Ying et al. 2012, Li et al. 2015, Yang et al. 2015).

Our results showed that the relationship between larvae performance and dietary protein/carbohydrate ratios was closely related. Clearly, altering the availability of quality diet can affect the performance of *H. vigintioctopunctata* larvae. Dietary carbohydrate levels, comparing with the total protein levels, seldom exerted a vital effect on survival, growth, and pupal weight. When the protein content was kept constant and the proportion of carbohydrates was gradually increased, the developmental duration of *H. vigintioctopunctata* larvae was significantly affected to the extent that they were unable to complete their development in the larval stage (Table 3 and Fig. 3). However, *H. vigintioctopunctata* larvae were not only able to complete their development, but the developmental period was shortened when the carbohydrate content was kept constant as the protein content percentage was increased (Table 2 and Fig. 1). The data demonstrated that protein deficits are more detrimental to *H. vigintioctopunctata* larval survival than carbohydrate deficits. This agrees with several previous reports from Simpson et al. (2004), Thompson and Redak (2000) and Wilkinson (2001) in grasshoppers, caterpillars, and aphids, respectively. However, because omnivores and herbivores regulate protein intake more intensely than they do carbohydrate, it is inevitable that they are more susceptible to insufficient protein quantities when fed on low p:c ratios (Simpson and Raubenheimer 2005).

During each animal's developmental stage, various tissues require a specific quantity and blend of nutrients (Simpson and Raubenheimer 1993). This requirement is applicable to insects as well, where the function and effect of protein and carbohydrate will vary depending on the insect's development, growth, and fecundity. It also can vary dramatically depending on the stage and sex where the demands made by larvae, and adult females and males for protein and carbohydrate are very different (Joern and Behmer 1997). An adult female insect cannot maximize both longevity and egg production rate on a single diet (Simpson and Raubenheimer 2009). The lifespan of both male and female insect are maximized on a high-carbohydrate low-protein diet. However, comparing the blend of nutrition necessary to maximized a female's lifespan, with the, blend needed to achieve the optimal female reproductive performance will often produce two ratios that are considerably different (Lee et al. 2008, Maklakov et al. 2008, Fanson et al. 2009). There is a sexual difference in the trade-off between lifespan and reproduction in females and males; the females require high levels of protein for reproduction (Wheeler 1996) while males require low-protein, high-lipid diets to build energy reserves to support them in their search for females (Stockhoff 1993).

Although proteins are very important resources for promoting host resistance and producing immunological components to resist infections by pathogens (Washburn et al. 1996, Trudeau et al. 2001, Lee et al. 2006), subsisting on pure or higher than optimal level of protein, inevitably results in adversely affecting an insect's growth and development and inducing a fitness cost (Wilkinson 2001, Cease et al. 2012). Although there was no significant difference between dietary p33:20 and p35:20 in larval survival rate and pupal mass, the survival rate (72%) and pupal mass (19.5 mg) of insects reared on dietary p33:c20 was higher than the survival rate (68%) and pupal mass (18.4 mg) for those reared on dietary p35:c20. These data suggest that the optimal p:c ratio for maximizing *H. vigintioctopunctata* larval performance is 33:20.

In addition, the present study found that females that were emerged from the experimental pupae were unable to produce offspring when fed on any of the artificial diets, regardless of the protein: carbohydrate ratio. This would may be that the protein and carbohydrate requirements differ considerably between the larvae and adults. Both larvae and adults of *H. vigintioctopunctata* feed on leaves of Solanaceae plants. *H. vigintioctopunctata* larvae have little, if any, opportunity to alter their diet by choosing between different host plants, so the beetle larvae are forced to feed within a relatively narrow p:c range. However, the situation is remarkably different for *H. vigintioctopunctata* adults, where the adults have the ability to manage imbalances by compensatory food selection. Wang et al. (2017) and Zhou et al. (2015) reported that the survival rate of *H. vigintioctopunctata* larvae was significantly higher on eggplant than it was on *S. nigrum*, while the fecundity of adults on eggplant was significantly lower than on *S. nigrum*. Their results indicate that, based on the reduced survival rates, the larvae were considerably less adapted on *S. nigrum*, than were the adults, where their fecundity was increased.

In conclusion, breeding *H. vigintioctopunctata* larvae using artificial diet was successful. Although the total length of the larval stage (22.95 d) when reared on the p33:c20 diet was longer than when reared on *S. nigrum* (14.14 d) (Wang et al. 2017), the highest s_{xj} for *H. vigintioctopunctata* larvae was 72% when feed on the p33:c20 diet which was much closer to the natural host plant, *S. nigrum* (72.5%) (Wang et al. 2017) and 75% (Huang et al. 2018). The reason for the prolonged larval period but approximately normal survival rate when compared to the host plant may be due to insufficient micronutrients such as cholesterol in the artificial diet (Svoboda et al. 1975). Our findings are a substantial improvement over previous attempts to rear *H. vigintioctopunctata* on blended artificial diets (see: Kono (1979), Murata et al. (1994) and Kawazu (2014)).

Although we were successfully in rearing *H. vigintioctopunctata* larvae, the artificial diet we used was not appropriate for rearing the adults because of their inability to produce offspring. Further refinements are needed in the breeding method, and additional studies will also be needed to determine the ideal nutrient ratio to formulate a successful artificial diet for the *H. vigintioctopunctata* adult.

Acknowledgments

References Cited

Altaye, S. Z., C. W. Pirk, R. M. Crewe, and S. W. Nicolson. 2010. Convergence of carbohydrate-biased intake targets in caged worker honeybees fed different protein sources. J. Exp. Biol. 213: 3311–3318.

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. Annu. Rev. Entomol. 47: 817–844.

Behmer, S. T. 2009. Insect herbivore nutrient regulation. Annu. Rev. Entomol. 54: 165–187.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects, vol. 2. pp. 7–9. Springer, New York.

Cease, A. J., J. J. Elser, C. F. Ford, S. Hao, L. Kang, and J. F. Harrison. 2012. Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. Science 335: 467–469.

Chi, H. 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. Environ. Entomol. 17: 26–34.

Chi, H. 2016. TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. National Chung Hsing University, Taichung, Taiwan. Available from http://140.120.197.173/Ecology/Download/Twosex-MSChart.zip

Chi, H., and H. Liu. 1985. Two new methods for the study of insect population ecology. Bull. Inst. Zool. Acad. Sin. 24: 225–240.

Dussutour, A., and S. J. Simpson. 2009. Communal nutrition in ants. Curr. Biol. 19: 740–744.

Efron, B., and R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman & Hall, New York, NY.

Fanson, B. G., C.W. Weldon, D. Pérez-Staplesn, S. J. Simpson, and P. W. Taylor. 2009. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). Aging Cell 8: 514–523.

Han, L., S. Li, P. Liu, Y. Peng, and M. Hou. 2012. New artificial diet for continuous rearing of *Chilo suppressalis* (Lepidoptera: Crambidae). Ann. Entomol. Soc. Am. 105: 253–258.

Hu, L. X., H. Chi, J. Zhang, Q. Zhou, and R. J. Zhang. 2010. Life-table analysis of the performance of *Nilaparvata lugens* (Hemiptera: Delphacidae) on two wild rice species. J. Econ. Entomol. 103: 1628–1635.

Huang, Y. B., and H. Chi. 2012. Assessing the application of the jackknife and bootstrap techniques to the estimation of the variability of the net reproductive rate and gross reproductive rate: a case study in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). J. Agri. Fore. 61: 37–45.

Huang, H. W., H. Chi, and C. L. Smith. 2018. Linking demography and consumption of *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) fed on *Solanum photeinocarpum* (Solanales: Solanaceae): with a new method to project the uncertainty of population growth and consumption. J. Econ. Entomol. 111: 1–9.

Jahn, G. C., L. P. Almazan, and J. B. Pacia. 2005. Effect of nitrogen fertilizer on the intrinsic rate of increase of *Hysteroneura setariae* (Thomas) (Homoptera: Aphididae) on rice (*Oryza sativa* L.). Environ. Entomol. 34: 938–943.

Joern, A., and S. T. Behmer. 1997. Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia 112: 201–208.

Karasov, W. H., and C. M. del Rio. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, Princeton.

Kawazu, K. 2014. Rearing the 28-spotted ladybird beetle, *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinelidae), with a switchover from host plant leaves to artificial diet. Appl. Entomol. Zool. 49: 359–362.

Kono, Y. 1979. Abnormal photoperiodic and phototactic reactions of the beetle, *Epilachna vigintioctopunctata*, reared on sliced potatoes. Appl. Entomol. Zool. 14: 185–192.

Lee, K. P., J. S. Cory, K. Wilson, D. Raubenheimer, and S. J. Simpson. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. Phil. Trans. R. Soc. Lond. B. 273: 823–829.

Lee, K. P., S. J. Simpson, F. J. Clissold, R. Brooks, J. W. O. Ballard, P. W. Taylor, N. Soran, and D. Raubenheimer. 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. Proc. Natl. Acad. Sci. USA. 105: 2498–2503.

Li, W., Y. Yang, W. Xie, Q. Wu, B. Xu, S. Wang, X. Zhu, S. J. Wang, and Y. Zhang. 2015. Effects of temperature on the age-stage, two-sex life table of *Bradysia odoriphaga* (Diptera: Sciaridae). J. Econ. Entomol. 108: 126–134.

Maklakov, A. A., S. J. Simpson, F. Zajitschek, M. D. Hall, J. Dessmann, F. Clissold, D. Raubenheimer, R. Bindurianshky, and R. C. Brooks. 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr. Biol. 18: 1062–1066.

Morris, R. F., and C. A. Miller. 1954. The development of life tables for the spruce budworm. Can. J. Zool. 32: 283–301

Murata, M., K. Iwabuchi, and J. Mitsuhashi. 1994. Partial rearing of a phytophagous lady beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinelidae). Appl. Entomol. Zool. 29: 116–119.

Nakamura, K. 1976. Studies on the population dynamics of the 28-spotted lady beetle, *Henosepilachna vigintioctopunctata* F., 1. Analysis of life tables and mortality process in the field population. Jap. J. Ecol. 26: 49–59.

Raubenheimer, D., and S. J. Simpson. 1993. The geometry of compensatory feeding in the locust. Anim. Behav. 45: 953–964.

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

Sharma, A., A. Thakur, S. Kaur, and P. K. Pati. 2012. Effect of *Alternaria alternata* on the coccinellid pest *Henosepilachna vigintioctopunctata* and its implications for biological pest management. J. Pest. Sci. 85: 513–518.

Simpson, S. J., and D. Raubenheimer. 1993. A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Phil. Trans. R. Soc. Lond. B. 342: 381–402.

Simpson, S. J., and D. Raubenheimer. 2005. Obesity: the protein leverage hypothesis. Obesity. Rev. 6: 133–142.

Simpson, S. J., and D. Raubenheimer. 2009. Macronutrient balance and lifespan. Aging 1: 875–880.

Simpson, S. J., and D. Raubenheimer, 2012. The nature of nutrition: a unifying framework from animal adaptation to human obesity. Princeton University Press, New Jersey.

Simpson, S. J., R. M. Sibly, K. P. Lee, S. T. Behmer, and D. Raubenheimer. 2004. Optimal foraging when regulating intake of multiple nutrients. Anim. Behav. 68: 1299–1311.

Stockhoff, B.A. 1993. Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. J. Insect. Physiol. 39: 677–686.

Svoboda, J. A., J. N. Kaplanis, W. E. Robbins, and M. J. Thompson. 1975. Recent developments in insect steroid metabolism. Annu. Rev. Entomol. 20: 205–220.

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

Trudeau, D., J. O. Washburn, and L. E. Volkman. 2001. Central role of hemocytes in *Autographa californica* M *nucleopolyhedrovirus* pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. J. Virol. 75: 996–1003.

Wang, Z. L., C. R. Li, J. J. Yuan, S. X. Li, X. P. Wang, and H. Chi. 2017. Demographic comparison of *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae) reared on three cultivars of *Solanum melongena* L. and a wild hostplant *Solanum nigrum* L. J. Econ. Entomol. 110: 2084–2091.

Washburn, J. O., B. A. Kirkpatrick, and L. E. Volkman. 1996. Insect protection against viruses. Nature 383: 767.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41: 407–431.

Wilkinson, T. L., L. B. Minto, and A. E. Douglas. 2001. Amino acids as respiratory substrates in aphids: an analysis of *Aphis fabae* reared on plants and diets. Physiol. Entomol. 26: 225–228.

Yang, Y., and A. Joern. 1994. Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. Physiol. Entomol. 19: 75–82

Yang, Y. T., W. X. Li, W. Xie, Q. J. Wu, B. Y. Xu, S. L. Wang, C. R. Li, and Y. J. Zhang. 2015. Development of *Bradysia odoriphaga* (Diptera: Sciaridae) as affected by humidity: an age–stage, two-sex, life-table study. Appl. Entomol. Zool. 50: 3–10.

Ying, W. D., W. T. Yan, G. S. Qiu, H. J. Zhang, and C. S. Ma. 2012. Age-stage two-sex life tables of the experimental population of *Panonychus ulmi* (Acari: Tetranychidae) on apples *Malus sieversii* subsp. *kirghisorum* and *M. domestica* golden delicious. Acta Entomol. Sinica. 55: 1230–1238.

Zhou, L., B. G. Xie, and X. P. Wang. 2015. Population dynamic of *Henosepilachna vigintioctopunctata* in different host plants in Jianghan Plain. China J. Northern. Hort. 11: 103–105.