Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly *Hermetia illucens*

**KAROL B. BARRAGAN-FONSECA**¹,², **GERRIT GORT**³, **MARCEL DICKE**¹ and **JOOP J. A. VAN LOON**¹

¹Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands, ²Departamento de Producción Animal, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, Colombia and ³Biometris, Wageningen University, Wageningen, The Netherlands

**Abstract.** We investigate how the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae) responds to dietary protein (P) and carbohydrate (C) contents and the P:C ratio in terms of both immature and adult life-history traits, as well as effects on larval body composition. Nine chicken-feed based diets varying in their P:C ratio are formulated. We test three protein concentrations (10%, 17% and 24%) and three carbohydrate concentrations (35%, 45% and 55%) and their combinations. All nine diets support the complete development and reproduction of this species. Survival is high on all diets. Development time, larval yield, larval crude fat and egg yield are more influenced by P and C contents than by the P:C ratio. Low contents result in a shorter development time. Larval yield is higher on diets with higher C-contents. Pupal development is faster on a low dietary P-content for all three C-contents. Egg yield only increases when P-content increases, although it also varies with the P:C ratio. Larval crude protein content is similar on all nine diets but increases when C-content is low (10%) in P10 and P17. Larval crude fat content is high at P24-diets irrespective of C-content. We conclude that a high macronutrient content combined with a low P:C ratio positively affects *H. illucens* performance. The diet P17:C55 supports the highest larval and adult performance and results in a high larval body protein content and an intermediate crude fat content.

**Key words.** Body nutrient composition, fecundity, food quality, larval performance, macronutrients, nutrition.

**Introduction**

For optimal performance, insects need not only sufficient food, but also a maintained diet that has the optimal content and balance of nutrients required by them over a stated time period (Simpson & Raubenheimer, 1995; Raubenheimer & Simpson, 1999). To compensate for an unbalanced diet and to obtain an optimal intake of energy and nutrients, insects have two mechanisms of regulation of body nutrient content: pre-ingestive mechanisms and/or post-ingestive mechanisms (Simpson & Raubenheimer, 1995; Raubenheimer & Simpson, 1997; Zudaire *et al.*, 1998; Raubenheimer & Simpson, 2003; Behmer, 2009). To study nutrient regulation in insects, Simpson & Raubenheimer (1993) developed the Geometric Framework (GF), a multidimensional ‘nutrient space’ model, which specifically explores how nutrients interact and the effects of a food’s nutrient content on animal performance (Roeder, 2010).

The ratio between the intake of protein and carbohydrate (P:C) provides a fruitful starting point for investigating regulatory trade-offs in macronutrient intake in insects (Simpson & Raubenheimer, 1993; Raubenheimer & Simpson, 1999). This is plausible because proteins and carbohydrates are the most important nutrients for insect survival, growth and reproduction (Aguila *et al.*, 2013; Nash & Chapman, 2014), particularly...
for species with a nonfeeding adult stage (Arrese & Soulages, 2010) and also for those holometabolous insect species that acquire most of their adult protein needs as larvae (Waldbauer et al., 1984).

Most studies on the balance between proteins and digestible carbohydrates and its effect on insect performance focus on grasshoppers, caterpillars, larval beetles, aphids and cockroaches (Waldbauer & Bhattacharya, 1973; Simpson et al., 1988; Raubenheimer & Simpson, 2003; Behmer, 2009; Roeder & Behmer, 2014). Such studies find that the optimal dietary P:C ratio varies among species because of their life-history strategy and phylogeny (Raubenheimer & Simpson, 1997). For example, insects with the lowest P:C requirements are those with endosymbiotic bacteria that contribute to nitrogen metabolism, such as aphids and generalist cockroaches (Raubenheimer & Simpson, 2003).

An optimal P:C ratio can also vary within species dependent on the developmental stage and even sex as a result of different physiological needs (Canato & Zucoloto, 1998). For example, the optimum P:C ratio for reproductive performance does not always coincide with that for other life-history traits. In field crickets (Gryllus veletis), adult weight gain and egg production is reported to be maximized on high-protein diets (3 : 1), whereas adult lifespan is maximized on high-carbohydrate diets (males: 1 : 3; females: 1 : 8) (Harrison et al., 2014). In the fruit fly Drosophila melanogaster, there are large differences in nutrient-dependent optima for lifespan and reproduction between females and males (Jensen et al., 2015). Indeed, some studies demonstrate the reproductive consequences of a persistent unbalanced diet in insects. Thus, in the Queensland fruit fly, Bactrocera tryoni, dietary P and C contents can affect sexual activity and longevity (Lee et al., 2008; Prabhu et al., 2008).

The nutritional studies available to date on the black soldier fly (Hermetia illucens L.; Diptera: Stratiomyidae) address the effects of diets on development and adult life-history traits by focussing on oligidic or natural diets containing many undefined components, such as organic waste or industrial by-products (Barragan-Fonseca et al., 2017). Cammack & Tomberlin (2017) report examining the impact of artificial diets (P + C content 42%) with three P:C ratios (1 : 5, 1 : 1 and 5 : 1) on the life-history traits of the fly and find a higher survival rate on a 1 : 1 ratio, although no conclusive effects on adult life-history traits. It is largely unknown how H. illucens responds to protein and carbohydrate contents and ratios and how this affects its body composition and adult performance. This saprophagous species exploits diverse diets, such that larval stages are likely to encounter a wide range of dietary macronutrient ratios. When analyzing the food sources on which this species is found in the wild (vegetable waste, manure, etc.), P:C ratios tend to be carbohydrate-biased (P:C ratios 1 : 1.5, 1 : 2) and present a wide range of crude protein and digestible carbohydrate contents [40–80% dry matter (DM)]. Larvae of the fly hide inside feed substrates and mix uneaten diet with their faeces, making it unsuitable for self-selection experiments. Based on its natural food sources, we can assume that its intake target might be carbohydrate biased.

It is clear that dietary macronutrient content is important to insects and even small departures from an optimal P:C ratio can have dramatic physiological effects and affect insect fitness (Prabhu et al., 2008; Le Gall & Behmer, 2014). However, most nutrient regulation studies in insects fail to address consequences for fitness (Roeder, 2010). By varying dietary P:C ratios and contents, it is possible to explore how performance, both within developmental stages and across the entire lifespan, is affected by these nutrients. Therefore, the present study explores the effects of dietary P and C contents and the P:C ratio within the range of its natural food sources under controlled conditions on both immature and adult life-history traits of H. illucens and on larval body macronutrient composition.

Materials and methods

Experimental insects

Hermetia illucens eggs were obtained from a colony maintained under constant conditions in a climate room (LD 12 : 12 h photocycle at 27 ± 1°C and 70% relative humidity) at the Laboratory of Entomology, Wageningen University (The Netherlands).

Experimental design

Nine diets varying in their P:C ratio and contents were formulated. We tested three protein concentrations (10%, 17% and 24%) and three concentrations of digestible carbohydrates (35%, 45% and 55%), with the total macronutrient (P and C) content of these diets ranging from 45–79%: (i) P10:C35 (10% protein and 35% carbohydrate; combined macronutrient content = 45%); (ii) P10:C45; (iii) P10:C55; (iv) P17:C35; (v) P17:C45; (vi) P17:C55; (vii) P24:C35; (viii) P24:C45; and (ix) P24:C55. The P:C ratios are listed in Table 1. The diets were formulated by including a standard proportion of chicken feed (Opfokmeel Farmfood; Agruniek Rijnvallei Voer BV, The Netherlands) in the diet (63% of the total dry mass of the experimental diet, which contributed 16.56% protein, 3.5% fat and 44% carbohydrate to each treatment). This was used because it was found to support the full life cycle of the fly during H. illucens rearing (K. Barragán-Fonseca, personal observations) and in previous experiments (Spranghers et al., 2016; Barragan-Fonseca et al., 2018). The remaining 37% of the experimental diet was composed of the protein casein (Bio-connect, The Netherlands) and the digestible carbohydrate starch (Duchefa Biochemie BV, The Netherlands), which served both as a nutrient and as a texturing agent. The α-sugar linkages in starch make it highly susceptible to digestion by insects (Cohen, 2004). The remaining proportion was cellulose (Alphacel non-nutritive bulk; Bio-connect), which is considered to be a poorly digestible bulk agent (Table 1). Ingredients were mixed thoroughly to avoid larval selection.

The larvae were reared on the nine experimental diets, each comprising 60% water. Each replicate consisted of 100 larvae (hatched from the egg less than 24 h before) per 750 mL container. A fixed ration of 0.6 g (DM basis) of food per larva was added once at the beginning of the experiment because a
Table 1. Composition of experimental diets.

| Ingredient (%) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Chicken feed   | 63  | 63  | 63  | 63  | 63  | 63  | 63  | 63  | 63  |
| Casein         | 0   | 0   | 0   | 7   | 7   | 7   | 7   | 14  | 14  |
| Starch         | 3   | 13  | 23  | 3   | 13  | 23  | 3   | 13  | 23  |
| Cellulose      | 34  | 24  | 14  | 27  | 17  | 7   | 21  | 10  | 0   |
| Nutrient (%)   |     |     |     |     |     |     |     |     |     |
| Crude protein  | 10  | 10  | 10  | 17  | 17  | 17  | 17  | 24  | 24  |
| Digestible    | 35  | 45  | 55  | 35  | 45  | 55  | 35  | 45  | 55  |
| carbohydrate   |     |     |     |     |     |     |     |     |     |
| Sum of crude   | 45  | 55  | 65  | 52  | 62  | 72  | 59  | 69  | 79  |
| P:C ratio      | 1 : 3.5 | 1 : 4.5 | 1 : 5.5 | 1 : 2.1 | 1 : 2.6 | 1 : 3.2 | 1 : 1.5 | 1 : 1.9 | 1 : 2.3 |

*Noncellulose carbohydrate.

preliminary experiment showed that larvae fed once rather than three times per week reached a higher biomass. The feeding experiments were conducted in a climatic room (4.5 m²) and maintained under constant conditions (LD 12 : 12 h photocycle at 27 ± 1 °C and 55 ± 5% relative humidity). To eliminate positional effects, all containers were randomly relocated three times per week.

**Larval life-history traits**

Per dietary treatment, nine replicates were set up. Larvae were harvested when at least 50% of the larvae were observed to have reached the pre-pupal stage, as indicated by the characteristic melanized cuticle of prepupae (May, 1961). Development time was considered to be the number of days between the start of the experiment and the day of harvesting. All animals from each container were harvested with forceps, counted and weighed collectively. Larvae were washed under running water to remove any residues consisting of left-over feed and faeces, their integument dried with paper tissue and then their weight was registered as *H. illucens* larval yield (g fresh matter) on a precision balance (Adventurer Pro AV313; Ohaus, Parsippany, New Jersey; precision ±0.001 g). To determine survival, the number of live *H. illucens* larvae at the day of harvesting was divided by the initial number of larvae per replicate (100). For the adult experiment, when at least 50% of the larvae were observed to have reached the pupal stage, individual pupal weight (g fresh matter) was determined in the same way as larval weight. The development time until the adult stage was considered as the number of days between the start of the experiment and the median day of adult emergence.

**Adult life-history traits**

Per dietary treatment four replicates were set up. Forty newly eclosed (<12 h old, 20 females and 20 males) adult *H. illucens* from each container were released into a BugDorm cage (30 × 30 × 30 cm) (MegaView Science Co., Ltd, Taiwan) and kept in a greenhouse compartment under controlled conditions (natural light at 25 ± 1 °C and 70 ± 5% relative humidity). Adults were provided with water and sugar *ad libitum* and, 2 days after eclosion, a mixture of sand (80 g), chicken feed (80 g) and water (100 mL) in a plastic container (10 × 10 × 6 cm) was offered for oviposition. On the surface of each container, four corrugated cardboard pieces (each with height 4 cm, length 9 cm) were placed as a substrate for egg laying. Every 3 days, the cardboard was removed from all containers, the sand–feed–water medium replaced and new cardboard pieces were also put into place. We then determined fecundity and the number of egg masses. Accordingly, we measured the surface of viable egg masses (mm²) [i.e. percentage of eggs from which larvae hatched (F1)] as a proxy of viable egg number; thus, we registered the surface of all the egg masses we collected, although we only took into account the surface of those egg masses from which F1 larvae hatched; this variable was called egg yield (mm²). To determine survival until the adult stage, the number of *H. illucens* adults eclosed at the end of the experiment was divided by the initial number of larvae per replicate.

**Proximate chemical analysis of larvae**

Samples were stored in a freezer (−25°C) until all replicates were harvested. Both the larvae and the diets were analyzed for DM, crude protein and crude fat contents at the Animal Nutrition Laboratory of Wageningen University. The samples were oven-dried at 70 °C until a constant weight was achieved and then homogenized by grinding the sample in an ultra-centrifugal mill (ZM 200; Retsch, Germany). Crude protein (6.25 × N-content) was determined using the Kjeldahl method (ISO, 2005) and crude fat was analyzed in accordance with the Berntop method (ISO, 2005). Proximate chemical analysis of larvae

**Statistical analysis**

To examine how protein and carbohydrate levels affected the response variables on insect performance and larval body content data, we used r, version 3.3.3 (R Core Team, 2017), specifically the package rsm, for response surface models. We
first fitted quadratic response surface models using the total content of P and C and their ratio (P:C) as regressors. For these regressions, sums of squares for specific components were obtained: (i) linear and quadratic effects of P + C (d.f. = 2); (ii) linear and quadratic effects of P:C (d.f. = 2); (iii) interactions among P + C and P:C (d.f. = 4); and (iv) residual errors (d.f. = 72 for larval data, d.f. = 45 for larval body composition data, d.f. = 27 for adult data). For components (i) and (ii), the sums of squares were obtained in two orders (using models with each component corrected or not corrected for the other but always without interaction terms) and the resulting sums of squares were averaged. The sums of squares obtained were divided by the total sum of squares, giving the relative importances of the four components, which next were visualized in a stacked barplot. The full analysis of variance (ANOVA) for all 11 response variables is given.

Next, we fitted quadratic response surface models using the actual P and C contents as regressors. Results were summarized with an overall F-test (testing for any effect at all) and with F-tests for linear effects of P and of C; for quadratic effects of P and of C (after linear effects of P and C); for interaction of P and C (after linear effects of P and C); and a lack of fit for the quadratic response surface model (indicating higher-order interactions). Furthermore, regression coefficients with SEs for all regressors were produced (Appendices I–III), using higher-order terms that were orthogonalized to lower-order terms. The advantage of this approach is that slopes for lower-order terms will not change if a higher-order term (e.g. an insignificant lack-of-fit term) is removed from the model. The full ANOVA for all of the response variables is given. If the overall F-test showed a significant result, we made contour plots showing the fitted response surfaces. For contour plots, the P–C interaction and quadratic terms were removed from the model if found to be unnecessary (P > 0.05) and, in the case of a significant lack of fit (P < 0.05), higher-order terms were added. For all fitted models, residual plots were made to check for potential outliers and deviations of assumptions (i.e. approximate normality and constant variance). The relationship between egg mass production and pupal weight was studied using the Pearson correlation coefficient.

**Results**

Table 2 shows sums of squares for sets of regressors per response variable with rows ordered by fraction variance explained by total P + C and (P + C)². Relative importances (Fig. 1) were calculated as sums of squares divided by total sum of squares. Pupal weight and larval yield were largely (~80%) and almost exclusively determined by linear and quadratic effects of P + C. Larval crude fat content, egg yield and development times for adults and larvae were explained with respect to 50–60% by P + C effects. Relatively large contributions of linear and quadratic effects of the ratio P:C (20–30%) were found for larval crude fat, adult development time, number of egg masses and larval crude protein. Hardly any variation was explained either by P + C or P:C for larval survival, adult emergence and larval hatching. Table 3 shows the full ANOVA for all 11 response variables.

P + C content had a stronger effect than P:C ratio on larval yield, larval development time, egg yield, development time until adult and larval crude fat content. Hardly any variation was explained either by P + C or P:C for larval survival, adult emergence and larval hatching. Some variation was explained by P + C and by P:C for number of egg masses and larval crude protein content (Fig. 1).

**Immature life-history traits**

**Performance.** Survival rate was not affected by protein or carbohydrate content (P = 0.36) (Table 4). Development time showed strong linear effects of protein and of carbohydrate content (P < 0.001), although some interaction (P = 0.003) and a quadratic effect of protein (P = 0.008) also occurred. (Table 2). Development time was shorter with lower protein and carbohydrate contents (Fig. 2a). Larval yield showed a very strong linear effect of carbohydrate content (P < 0.001) and less

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### Table 2. Sums of squares for larval (L), adult (A) and body compositional (BC) response variables.

| Response Variable | SS(s1) (d.f. = 2) | SS(s1 + r) (d.f. = 2) | SS(s1 + r) (d.f. = 4) | SS(s1 + r) (d.f. = 4) | SS(error) (d.f. = 72/45/27) | SS(total) (d.f. = 80/53/35) |
|-------------------|------------------|----------------------|----------------------|----------------------|-----------------------------|-----------------------------|
| Pupal weight (A)  | 0.02062          | 0.00022              | 0.02080              | 0.00004              | 0.00140                     | 0.00321                     | 0.02548                     |
| Larval yield (L)  | 63.00            | 2.46                 | 64.57                | 0.90                 | 2.50                        | 12.37                       | 80.33                       |
| Crude fat (BC)    | 776.2            | 306.6                | 692.8                | 390.0                | 32.5                        | 117.9                       | 1233.2                      |
| Egg yield (A)     | 0.711            | 0.092                | 0.632                | 0.172                | 0.124                       | 0.233                       | 1.160                       |
| Adult development time (A) | 285.5 | 89.6                | 235.6                | 139.6                | 16.9                        | 58.8                        | 450.8                       |
| Larval development time (L) | 133.7 | 33.2                | 130.2                | 36.8                 | 1.2                         | 82.0                        | 250.2                       |
| Egg mass number (A) | 581.3        | 536.5                | 420.5                | 697.3                | 389.7                       | 1335.5                      | 2843.0                      |
| Crude protein (BC) | 20.71           | 16.63                | 14.10                | 23.23                | 7.73                        | 65.40                       | 110.46                      |
| Larval survival (L) | 45.2           | 59.2                 | 47.7                 | 54.6                 | 35.2                        | 1111.6                      | 1249.1                      |
| Adult emergence (A) | 7.7             | 14.2                 | 6.1                  | 15.8                 | 31.0                        | 511.0                       | 563.9                       |
| Larval hatching (A) | 10.3           | 46.8                 | 15.7                 | 41.4                 | 423.8                       | 1999.9                      | 2480.7                      |

Showing sums of squares for combined effect of sum P + C and (P + C)² (labelled as ‘s’, d.f. = 2), combined effect of ratio P:C and (P:C)² (labelled as ‘r’, d.f. = 2), interaction terms for ‘s’ and ‘r’ (d.f. = 4), residual (d.f. for L:72, A:27, BC:45) and total (d.f. for L:80, A:35, BC:53). Sums of squares for ‘s’ and for ‘r’ are shown in two orders: e.g. SS(s1) indicates the sum of squares for ‘s’, which is added to a model that only contains an intercept (1), whereas SS(s1 + r) is the sum of squares for ‘s’, which is added to a model that contains both an intercept and ‘r’.

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Table 3. Analysis of variance for larval (L), adult (A) and body compositional (BC) response variables.

|                     | SS     | d.f. | MS    | F      | P      |
|---------------------|--------|------|-------|--------|--------|
| Pupal weight (A)    |        |      |       |        |        |
| PC total (L + Q)    | 0.02080| 2    | 0.01040| 87.45  | 0.0000 |
| PC ratio (L + Q)    | 0.00022| 2    | 0.00011| 0.94   | 0.4042 |
| Higher-order and interaction | 0.00143| 4    | 0.00036| 3.01   | 0.0354 |
| Residual            | 0.00321| 27   | 0.00012|        |        |
| Larval yield (L)    |        |      |       |        |        |
| PC total (L + Q)    | 64.566 | 2    | 32.283 | 187.94 | 0.0000 |
| PC ratio (L + Q)    | 2.462  | 2    | 1.231  | 7.17   | 0.0015 |
| Higher-order and interaction | 2.503  | 4    | 0.626  | 3.64   | 0.0093 |
| Residual            | 12.368 | 72   | 0.172  |        |        |
| Crude fat (BC)      |        |      |       |        |        |
| PC total (L + Q)    | 692.80 | 2    | 346.40 | 132.17 | 0.0000 |
| PC ratio (L + Q)    | 306.59 | 2    | 153.29 | 58.49  | 0.0000 |
| Higher-order and interaction | 32.49  | 4    | 8.12   | 3.10   | 0.0246 |
| Residual            | 117.94 | 45   | 2.52   |        |        |
| Egg yield (A; log transformed) |      |      |        |        |        |
| PC total (L + Q)    | 0.6319 | 2    | 0.3160 | 36.69  | 0.0000 |
| PC ratio (L + Q)    | 0.0920 | 2    | 0.0460 | 5.34   | 0.0111 |
| Higher-order and interaction | 0.1236 | 4    | 0.0309 | 3.59   | 0.0180 |
| Residual            | 0.2325 | 27   | 0.0086 |        |        |
| Adult development time (A) |      |      |        |        |        |
| PC total (L + Q)    | 235.60 | 2    | 117.80 | 54.14  | 0.0000 |
| PC ratio (L + Q)    | 89.58  | 2    | 44.79  | 20.59  | 0.0000 |
| Higher-order and interaction | 16.93  | 4    | 4.23   | 1.95   | 0.1318 |
| Residual            | 58.75  | 27   | 2.18   |        |        |
| Larval development time (L) |      |      |        |        |        |
| PC total (L + Q)    | 130.16 | 2    | 65.08  | 57.14  | 0.0000 |
| PC ratio (L + Q)    | 33.22  | 2    | 16.61  | 14.58  | 0.0000 |
| Higher-order and interaction | 1.25   | 4    | 0.31   | 0.27   | 0.8937 |
| Residual            | 82.00  | 45   | 1.84   |        |        |
| Egg mass number (A) |        |      |       |        |        |
| PC total (L + Q)    | 420.50 | 2    | 210.23 | 4.25   | 0.0248 |
| PC ratio (L + Q)    | 536.50 | 2    | 268.24 | 5.42   | 0.0105 |
| Higher-order and interaction | 389.70 | 4    | 97.42  | 1.97   | 0.1277 |
| Residual            | 1385.50| 45   | 29.64  |        |        |
| Crude protein (BC)  |        |      |       |        |        |
| PC total (L + Q)    | 14.10  | 2    | 7.05   | 4.85   | 0.0124 |
| PC ratio (L + Q)    | 16.63  | 2    | 8.31   | 5.72   | 0.0061 |
| Higher-order and interaction | 7.73   | 4    | 1.93   | 1.33   | 0.2739 |
| Residual            | 65.40  | 45   | 1.45   |        |        |
| Larval survival (L) |        |      |       |        |        |
| PC total (L + Q)    | 47.71  | 2    | 23.85  | 1.55   | 0.2203 |
| PC ratio (L + Q)    | 59.17  | 2    | 29.59  | 1.92   | 0.1546 |
| Higher-order and interaction | 35.23  | 4    | 8.81   | 0.57   | 0.6849 |
| Residual            | 1111.56| 72   | 15.44  |        |        |
| Adult emergence (A) |        |      |       |        |        |
| PC total (L + Q)    | 6.09   | 2    | 3.05   | 0.16   | 0.8522 |
| PC ratio (L + Q)    | 14.24  | 2    | 7.12   | 0.38   | 0.6900 |
| Higher-order and interaction | 30.96  | 4    | 7.44   | 0.41   | 0.8006 |
| Residual            | 511.00 | 27   | 18.93  |        |        |
| Larval hatching (A) |        |      |       |        |        |
| PC total (L + Q)    | 15.67  | 2    | 7.84   | 0.11   | 0.9000 |
| PC ratio (L + Q)    | 46.83  | 2    | 23.42  | 0.32   | 0.7316 |
| Higher-order and interaction | 423.77 | 4    | 105.94 | 1.43   | 0.2510 |
| Residual            | 1999.85| 27   | 74.07  |        |        |

Showing sums of squares for combined linear (L) and quadratic (Q) effects of sum P+C, combined linear and quadratic effects of ratio P:C, higher-order and interaction terms (d.f. = 4), and residual (d.f. for L:72, A:27, BC:45).

Type II sums of squares are given. Tables are ordered by the fraction of variation explained total P+C (L+Q).

Fig. 1. Relative importance of summed protein and carbohydrate (P+C) content and ratio (P:C) on performance and larval body content variables of black soldier fly.

Larval body composition. Larval crude protein was affected in a linear fashion by dietary protein (P < 0.001) (Table 5) and by the interaction of protein × carbohydrate contents (P = 0.004) (Table 5). At a low carbohydrate content, larval crude protein decreased with an increasing protein level in the diet. At a high carbohydrate content, larval crude protein did not change with increasing protein content (Fig. 3a). Larval crude fat was very well explained by the model (r² = 0.90) and showed the strongest linear trend for protein (P < 0.001) (Table 5), although less so for carbohydrate (P < 0.001) (Table 5); smaller interaction and quadratic protein effects were also significant (Table 4). At a high protein content for the diet, the dietary carbohydrate concentration decreased (Fig. 2b, c).

Adult life-history traits

Performance. Development time from egg until adulthood was affected in a strongly linear fashion, first in size by protein (P < 0.001) (Table 6) and next by carbohydrate content (P < 0.001) (Table 6); a considerable protein–carbohydrate interaction was found (P < 0.001) (Table 6), as well as a relatively small quadratic protein effect (P = 0.01) (Table 6). Development was fastest on diets with the lowest protein content for all carbohydrate contents, and these then decreased in a linear fashion as the dietary carbohydrate concentration decreased (Fig. 2b, c).
Table 4. Response surface model significance shown as P-values (F-values in parentheses) for the full model (F with 8 and d.f. = 72; for pupal weight, F with 8 and d.f. = 27), linear effects (P is protein content and C is carbohydrate content), their interactions and quadratic terms for larval performance measurements (F with 1 and d.f. = 72; for pupal weight, F with 1 and d.f. = 27).

| Source           | Survival rate (%) | Development time (days) | Larval yield (g dry matter) | Pupal weight (g fresh matter) |
|------------------|-------------------|-------------------------|-----------------------------|-------------------------------|
| Full model       | 0.364 (1.11)      | <0.001 (18.46)          | <0.001 (49.5)               | <0.001 (23.4)                 |
| P                | 0.192 (1.73)      | <0.001 (86.7)           | <0.001 (57.7)               | <0.001 (35.2)                 |
| C                | 0.242 (1.39)      | <0.001 (42.3)           | <0.001 (305.7)              | <0.001 (119.1)                |
| P × C            | 0.142 (2.20)      | 0.003 (9.75)            | <0.001 (12)                 | <0.001 (14.4)                 |
| P²               | 0.310 (1.10)      | 0.008 (7.42)            | 0.447 (0.58)                | 0.060 (3.96)                  |
| C²               | 0.750 (1.10)      | 0.61 (0.27)             | 0.022 (5.49)                | 0.063 (3.73)                  |
| Lack of fit      | 0.496 (0.80)      | 0.73 (0.43)             | 0.005 (4.71)                | 0.025 (3.64)                  |
| r²               | 0.11              | 0.67                    | 0.84                        | 0.85                          |

(a) Development time (d); (b) larval yield per container [g dry matter (DM)]; and (c) individual pupal weight [g fresh matter (FM)]. A fitness landscape corresponding to insect response to each diet has been fitted over nutrient space; contour lines delimit colour areas going from the lowest (green) to the highest (orange) values calculated for each performance variable. Black points indicate the nine experimental diets. [Colour figure can be viewed at wileyonlinelibrary.com].

Fig. 2. Response surfaces for black soldier fly larval performance variables on diets of different protein and carbohydrate contents. (a) Development time (d); (b) larval yield per container [g dry matter (DM)]; and (c) individual pupal weight [g fresh matter (FM)]. A fitness landscape corresponding to insect response to each diet has been fitted over nutrient space; contour lines delimit colour areas going from the lowest (green) to the highest (orange) values calculated for each performance variable. Black points indicate the nine experimental diets. [Colour figure can be viewed at wileyonlinelibrary.com].

masses produced per female showed a strong linear effect of protein (P < 0.001) (Fig. 4b and Table 6). Egg yield, which was log-transformed, was affected in a linear fashion by protein and carbohydrate (both P < 0.001) (Table 6) and also showed second- and higher-order effects (Table 6). Egg yield was highest on P17:C55 and lowest on P10:C35 (Fig. 4c); however, there are minor differences compared with the other seven diets. There was a significant relationship between pupal weight and egg yield (Pearson correlation coefficient: r = 0.63, P < 0.05).

Discussion

Our data suggest that H. illucens performance is affected by the total concentration of dietary P+C content rather than by the P:C ratio. The P:C ratios tested are probably within the wide range of P:C ratios that are found in the diverse substrates utilized by the fly in nature. Cammack & Tomberlin (2017) report an examination of the impact of artificial diets (P+C content 42%) with three P:C ratios (1:1, 1:5 and 5:1) and two moisture levels, finding a greater effect of diet moisture than P:C ratio, with larval performance being quite similar among all the P:C ratios. In herbivorous insect species, such as the grasshopper Melanoplus differentialis, the contents of dietary macronutrients also have more pronounced effects than the P:C ratio (Le Gall & Behmer, 2014). However, the impact of low quality food can be amplified by a suboptimal P:C ratio, as is found for S. littoralis for which the negative impact of a low quality protein diet on larval performance is amplified as the P:C ratio of the diet decreases (Lee, 2007).

Larval life-history traits

Performance. Because this species can be found in manure (Sheppard et al., 1994) and in carrion (Martinez-Sanchez et al.,
Table 5. Response surface significance shown as P-values (F-values between parentheses) for the full model (F with 8 and d.f. = 45), linear effects (P is protein and C is carbohydrate content), their interactions and quadratic terms for larval body protein and fat contents (F with 1 and d.f. = 45).

| Source        | Larval crude protein(%) | Larval crude fat(%) |
|---------------|-------------------------|---------------------|
| Full model    | 0.002 (3.87)            | < 0.001 (53.2)      |
| P             | < 0.001 (13.4)          | < 0.001 (309.1)     |
| C             | 0.543 (9.23)            | < 0.001 (66.1)      |
| P × C         | 0.004 (9.1)             | < 0.001 (25.9)      |
| P²            | 0.565(0.36)             | < 0.001 (27.7)      |
| C²            | 0.945 (0.005)           | 0.565(1.30)         |
| Lack of fit   | 0.176 (1.72)            | 0.820 (0.31)        |
| r²            | 0.41                    | 0.90                |

2011), it experiences a high degree of nutritional heterogeneity in its dietary protein and carbohydrate contents. In the present study, we use diets containing macronutrient contents and ratios similar to those found for nutrient sources exploited by the fly in nature; in particular, chicken, pig and cow manure (K. Barragán-Fonseca, unpublished data). We therefore expect that this fly would perform well in terms of survival and growth. This is confirmed by the high survival rate on all diets. However, differences in performance among diets are significant in terms of development time and larval weight gain. Our analysis of the relative importance of contents and ratio of macronutrients (Fig. 1) demonstrates that duration of development is a function of protein and carbohydrate content (> 50%) and their interaction (~20%), whereas larval and pupal weight are mostly a function of protein and carbohydrate content (> 80%). We observe a longer development time at higher carbohydrate contents; similarly, Cammack & Tomberlin (2017) report that a carbohydrate-biased diet increases the development time in H. illucens larvae. In herbivorous insects such as Melanoplus differentialis, the duration of development is a function of an interaction between plant protein and carbohydrate contents; however, weight gain is a function of only carbohydrate content (Le Gall & Behmer, 2014). Our results for H. illucens clearly demonstrate an effect of carbohydrate content on body mass.

The highest larval weights are achieved by larvae fed on the diets with the highest contents of both protein and carbohydrate. Because H. illucens larvae are restricted to one diet, they are unable to regulate the intake of one component without simultaneously altering intake of all others. The high larval weight associated with a high dietary carbohydrate concentration, even on the low and medium protein diets, may indicate that larval and pupal weight are more affected by dietary carbohydrate than by dietary protein content. This could result in the high body fat contents that we find in the present study, especially when the protein concentration in food is low (Le Gall & Behmer, 2014). Deposition of lipids has important physiological costs: theoretical estimates suggest that the conversion of hexoses to storage fat can take up to 20–25% of the energy content of the food supplied (Westerterp, 1994; Le Gall & Behmer, 2014).

We find that H. illucens larvae may trade-off developing faster over growing bigger because faster development results

Fig. 3. Black soldier fly larval body composition when feeding on diets differing in P and C contents. (a) Larval crude protein (%) and (b) larval crude fat (%). A fitness landscape corresponding to insect response to each diet has been fitted over nutrient space; contour lines delimit colour areas going from the lowest (green) to the highest (orange) values for each performance variable. Black points indicate the nine experimental diets. [Colour figure can be viewed at wileyonlinelibrary.com].
Table 6. Response surface significance shown as P-values (F-values between parentheses) for the full model (F with 8 and d.f. = 27), linear effects (P is protein content and C is carbohydrate content), their interactions and quadratic terms for adult performance measurements (F with 1 and d.f. = 27).

| Source          | Adult emergence (%) | Development time until adult (days) | Number of egg masses* | Larval hatching (%) | Egg yieldb (mm²) |
|-----------------|---------------------|-------------------------------------|-----------------------|--------------------|------------------|
| Full model      | 0.940 (0.35)        | <0.001 (22.5)                       | 0.005 (3.81)          | 0.599 (0.81)       | <0.001 (13.5)    |
| P               | 0.356 (0.88)        | <0.001 (119)                        | <0.001 (20.8)         | 0.834 (0.04)       | <0.001 (54.1)    |
| C               | 1.0 (< 0.001)       | <0.001 (22.1)                       | 0.44 (0.61)           | 0.762 (0.09)       | <0.001 (25.2)    |
| P × C           | 0.820 (0.06)        | <0.001 (27.6)                       | 0.46 (0.55)           | 0.638 (0.23)       | 0.02 (6.32)      |
| P²              | 0.670 (0.19)        | 0.014 (6.95)                        | 0.16 (2.12)           | 0.652 (0.21)       | 0.009 (7.73)     |
| C²              | 0.915 (0.01)        | 0.635 (0.23)                        | 0.80 (0.06)           | 0.454 (0.57)       | 0.062 (3.80)     |
| Lack of fit     | 0.649 (0.55)        | 0.314 (1.24)                        | 0.121 (2.11)          | 0.175 (1.78)       | 0.028 (3.53)     |
| r²              | 0.01                | 0.87                                | 0.53                  | 0.2                 | 0.53             |

*a Square root transformation because of count data.

*b Log₁₀ transformation.

Fig. 4. Black soldier fly adult performance when feeding on diets differing in protein and carbohydrate contents. (a) Development time until adult stage (d); (b) number of egg masses per treatment group; and (c) egg yield (mm²) (log₁₀ transformation). A fitness landscape corresponding to insect response to each diet has been fitted over nutrient space; contour lines delimit colour areas going from the lowest (green) to the highest (orange) values for each performance variable. Black points indicate the nine experimental diets. [Colour figure can be viewed at wileyonlinelibrary.com].

in lighter larvae. It is possible that the positive relationship between development time and final body mass has a fitness benefit only when the constraints for long development are relaxed (Kause et al., 2001). In the case of those species that feed on diets with varying resource qualities with an increasing level of competition, they might gain higher fitness by rapid development, aiming to avoid the possible cost of increased mortality as a result of larval predation and parasitism and to start adult foraging sooner (Kaspi et al., 2001; Lee et al., 2006; Dmitriew, 2011). This trend is reported for other species, such as the autumnal moth (Epirrita autumnata), the birch-feeding sawfly (Amauronematus amplus) (Kause et al., 2001) and the larval stages of the Mediterranean fruit fly (Ceratitis capitata) (Kaspi et al., 2002). A shorter development time without a reduction in body size could be considered as an indicator of a high-quality resource. For example, larvae of the spotted-wing fruit fly (Drosophila suzukii) are able to develop faster without a reduction in adult body size on low P:C ratio diets (Jaramillo et al., 2015). An alternative interpretation, however, is that adaptation to a protein-poor food constrains the ability to respond plastically when presented with a higher quality resource (Hardin et al., 2015; Jaramillo et al., 2015).

Larval body composition. The present study shows that only a small fraction of variation in larval crude protein is explained by dietary protein and carbohydrate contents and their interaction (approximately 20%). In our study, larval crude protein

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content (range 41.4–44.2% DM) is high and similar on all nine diets. Similar values for protein content are found in previous studies for larvae fed on chicken feed, by-products of food manufacturing and manure (43.2% ± 2.7% DM) (Barragan-Fonseca et al., 2017). We find that larval crude protein content is higher when dietary P-content is lower (P10 and P17). This is in line with the findings of Barragan-Fonseca et al. (2018) and Tschirner & Simon (2015) who report that body crude protein content is higher in larvae fed on the diet with the lowest crude protein content. However, it is also argued that this content has been overestimated so far by approximately 20% as a result of the presence of nonprotein nitrogen in insects (Janssen et al., 2017). Our nitrogen analysis data do not allow a distinction between protein- and nonprotein-nitrogen. A high level of larval crude protein on low quality diets could explain the shorter larval development time of H. illucens larvae because they may use protein to develop faster into prepupae.

Low P+C content diets result in lower larval weights. This indicates that H. illucens larvae feeding on low protein diets use dietary protein efficiently, as is seen for insects with symbionts (Raubenheimer & Simpson, 2003). Hermetia illucens larvae have a diverse microbial community in their digestive tract that is likely to harbour such symbionts (Jeon et al., 2011). The high larval weight that we record on diets with a high protein content is associated with increased lipid contents, as explained previously. However, in other studies on this species, larvae feeding on high protein substrates, such as liver or meat, have higher protein contents (60.3% ± 3.3% DM) and larvae feeding on vegetable waste have lower protein contents (38.5% DM) (Nguyen et al., 2015). This suggests that the quality of dietary protein affects larval protein content.

Larval crude fat content is also affected by the protein and carbohydrate contents of the substrate, and their interaction, and strong differences are observed between treatments. Larval crude fat content increases when C-content was high, and it is also high for all P24-diets. The high larval crude fat content at high dietary protein and carbohydrate levels may stimulate lipogenic activity and lipid incorporation in reserves (Nestel & Nemny-Lavy, 2008). These fat contents are in the range of values reported in the literature on H. illucens larvae, which vary substantially among substrates (ranging from 7% to 39% DM) (Barragan-Fonseca et al., 2017). The wide range in larval crude fat content can likely be explained by the differences in energy content of the feed, and diets high in energy content would increase larval crude fat (Barragan-Fonseca et al., 2018). For example, substrates high in fat and carbohydrate contents increase larval crude fat contents (Zheng et al., 2012). By contrast to this species, providing the fruit fly Ceratitis capitata with diets with excess low dietary carbohydrates results in pupating larvae with lower lipid and also low protein contents. High P:C ratios in the diet yield C. capitata larvae with high protein and lipid contents. Differences between this fly and other species may be a result of differential post-ingestion regulation, where a high dietary carbohydrate diet reduces the lipogenic activity of the larvae, and induces a shift from lipid to glucose oxidation, resulting in a relatively low incorporation of lipid reserves (Nestel & Nemny-Lavy, 2008).

In the present study, we also find that heavier H. illucens larvae have a higher crude fat content, which could be important for adult fitness because an increase in weight generally increases the fecundity of flies (Gobbi et al., 2013). In most insects, carbohydrates that are ingested in excess of requirement are converted to lipids and stored (Zanotto et al., 1993). For example, in locusts that are fed diets high in carbohydrates and low in protein, lipogenesis increases (Zanotto et al., 1993), which could be more beneficial for females because lipids are an important egg component, with triacylglycerol comprising approximately 40% of a mature oocyte (Chapman et al., 2013).

The results of the present study clearly demonstrate that a high larval weight is associated with a high P+C content, as well as indicating a key role for digestible carbohydrates. A similar trend is found in M. differentialis (Le Gall & Behmer, 2014) and H. virescens (Roeder, 2010) where high dietary carbohydrate levels increase insect weight. Moreover, an excess of carbohydrates leads to high lipid levels, especially when the protein concentration in their food is low (Le Gall & Behmer, 2014). Similarly, in the grasshopper S. americana, nymphs feeding on diets with an intermediate to high nutrient content are significantly larger and contain a significantly greater proportion of lipid stores at adult eclosion, although not protein or carbohydrate stores, than individuals feeding on low nutrient content diets. It is suggested that larva-derived lipid stores may be more important to fitness than carbohydrate or protein stores (Hahn, 2005). However, a high dietary crude protein content might be used by H. illucens larvae for energy production (Waldbauer et al., 1984) because it also directly impacts body mass on high P-diets.

**Adult life-history traits**

Suboptimal nutrition during larval development can significantly affect physiological processes later in life in insects (Jones & Raubenheimer, 2001; Hahn, 2005; Prabhu et al., 2008; Roeder & Behmer, 2014). The results of the present study show that food macronutrient contents during the larval phase significantly affect development time until adult and egg yield, with protein content having the main effect. Protein content certainly is the limiting factor with respect to development rate. A high dietary nutrient concentration results in heavy larvae and adults compared with larvae fed on low P and C contents that are smaller and have a lower fecundity.

Development time until the adult stage is affected by P- and C-content and the P × C interaction and, similar to larval development, it is shorter on a low P-content for all C-contents. An important cost of the short larval developmental time is the limited opportunities to build up metabolic reserves for adult life (Hanski, 1987). The number of egg masses produced per female is higher on P17:C55 and P24:C35 diets, being influenced only by protein content. As with larval survival rate, adult emergence is not affected by P or C levels, does not show differences among treatments and is high for all treatments (83–93%). Similarly, our experiments indicate that the fly follows an equal distance rule (Raubenheimer & Simpson, 1997) because it could manage the variable dietary P and C levels tested in the present study.
However, we still need to investigate the effects of extreme contents of digestible carbohydrates and protein to be able to be more conclusive.

In general, the results of the present study reveal that dietary protein and carbohydrate contents are more important than P:C ratio and affect larval performance, larval body composition and fecundity of this species, with high P + C-contents tending to improve *H. illucens* larval performance over a wide range of carbohydrate-biased P:C ratios. Even though all P + C-contents tested are suitable for the complete life cycle of this species, because diet P17:C55 (P:C = 1:3; P + C = 72) supports the highest larval and adult performance and results in a high body crude protein content (42.3%) and an intermediate crude fat content (27.6%) on the diets tested, this would be the intake target for the fly feeding on chicken feed-based diets, which matches with the P + C content of those resources that this species colonizes in the wild. Additionally, we also confirm our previous findings concerning the regulation of larval protein content within narrow limits, whereas larval crude fat content is strongly affected by nutrient concentration (Barragan-Fonseca et al., 2018).

We cannot conclude from the present study how this species deals with protein or carbohydrate excess because all diets used here are likely to be in the range of its food under natural conditions. Future studies addressing diets with a broader range of P:C ratios at different P + C contents and defined diet components are warranted to gain information on the physiological mechanisms used by the fly on nutritionally unbalanced diets. From the perspective of quality of *H. illucens* as an animal feed, a broad perspective on dietary macronutrient efficiency is desirable. Such information will improve our understanding of the effects of diets on *H. illucens* development, growth and body composition, and also provide tools for economic decisions.

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**Appendix I. Regression coefficients (SE) for larval performance measurements.**

| Source | Survival rate | Development time | Larval yield | Pupal weight |
|--------|---------------|------------------|--------------|--------------|
| P      | −0.101        | 0.193            | 0.061        | 0.00189      |
|        | (0.076)       | (0.021)          | (0.0081)     | (0.00032)    |
| C      | 0.063         | 0.094            | 0.099        | 0.00243      |
|        | (0.053)       | (0.015)          | (0.0056)     | (0.00022)    |
| P × C  | 0.0139        | −0.0079          | −0.0034      | −0.000148    |
|        | (0.0094)      | (0.0025)         | (0.00099)    | (0.000039)   |
| P²     | 0.020         | 0.0152           | −0.0015      | −0.000114    |
|        | (0.022)       | (0.0059)         | (0.0023)     | (0.000090)   |
| C²     | 0.0029        | 0.00189          | −0.0023      | −0.000054    |
|        | (0.0106)      | (0.00289)        | (0.0011)     | (0.000044)   |
| P² × C | −0.00096      | −0.00034         | 0.00073      | −0.0000095   |
|        | (0.00231)     | (0.00063)        | (0.00024)    | (0.0000096)  |
| P × C² | 0.0024        | 0.00040          | 0.00039      | 0.000203     |
|        | (0.0016)      | (0.00044)        | (0.00017)    | (0.000067)   |
| P² × C²| 0.000006      | −0.000045        | −0.000024    | −0.000016    |
|        | (0.000401)    | (0.00011)        | (0.000042)   | (0.000017)   |

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### Appendix II. Regression coefficients (SE) for larval body protein and fat contents.

| Source     | Larval crude protein | Larval crude fat |
|------------|----------------------|------------------|
| P          | −0.115 (0.029)       | 0.678 (0.039)    |
| C          | −0.012 (0.020)       | 0.219 (0.027)    |
| P × C      | 0.0106 (0.0035)      | −0.0240 (0.0047) |
| P²         | −0.0069 (0.0082)     | 0.044 (0.011)    |
| C²         | −0.0011 (0.0040)     | −0.0056 (0.0054) |
| P² × C     | 0.00156 (0.00087)    | 0.00024 (0.00117)|
| P × C²     | 0.00074 (0.00061)    | 0.00076 (0.00082)|
| P² × C²    | 0.00010 (0.00015)    | 0.00020 (0.00020)|

### Appendix III. Regression coefficients (SE) for adult performance measurements.

| Source     | Adult emergence (%) | Development time until adult | Number of egg masses | Larval hatching (%) | Egg yield |
|------------|---------------------|-----------------------------|----------------------|---------------------|-----------|
| P          | −0.12 (0.13)        | 0.470 (0.043)               | 0.93 (0.21)          | 0.053 (0.251)       | 0.0199    |
| C          | 0.000 (0.089)       | 0.142 (0.030)               | 0.11 (0.14)          | 0.054 (0.176)       | 0.0019    |
| P × C      | 0.004 (0.016)       | −0.0277 (0.0053)            | −0.019 (0.025)       | 0.015 (0.031)       | −0.00083  |
| P²         | −0.000 (0.036)      | −0.035 (0.012)              | −0.063 (0.058)       | 0.048 (0.071)       | −0.00170  |
| C²         | 0.0005 (0.018)      | −0.0060 (0.0060)            | −0.0008 (0.0286)     | 0.014 (0.035)       | −0.00056  |
| P² × C     | −0.0031 (0.0038)    | −0.0014 (0.0013)            | −0.0142 (0.0062)     | −0.0057 (0.0076)    | −0.00024  |
| P × C²     | −0.0018 (0.0027)    | −0.00098 (0.00091)          | 0.0044 (0.0044)      | 0.00002 (0.00053)   | 0.000072  |
| P² × C²    | 0.00051 (0.00067)   | 0.00027 (0.00023)           | −0.0004 (0.0011)     | −0.0029 (0.0013)    | −0.000006 |

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