A THESIS
FOR THE DEGREE OF MASTER OF SCIENCE

The Impacts of Dietary Macronutrients on Performance and Body Composition in the Black Soldier Fly, *Hermetia illucens* L. (Diptera: Stratiomyidae)

음식물의 거대영양소가 아메리카동애등에(*Hermetia illucens*)의 발육성적과 체성분 조성에 미치는 영향에 대한 연구

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ABSTRACT

The Impacts of Dietary Macronutrients on Performance and Body Composition in the Black Soldier Fly, *Hermetia illucens* L. (Diptera: Stratiomyidae)

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The production of animal-derived protein requires substantial conventional feed ingredients, such as grains, soybean meal, and fish meal, will pose a major challenge for food security around the globe. In recent years, insects have been regarded as a promising and environmentally-friendly solution for this impending crisis because they convert low-grade organic waste into high-quality protein sources efficiently, are cost effective to produce, and emit less greenhouse gas. The black soldier fly (BSF, henceforth), *Hermetia illucens* (Diptera: Stratiomyidae), has emerged as one of the most economically important insects that can be an excellent agent for reducing organic food waste and other organic by-products and also a nutritionally suitable replacement for traditional feed for swine, poultry and fish. The larvae of BSF are extremely polyphagous detritivores that consume a wide variety of plant- and animal-derived foods. Despite its economic importance, studies examining the exact nutritional requirement of BSF have been scarce.

Nutrient governs nearly all aspects of biology in insects. It is widely held that protein and carbohydrate are the two major macronutrients that have profound consequences for performance and fitness in insects with herbivorous and omnivorous feeding habits. The purpose of this thesis was to explore the separate and combined effects of dietary protein and carbohydrate content of food on multiple preadult life-history traits and body composition in BSF through the application of a powerful state-space modelling framework, the Nutritional Geometry (NG). To this end, various traits related to the evolutionary fitness in BSF larvae were recorded from male and female BSF individuals assigned to one of 32 chemically defined diets containing one of eight ratios of protein-to-carbohydrate (P:C= 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, or 8:1) and one of four concentrations of protein plus carbohydrate (P+C= 60, 120, 180, or 240 g l^{-1}) throughout their larval feeding stage. The performance data obtained from insects reared on these 32 diet treatments were used to construct the nutritional performance landscape superimposed over the gradients of protein and carbohydrate concentration.

Compared to male BSF, female BSF grew slowly but developed into larger adults. Despite such sex difference in development time and body size at sexual maturity, there were no major sexual dimorphisms in the shape of the nutritional performance landscapes for all traits measured. This study however found that the overall topography of the landscapes differed significantly among all key parameters of fitness measured for BSF larvae, with the nutritional optima, which were represented as the summit of the fitness mountain, for all traits being located at the different coordinate in the nutrient space. Preadult survivorship was maintained high (>60%) as long as the concentration of protein in the diet was lower than 120 g l^{-1}, and fell as protein concentration exceeded 120 g l^{-1}. Notably, BSF larvae exhibited a high tolerance to extreme protein limitation. The optimal P:C for preadult survivorship was 1:1.71. The duration of preadult development was the shortest at the P:C ratios of 1:1.11–1:1.56 and extended profoundly as dietary protein concentration decreased below 35 g l^{-1}. The body weight of adult flies recorded at emergence peaked at the carbohydrate-biased P:C ratios of 1:3.16–1:3.36 and fell gradually as dietary carbohydrate concentration decreased. Growth rate had a convex relationship with dietary protein concentration, with the peak for growth rate being centered around the intermediate P:C ratios of 1:1.40–1:1.83. Most importantly, the proxy measure of preadult fitness, which was computed as the product of preadult survivorship and growth rate, was maximized at the intermediate P:C ratios of 1:1.37–1:1.43, suggesting that this particular composition can be the most suitable mixture for mass rearing this species. This fitness proxy decreased as the concentration of dietary protein and carbohydrate either increased or decreased from the optimal level. Across 32 diet treatments, lean body weight remained relatively stable across 32 diet treatments whereas lipid content was high variable depending upon dietary carbohydrate content.

The present results revealed that dietary protein and carbohydrate availability in the diet had strong influence over BSF performance. Dietary protein was established to be the most influential dietary determinant of most life-history traits, except for body weight and lipid content. Different life-history traits responded to dietary protein and carbohydrate concentration differently, leading to significant divergences in nutritional optima among them. The Euclidean distance between the nutritional optima was the farthest between body weight and development time, suggesting that life-history trade-off between these two key preadult traits may be strongly mediated by macronutrients in BSF.

The results reported in this thesis will provide valuable insights for improving the process and efficacy of the mass production of this economically important species through designing an optimal dietary formula and also offer a promising opportunity to customize the production of BSF phenotype depending on its intended purpose.

Key words: Body composition, Dietary macronutrient, *Hermetia illucens*, Insect mass production, Nutritional optima, Preadult life-history traits, Protein

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INTRODUCTION

The global demand for animal-derived proteins from livestock, poultry, and fish has been on a steady rise over the recent years, mainly driven by increasing meat consumption in developing countries as living standards improve (Msangi and Rosegrant, 2011; van Huis, 2013). The production of meat requires substantial amounts of animal feed ingredients, such as grains, maize, soybean meal, fish meal, and fish oil, but the supply of these conventional feed ingredients is unlikely to match the growing demand for meat production because of the combinations of reduced agricultural productivity, finite land use, deforestation, water shortage, climate warming, etc (van Huis, 2013). As the prices of these feed ingredients are projected to increase rapidly, the provision of sufficient and high-quality feed sources for meat production will be a major challenge for assuring food security (FAO, 2009). Insects have emerged as a sustainable and promising solution to this imminent crisis because they are highly efficient at converting low-grade organic waste into high-quality protein sources and also cost effective to produce. Several insect species are currently used across the globe as an alternative source of protein for livestock, poultry and aquaculture and these include the yellow mealworm (*Tenebrio molitor* Linnaeus), the lesser mealworm (*Alphitobius diaperinus* Panzer), the silkworm (*Bombyx mori* Linnaeus), the common housefly (*Musca domestica* Linnaeus), and several orthopteran species (reviewed by van Huis, 2013). However, no insect species has received more attention than the black soldier fly (BSF, henceforth), *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae), over the recent years as a potential replacement for conventional protein-rich animal feed (reviewed by Barragan-Fonseca et al., 2017; Wang and Shelomi, 2017).

BSF is native to the tropical and warm temperate regions of the Americas, but is now widespread worldwide, possibly due to human transportation (Sheppard et al., 1994). BSF passes through six larval instars before entering the prepupal stage upon which it ceases to feed and leave the food substrates to pupate. Under ideal conditions, this species completes its development in 40-43 days (Tomberlin et al., 2002). As a capital breeder, BSF does not feed as adult and uses nutritive reserves acquired and stored during the larval stage to produce eggs. The larvae of BSF are extremely polyphagous detritivores that are able to consume a wide variety of foods, including agricultural by-products (Oonincx et al., 2015a; Spranghers et al., 2016), animal carrion and fish offal (St-Hilaire et al., 2007; Nguyen et al., 2013), animal manure (Newton et al., 2005; Oonincx et al., 2015b), human feces (Banks et al., 2014), food waste (Nguyen et al., 2015), etc. Because of their versatile feeding habit, BSF larvae are currently used as the agent for reducing organic food waste and managing other organic by-products worldwide (Diener et al., 2011; Nguyen et al., 2015). Besides this benefit as an agent for waste management and recycling, BSF larvae are also highly efficient at converting these organic waste products into insect biomass, which in turn can be a high-quality protein source for larger animals, such as swine, poultry, and fish. For example, the body of BSF larvae is rich in protein (ca. 37–63%) and fat (7–39%), making them highly nutritious diet for various domestic and captive animals. Due to its increasing economic importance, considerable efforts have been made over the last decades to establish the optimal growing conditions, such as humidity, density, and temperature, required for the mass production of BSF on an industrial scale (reviewed by Barragan-Fonseca et al., 2017). There has been a wealth of studies investigating the nutritional suitability of various organic by-products as a food source for mass rearing BSF (e.g., Lalander et al., 2019), but our basic understanding of the nutritional requirement and optimal diet of BSF is still in its infancy.

Nutrition dictates nearly all aspects of biology in all organisms, with protein and carbohydrate being the two most important macronutrients that have profound influences over individual performance and Darwinian or evolutionary fitness (Simpson et al., 2015). Carbohydrate is the primary substrate for metabolic energy and activity whereas protein and its constituent amino acids are the major building blocks for somatic growth and reproduction. In insects and other organisms, the expression of various life-history traits closely linked to fitness, such as survival, growth, and reproductive success, is maximized when ingesting a diet comprising an optimal balance of these two macronutrients, which varies across species and also within species depending on life stage, sex, and genotype (Simpson and Raubenheimer, 2012). The nutritional goal of foraging is thus geared toward achieving the balanced mixture of multiple nutrients. The importance of the macronutrient balance as a key determinant of fitness in insect is further mirrored by the fact that insects have evolved a suite of physiological and behavioral mechanisms that enable them to balance the intake of these two macronutrients (Simpson and Raubenheimer, 1993, 2012; Raubenheimer and Simpson, 1999). It is important to note that the effects of these two macronutrients on insect performance are far from simple because they are often nonlinear and the interactions between them are complicated. These nutritional complexities have long hampered our complete understanding of the role played by these macronutrients in shaping organismal phenotype, ecological interactions, and evolutionary processes in organisms.

The Nutritional Geometry (NG) is an integrative state-space modelling framework that is developed to resolve these major complexities in nutritional research by visually representing the current nutritional status of an organism, its optimal nutritional requirement, and the nutritional compositions of the food as coordinates in a *n*-dimensional space (reviewed by Simpson and Raubenheimer, 2012; Simpson et al., 2015). The NG was initially applied to identify the nutrient regulatory responses in insects (Raubenheimer and Simpson, 1999; Lee et al., 2002, 2003), but it is now widely used as the standard methodology for exploring the relationship between macronutrient intake and organismal performance in a wide variety of organisms spanning unicellular slime molds to rodents (Dussutour et al., 2010; Solon-Biet et al., 2014). One of the most important advantages of using the NG is that it has offered us a powerful means to partition the separate and interacting effects of multiple macronutrients on animal performance, which was infeasible in the traditionally approaches (Simpson et al., 2015). Using more than 1000 individual flies confined to one of 28 diets that varied systematically in protein and carbohydrate content, for example, Lee et al. (2008) clearly identified that the most decisive nutritional factor for the phenotypic expression of lifespan and fecundity was the dietary ratio of protein-to-carbohydrate in *Drosophila melanogaster*, not the total amount of calories consumed as had been previously believed. Furthermore, in this species, the optimal P:C ratio supporting maximal lifespan was much lower than that for maximal female reproductive outcome (Lee et al., 2008; Jensen et al., 2015; Lee, 2015; Jang and Lee, 2018), suggesting that these two key life-history traits are not simultaneously maximized at the same protein and carbohydrate intake. Such diverging nutritional optima between lifespan and reproduction has been repeatedly documented from a number of species, including Queensland fruit flies (Fanson and Taylor, 2012), crickets (Maklakov et al., 2008; Harrison et al., 2014), and even rodents (Solon-Biet et al., 2014, 2015), thereby providing empirical support for the emerging notion that the occurrence of the fundamental trade-offs between these two components of fitness is mediated by the balance of macronutrients consumed by an organism and not necessarily by the altered allocation of limited resources between two competing traits. Evidence for this ‘nutrient-mediated or nutrient-space based life-history trade-offs’ (Rapkin et al., 2018; Morimoto and Lihoreau, 2019) is not restricted to the relationship found between lifespan and reproduction but can be broadly applied to other life-history and physiological traits expressed within and across life stages in insects (Jang and Lee, 2018; Rapkin et al., 2018).

In this study, I used NG to explore the separate and combined effects of dietary protein and carbohydrate content on various preadult life-history traits and body composition in BSF. Several previous studies have investigated how the balance between dietary protein and carbohydrate intake influences BSF performance, but most of these studies were undertaken using semi-synthetic diets that comprised a large portion of undefined ingredients, such as chicken feed (Gligorescu et al., 2018; Beniers and Graham, 2019; Barragan-Fonseca et al., 2019; but see Cammack and Tomberlin, 2017; Barragan-Fonseca et al., 2020). To determine the impact of dietary protein and carbohydrate with high precision, I used a total of 32 chemically defined diets that varied systematically in their protein-to-carbohydrate balance and total protein plus carbohydrate (P+C) content, which were formulated to encompass the natural range of these macronutrients encountered by BSF individuals during their larval feeding stage. The use of a large number of these chemically defined diets also enabled me to gain a comprehensive understanding of how these nutrients act in concert to shape the phenotype of BSF larvae across a wide range of dietary protein and carbohydrate.

The major goal of this study was to identify the optimal protein and carbohydrate composition in the diet required for maximizing individual performance and fitness in BSF. To achieve this aim, I recorded various measures of preadult life-history trait and body composition from BSF individuals confined to one of 32 synthetic diets that varied systemically in protein and carbohydrate content throughout their larval feeding stage. The phenotypic expression of each aspect of measured performance was then plotted as nutritional performance landscape (simply, nutritional landscape, henceforth) superimposed on the two-dimensional nutrient plane where the gradients of protein and carbohydrate concentration were represented on the x and y axis, respectively. The location of the summit of the performance mountain was identified as the nutritional optimum for a specific trait in question. I compared the position of the nutritional optima among different traits so as to examine the occurrence and strength of nutrient mediated trade-offs between life-history traits (Rapkin et al., 2018; Morimoto and Lihoreau, 2019). Furthermore, the pattern of the isoclines fitted along the nutritional landscapes was inspected to indicate the key nutritional driver of BSF performance. In this study, I investigated the effects of dietary protein and carbohydrate in male and female BSF separately.

MATERIALS AND METHODS

1. Experimental insects

The newly laid eggs of BSF used in this study were obtained from EntomoInnovation Co. (http://entomostore.kr/23), Chungju, Chungcheongbuk-do, Republic of Korea. Upon arriving at Seoul National University, these eggs were carefully placed in 5 diameter Petri dishes provided with the mixture of moistened sawdust and food waste at a density of 250–300 eggs per dish. Prior to being assigned to the experimental treatments (described below), newly hatched larvae were allowed to feed ad libitum on the provided food mixture for three days in an environmental chamber set at 25°C under a 16 h:8 h light:dark photoregime and 70% relative humidity.

2. Experimental diets

I prepared a total of 32 chemically defined diets following the protocol described in detail by Jang and Lee (2018). These synthetic diets were originally used for investigating the effects of macronutrient intake on various life-history traits in *Drosophila melanogaster* (Lee et al., 2013; Lee and Jang, 2014; Lee, 2015; Jang and Lee, 2018). Pilot studies confirmed that these diets were also suitable for supporting the development and growth of BSF. Thirty two experimental diets contained one of eight ratios of protein-to-carbohydrate (P:C= 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, or 8:1) and one of four concentrations of protein plus carbohydrate (P+C= 60, 120, 180, or 240 g l^{-1}). The exact protein and carbohydrate concentrations of these 32 experimental diets are outlined in Fig. 1. Since protein and carbohydrate contain similar amounts of calories per gram (4 kcal g^{-1}), I considered that those diets with the same P+C concentration are isocaloric. In these diets, sodium caseinate (Sigma C8654) and sucrose (Sigma S9378) were used as the source of protein and carbohydrate, respectively. All diets comprised fixed concentrations of dietary lipids (0.3 g l^{-1} cholesterol, 4 g l^{-1} lecithin), salts (0.71 g l^{-1} KH_2PO_4, 3.73 g l^{-1} K_2HPO_4, 0.62 g l^{-1} $MgSO_4$, 1 g l^{-1} $NaHCO_3$), nucleic acids (0.57 g l^{-1} uridine, 0.64 g l^{-1} inosine), vitamins (0.002 g l^{-1} thiamine, 0.01 g l^{-1} riboflavin, 0.012 g l^{-1} nicotinic acid, 0.0167 g l^{-1} calcium pantothenate, 0.0025 g l^{-1} pyridoxine, 0.0002 g l^{-1} biotin, 0.003 g l^{-1} folic acid), and preservatives (1 g l^{-1} nipagin, 0.3% propionic acid) (Jang and Lee, 2018). Diets were prepared by homogeneously dissolving all pre-weighed ingredients except vitamins and preservatives in 2% agar solution. The suspension was autoclaved at 121°C for 10–15 min. Vitamins and preservatives were later added to the autoclaved suspension when it had cooled down to <50°C and distilled water was added to adjust the final volume of the medium. After vigorous stirring, the agar-gelled medium was dispensed into plastic storage containers (15 cm × 10 cm × 5 cm), stabilized at room temperature for 6 h, and stored at 4°C until use.

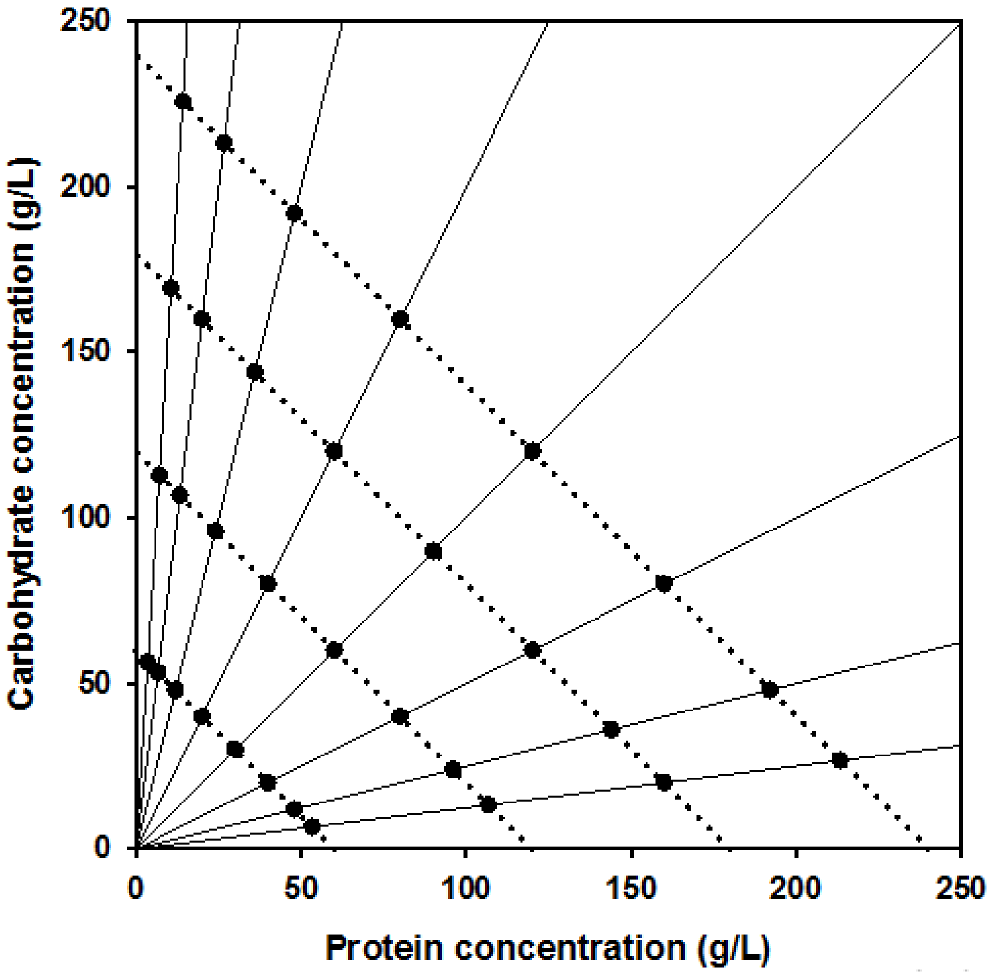


Figure 1. Graphic description of the 32 chemically defined diets used in this thesis. Each point describes the amount of protein and carbohydrate present in 1 liter of agar-gelled medium in each of 32 experimental diets that varied in protein:carbohydrate (P:C) ratio and in protein plus carbohydrate (P+C) concentration. Nutritional rails (solid lines) radiating from the origin represent eight P:C ratios (from left to right: 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1). Along each rail, there are four different diets, representing different P+C concentrations (ascending from the origin: 60, 120, 240 and 360 g l^{-1}). Across different nutritional rails, those diets with the same P+C concentration are connected by isocaloric lines (dotted lines).

3. Experimental procedure

In this study, the larvae of BSF were provided ad labium access to one of 32 chemically defined diets that varied systematically in P:C ratio and in P+C concentration throughout their preadult stages (larval and pupal). Three days after hatching, BSF larvae were collected and randomly grouped into cohorts of ten individuals. Each cohort was weighed to the nearest 0.1 mg using a microbalance (Ohaus Co., Parsippany, NJ) and then placed in an insect breeding dish (10 cm diameter × 4 cm) containing sufficient amounts (4–5 g) of one of 32 experimental diets. The fresh weight of BSF larvae at the start of the experiment (w_0) was estimated by dividing the fresh weight of each cohort by ten. The experimental diets were replaced every two days and the insects were transferred into new breeding dishes every four days. There were four replicate dishes per diet treatment, resulting in a total of 128 replicate dishes and 1,280 experimental insects used in this study. These replicate dishes were maintained in an incubator set at 25°C under a 16 h:8 h light:dark photoregime and 70% relative humidity. To eliminate any positional effect, I randomly relocated the replicate dishes inside the chamber more than twice a day.

The individual larvae housed in each dish were monitored daily for whether they entered the prepupal stage by inspecting the melanized cuticle, which is characteristic of the prepupae of BSF (May, 1961). Upon entering the prepupal stage, the insects were collected, rinsed with tap water, gently dried with paper towels, and then individually weighed to the nearest 0.1 mg. Weighed prepupae were individually placed in 5 cm diameter Petri dish, returned to the environmental chamber, and allowed to pupate and develop into adults. The date of adult emergence was checked daily. Newly emerged BSF adults were sexed, weighed, and frozen to death at -20°C. Dead carcasses were dried in an oven set at 65°C for 48 h, weighed, and subjected to lipid extraction. To extract body lipid, dried carcasses were sealed with plastic tea bags and placed in a 10-ml glass vial containing 7 ml of chloroform for 48 h. Lipid-extracted carcasses were redried and reweighed. The lipid content of each adult was determined by weight difference before and after lipid extraction. Preadult survivorship was determined as the proportion of larvae that successfully developed into adults for each replicate dish. The duration of preadult development (i.e., development time) was measured as the days elapsed from the start of the experiment (day 3 post hatching) to the point of adult emergence for each individual. For each individual, preadult growth rate (gr) was calculated using the following formula:

$$(1) \quad gr = \frac{\ln(w_t \ / \ w_0)}{t}$$

where t is development time, w_t is the fresh weight of insects recorded at the start of prepupal or adult stage, and w_0 is the fresh weight of larvae recorded at the start of the experiment (day 3 post hatching). The composite index of preadult performance was computed as the product of preadult survivorship and growth rate (Simpson et al., 2004). This performance index was constructed based on the general notion that the evolutionary fitness of an immature insect is positively correlated with high probability of survival to adulthood, large body size at maturity, and faster juvenile development (Honěk, 1993; Nylin and Gotthard, 1998).

4. Statistical analysis

I took the multivariate response surface approach to estimate the linear and nonlinear (i.e., quadratic and correlational) effects of protein (P) and carbohydrate (C) concentration in the diet on each measured trait using the generalized linear mixed models (PROC GLIMMIX in SAS v 9.2) with an identity link function and a Gaussian error distribution. Replicate dish (simply ‘dish’) nested within diet treatment was included as a random factor in the models fitted for all measured traits except preadult survivorship. Following the advice of Lande and Arnold (1983), I first ran a model containing only the linear terms of P and C concentration as fixed factors and ‘dish’ as a random factor. From this model, I estimated the linear effects of these nutrients. Next, I ran a second model in which the quadratic and correlational terms of both macronutrients were added to the first model. The gradients representing the quadratic (P^2, C^2) and correlational ($P \times C$) effects of these nutrients were estimated from the second model. These models were fitted using the restricted maximum likelihood method.

To test any significant shape difference between two different response surfaces, I first standardized each response variable using a z-transformation and then performed the likelihood ratio test (LRT) comparing the models with and without the all possible interaction terms between the trait or sex under comparison and the linear and nonlinear effects of P and C concentration. In this test, twice the difference in log likelihood ratio between these two models was compared with a χ^2 distribution with five degree of freedom, with $P<0.05$ indicating the shape difference between the two response surfaces under comparison.

Non-parametric thin-plate splines methodology was used to construct the two-dimensional nutrient response surfaces or landscapes visualizing how each trait was expressed over a grid of dietary P and C concentration using the Fields package (Nychka et al., 2017) in R v 4.0.2. (R Development Core Team, 2012). When plotting these response surfaces, I used the smoothing parameter (λ) that minimized the generalized cross-validation score (GCV) for each trait. One advantage of this method is that it allowed a realistic description of the effects of P and C on each trait by not constraining the shape of the response surfaces (Blows and Brooks, 2003). For each response surface, the coordinate in the nutrient plane where the trait in question was expressed in its maximum (global maximum) or its minimum (global minimum) was estimated using the OptimaRegion package (del Castillo et al., 2016) in R v 4.0.2. The vector of position directed from the origin to the P and C coordinate of the trait maximum (or minimum) was then defined as follows:

$$(2)\ \overrightarrow{v_i} = (P_i, \quad C_i)$$

where i is the trait for each sex. The distance (*d*) and angle (*θ*) between the two position vectors for trait A and B were calculated using the following formula provided by Morimoto and Lihoreau (2019):

$$(3) \quad \theta = \cos^{-1}\left(\frac{\overrightarrow{v_A} \cdot \overrightarrow{v_B}}{|\overrightarrow{v_A}||\overrightarrow{v_B}|}\right)$$

$$(4) \quad d = \sqrt{\quad \overrightarrow{v_A}^2 \ + \ \overrightarrow{v_A}^2 - 2 \quad \overrightarrow{v_A} \ \cdot \ \overrightarrow{v_B} \ \cos\theta}$$

The distance (*d*) and angle (*θ*) between the two position vectors for males and females in each trait were calculated accordingly.

All statistical analyses except the thin plate response surface methods were performed using SAS v 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

1. Preadult life-history traits

The proportion of larvae successfully reached the adult stage was significantly affected by a negative quadratic gradient of P concentration but was largely independent of C concentration (Table 1). This suggests that preadult survivorship was associated with dietary P concentration in a convex manner, with the peak being centered around the P:C ratio of 1:1.71 (global maximum: P=67.1 g l^{-1}, C=114.7 g l^{-1}). Preadult survivorship was maintained high (>60%) at P concentration below ca. 120 g l^{-1}, but fell rapidly at P concentration higher than 120 g l^{-1} (Fig. 2A). The proportion of pupae that failed to emerge into adults was similar across a range of P and C concentration although it tended to increase as a function of decreasing P content in the diet (Fig. 2B). I found that the nutritional landscape plotted for the survivorship recorded at the point of entering the prepupal stage (Fig. 2C) closely resembled that fitted for the survivorship at adult emergence (LRT: χ^2= 9.83, df=5, P=0.080). Collectively, these results suggest that the effect of dietary P and C concentration on preadult survivorship was determined during the larval feeding stage.

Apart from preadult survivorship, all other aspects of preadult life-history traits were quantified separately from male and female individuals. In all cases, I found no major sex differences in the topography of the response surfaces fitted for each trait (LRT results summarized in Table 2), suggesting that males and females responded similarly to dietary variation in P and C concentration.

Females took ca. 2.7 more days to complete their preadult (larval and pupal) development than males across all diet treatments (mean ± SE, male: 48.4 ± 1.68 days; female: 51.1 ± 1.85 days; t_{31}=4.05, P=0.003). For both sexes, there was a significant positive quadratic effect of P concentration on preadult development time (Table 1), suggesting a concave relationship between dietary P concentration and preadult development time (Fig. 3A, B). Preadult development time was the shortest at the P:C ratio of 1:1.56 (P=60.4 g l^{-1}, C=94.3 g l^{-1}) for males and 1:1.06 (P=48.0 g l^{-1}, C=51.0 g l^{-1}) for females, but extended greatly as P concentration fell below 35 g l^{-1} and also slightly as P concentration rose above 100 g l^{-1}. The distance and angle between the male and female minimum position vectors for preadult development time were 45.0 g l^{-1} and 10.7 °, respectively.

Female adults were ca. 28% heavier than male adults across all diet treatments (fresh weight, male: 83.3 ± 1.42 mg; female: 106.4 ± 1.84 mg; t_{31}=17.86, P<0.001). For both males and females, the body weight of BSF adults at emergence increased linearly with increasing dietary C concentration, as indicated by a significant positive linear gradient for C concentration (Fig. 4A, B; Table 1). I detected a significant negative quadratic gradient for C concentration in the diet in females but not in males. Adult body weight was largely insensitive to variation in dietary P concentration for both sexes (Table 1). The maximum body weight at adult emergence occurred around the P:C ratio of 1:3.36 (P=51.5 g l^{-1}, C=173.1 g l^{-1}) for males and 1:3.16 (P=49.5 g l^{-1}, C=156.4 g l^{-1}) for females. The distance and angle between the male and female maximum position vectors for adult body weight were 26. 9 g l^{-1} and 7.0 °, respectively.

The rate of body growth until adult emergence was ca. 5% higher in females than in males across all diet treatments (male: $4.6\times10^{-2} \pm 1.23\times10^{-3}$ ln mg d^{-1}; female: $4.9\times10^{-2} \pm 1.31\times10^{-3}$ ln mg d^{-1}; t_{31}=3.93, P<0.001). For both sexes, the relationship between dietary P concentration and growth rate was convex, as indicated by a significant negative quadratic term for P concentration (Fig. 5A, B; Table 1). Growth rate peaked at the P:C ratio of 1:1.40 (P=72.9 g l^{-1}, C=101.8 g l^{-1}) for males and 1:1.83 (P=48.9 g l^{-1}, C=89.6 g l^{-1}) for females, but decreased significantly as the concentration of P either increased or decreased. The distance and angle between the male and female maximum position vectors for adult body weight were 16.8 g l^{-1} and 1.0 °, respectively.

The composite index representing the overall fitness was ca. 6% higher in females than in males (male: $3.0\times10^{-2} \pm 1.43\times10^{-3}$; female: $3.2\times10^{-2} \pm 1.58\times10^{-3}$; t_{31}=4.18, P<0.001). For both sexes, this index of fitness was associated with dietary P and C concentration in a convex fashion, as indicated by significant negative quadratic gradients for both P and C concentration (Fig. 6A, B; Table 1). The fitness proxy was maximized at the P:C ratio of 1:1.37 (P=82.1 g l^{-1}, C=112.2

g l^{-1}) for males and 1:1.43 (P=78.8 g l^{-1}, C=112.6 g l^{-1}) for females, but decreased as the concentration of both P and C either increased or decreased from their respective optimal concentration. The distance and angle between the male and female maximum position vectors for adult body weight were 3.3 g l^{-1} and 1.2 °, respectively.

The pairwise comparisons between the nutritional landscapes plotted for preadult life-history traits within each sex are summarized in Table 3. For both sex, the results of pairwise comparisons revealed that there were significant shape difference among the nutritional landscapes fitted for preadult survivorship, development time, body weight, growth rate, and fitness (Table 3). The distance (*d*) and angle (θ) between any of the pairs of five position vectors for survivorship, development time, body weight, growth rate and fitness proxy in each sex are summarized in Table 4 and 5, respectively. For both sexes, the pairwise camparisons the distance between the nutritional optima was farthest between preadult development time and adult body weight (Table 4). The angle (θ) between the nutritional optima was the largest between preadult performance and adult body weight for males and between preadult development time and adult body weight for females (Table 5).

Table 1. Summary of the generalized linear mixed model testing the significance of the linear, quadratic, and cross product gradients for dietary protein (P) and carbohydrate (C) concentration on preadult life-history traits and body composition in BSF.

Response trait	Linear gradients		Quadratic gradients		Cross product gradients
	P	C	P^2	C^2	P × C
Both sexes					
Preadult survivorship					
Gradient ± SE	-5.7E-04±3.18E-04	1.98E-04±2.86E-04	-2E-05±5.78E-06	-6.99E-06±5.16E-06	6.11E-06±9.77E-06
$F_{1, 117,114}$	3.17	0.48	9.05	1.84	0.39
P	0.078	0.490	0.003	0.178	0.533
Males					
Development time					
Gradient ± SE	-5.59E-02±1.75E-02	2.31E-02±1.53E-02	2.00E-03±2.79E-04	4.29E-04±2.44E-04	9.52E-04±4.68E-04
$F_{1, 268,267}$	10.23	2.26	51.18	3.1	4.13
P	0.002	0.134	<0.001	0.080	0.043
Adult body weight					
Gradient ± SE	-2.22E-02±1.45E-02	6.57E-02±1.23E-02	-3E-05±2.69E-04	-3.2E-04±2.28E-04	8.08E-04±4.34E-04
$F_{1, 268,267}$	2.34	28.41	0.01	1.93	3.47
P	0.128	<0.001	0.908	0.166	0.064
Growth rate					
Gradient ± SE	3.4E-05±1.4E-05	-6.33E-06±1.2E-05	-1.61E-06±0	-3.71E-07±0	-4.20E-07±0
$F_{1, 268,267}$	6.21	0.29	61.29	4.3	1.5
P	0.013	0.593	<0.001	0.039	0.222
Fitness proxy					
Gradient ± SE	-3.34E-06±1.5E-05	5.22E-06±1.3E-05	-1.76E-06±0	-5.01E-07±0	-3.41E-08±0
$F_{1, 268,267}$	0.05	0.17	79.19	8.37	0.01
P	0.818	0.684	<0.001	0.004	0.918
Lean body weight					
Gradient ± SE	3.86E-04±3.35E-03	1.25E-02±2.83E-03	-5E-05±6.3E-05	-5E-05±5.3E-05	1.25E-04±1.01E-04
$F_{1, 268,267}$	0.01	19.53	0.7	0.76	1.53
P	0.908	<0.001	0.403	0.383	0.217
Lipid content					
Gradient ± SE	-1.34E-02±3.59E-03	2.28E-02±3.03E-03	1.96E-04±6.6E-05	-8E-05±5.5E-05	1.85E-04±1.05E-04
$F_{1, 268,267}$	13.94	56.6	8.92	2.36	3.11
P	<0.001	<0.001	0.003	0.126	0.079

Females					
Development time					
Gradient ± SE	-2.97E-02±1.84E-02	4.00E-02±1.66E-02	1.85E-03±3.12E-04	3.28E-04±2.75E-04	4.13E-04±5.12E-04
$F_{1, 241,240}$	2.6	5.78	35.17	1.43	0.65
P	0.108	0.017	<0.001	0.233	0.422
Adult body weight					
Gradient ± SE	-1.99E-02±2.14E-02	9.00E-02±1.88E-02	4.52E-04±3.92E-04	-1.1E-03±3.31E-04	4.79E-04±6.31E-04
$F_{1, 241,240}$	0.86	23.01	1.33	11.04	0.58
P	0.355	<0.001	0.250	0.001	0.449
Growth rate					
Gradient ± SE	1E-05±1.4E-05	-2E-05±1.3E-05	-1.42E-06±0	-3.60E-07±0	-1.89E-07±0
$F_{1, 241,240}$	0.53	2.03	36.51	3.03	0.24
P	0.469	0.156	<0.001	0.083	0.624
Fitness proxy					
Gradient ± SE	-2E-05±1.6E-05	4.06E-06±1.4E-05	-1.65E-06±0	-4.80E-07±0	2.23E-07±0
$F_{1, 241,240}$	2.02	0.08	48.7	5.22	0.33
P	0.157	0.777	<0.001	0.023	0.569
Lean body weight					
Gradient ± SE	6.97E-03±5.30E-03	1.67E-02±4.63E-03	-1E-05±9.9E-05	-2.7E-04±8.3E-05	1.2E-04±1.59E-04
$F_{1, 241,240}$	1.73	12.92	0.02	10.26	0.57
P	0.190	<0.001	0.888	0.002	0.451
Lipid content					
Gradient ± SE	-2.34E-02±6.00E-03	3.38E-02±5.26E-03	3.32E-04±1.01E-04	-3.5E-04±8.5E-05	1.85E-04±1.63E-04
$F_{1, 241,240}$	15.24	41.21	10.76	17.05	1.29
P	<0.001	<0.001	0.001	<0.001	0.258

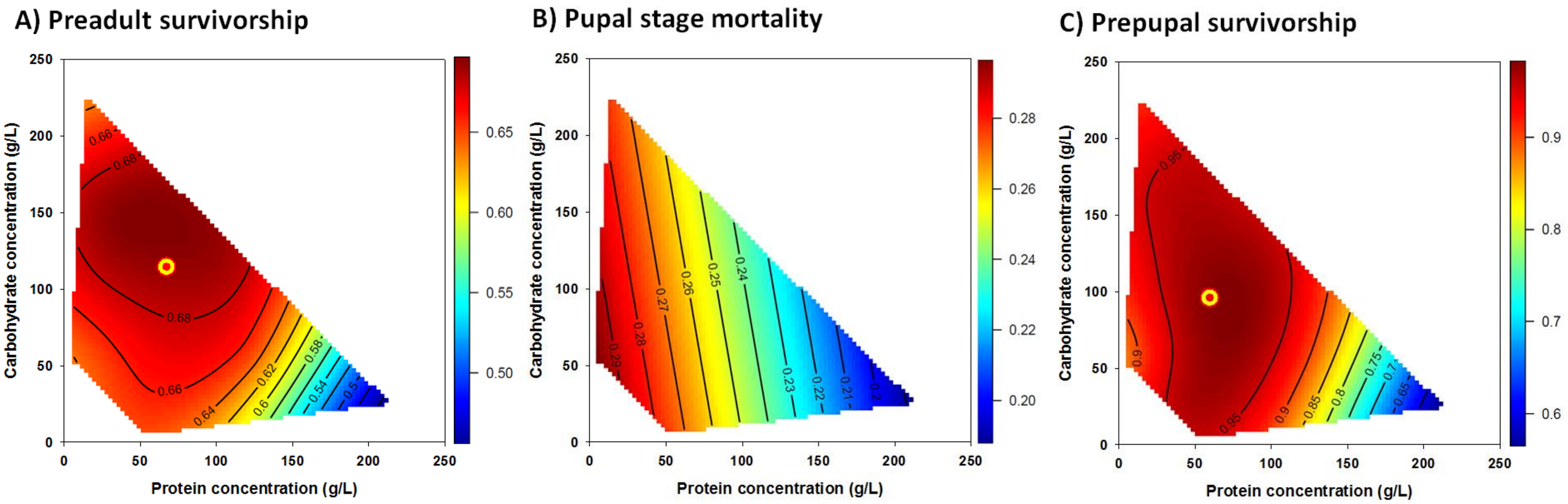
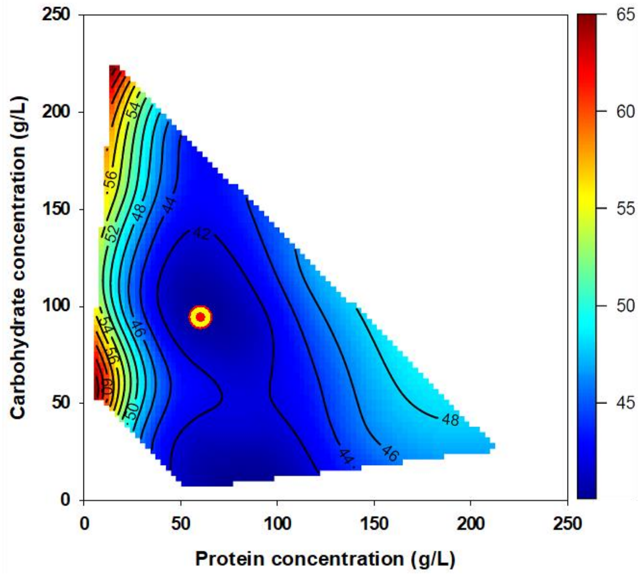


Figure 2. Nutritional landscapes for (A) preadult survivorship, (B) pupal stage mortality and (C) prepupal survivorship in BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

Table 2. Results of likelihood ratio test testing the shape difference in nutritional landscapes plotted for each trait between the two sexes.

Traits	Likelihood ratio tests	
	2 log likelihood (χ^2)	*P* value
Development time	4.004	0.549
Body weight	6.138	0.293
Growth rate	4.059	0.541
Fitness proxy	7.708	0.173
Lean body weight	6.693	0.245
Lipid content	6.308	0.277

A) Male



B) Female

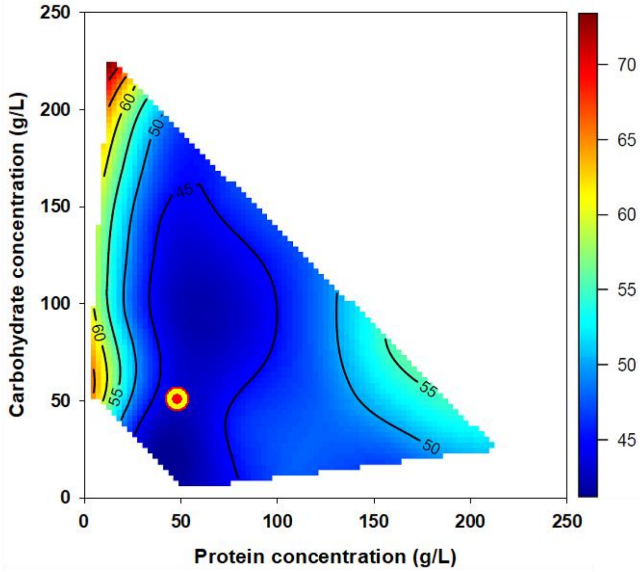


Figure 3. Nutritional landscapes for preadult development time in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull’s-eyes.

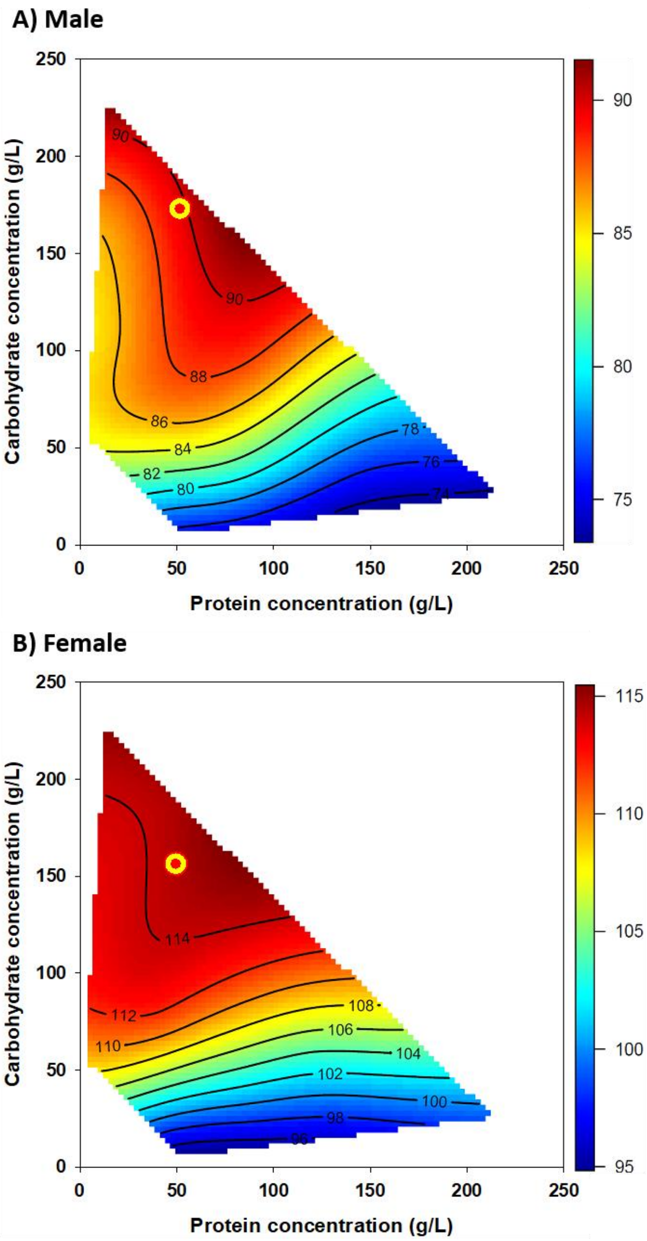


Figure 4. Nutritional landscapes for adult body weight in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

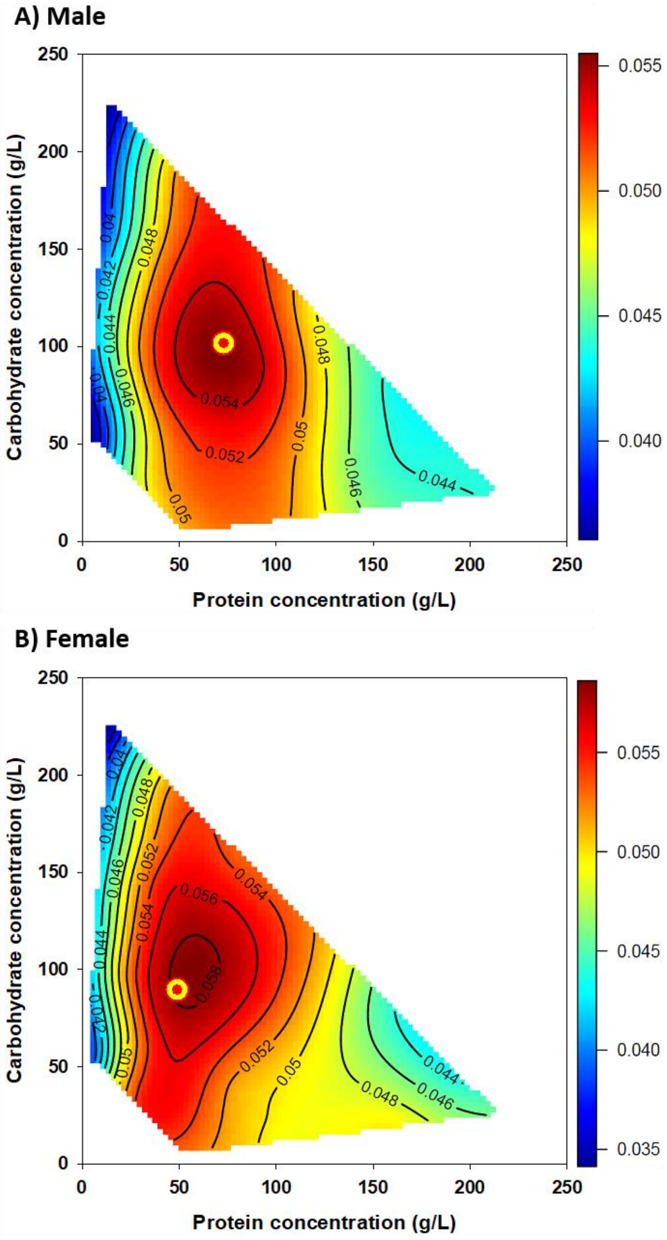
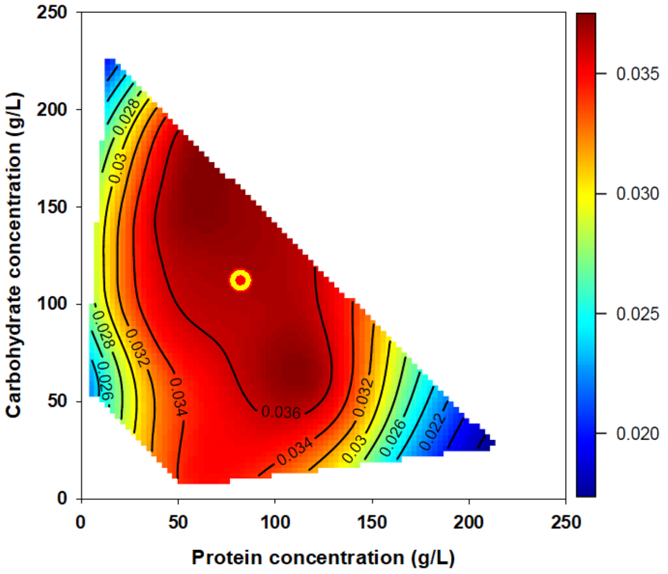


Figure 5. Nutritional landscapes for growth rate in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

A) Male



B) Female

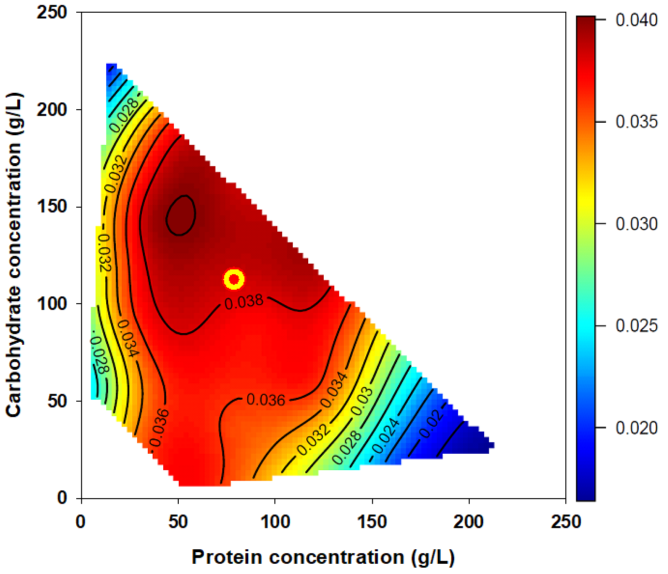


Figure 6. Nutritional landscapes for the proxy measure of preadult fitness in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

Table 3. Pairwise comparisons between the nutritional landscapes plotted for preadult life-history traits and body composition within each sex. The results for males and females are represented below and above the diagonal in the table, respectively. Values are twice the difference in log likelihood ratio between the models with and without all possible interaction terms between the trait and linear and nonlinear effects of dietary protein and carbohydrate under comparison.

Female Male	Survivorship	Development time	Body weight	Growth rate	Fitness proxy	Lean body weight	Lipid content
Survivorship		74.99***	22.17***	16.11**	12.73*	15.28**	47.25***
Development time	68.81***		43.42***	213.81***	256.02***	59.16***	49.92***
Body weight	13.03*	58.85***		117.59***	120.81***	7.70$^{\text{N.S.}}$	12.81*
Growth rate	20.31**	230.22***	109.18***		26.67***	76.10***	199.87***
Fitness proxy	16.72**	263.31***	108.38***	15.36**		91.19***	192.78***
Lean body weight	9.63$^{\text{N.S.}}$	63.09***	3.91$^{\text{N.S.}}$	75.72***	82.41***		40.44***
Lipid content	35.32***	33.18***	12.09*	188.35***	194.79***	24.58***	

*$0.01<P<0.05$; **$0.001<P<0.01$; ***$P<0.001$; N.S. not significant

Table 4. Summary of the Euclidean distance (*d*) between the nutritional optima for all pairs of traits in each sex. The distances for males and females are represented below and above the diagonal in the table, respectively.

Female / Male	Survivorship	Development time	Body weight	Growth rate	Fitness proxy	Lean body weight	Lipid content
Survivorship		66.51	45.27	31.00	11.89	33.71	59.14
Development time	21.49		105.44	38.63	68.91	99.53	93.20
Body weight	60.42	79.30		66.81	52.68	37.13	41.88
Growth rate	14.17	14.57	74.45		37.75	64.64	59.91
Fitness proxy	15.21	28.18	68.09	13.95		31.50	70.81
Lean body weight	29.69	50.67	33.10	42.88	35.09		72.31
Lipid content	117.80	134.07	59.61	131.97	127.18	92.55	

Table 5. Summary of the angles (θ) between the nutritional optima for all pairs of traits in each sex. The angles for males and females are represented below and above the diagonal in the table, respectively.

Female Male	Survivorship	Development time	Body weight	Growth rate	Fitness proxy	Lean body weight	Lipid content
Survivorship		12.97	12.77	1.70	4.65	0.10	25.18
Development time	2.31		25.74	14.67	8.32	12.87	38.15
Body weight	13.75	16.06		11.06	17.42	12.87	12.41
Growth rate	5.29	2.97	19.03		6.36	1.80	23.48
Fitness proxy	5.86	3.55	19.61	0.57		4.55	29.83
Lean body weight	5.10	7.42	8.64	10.39	10.96		25.28
Lipid content	26.28	28.59	12.54	31.57	32.14	21.18	

2. Body composition

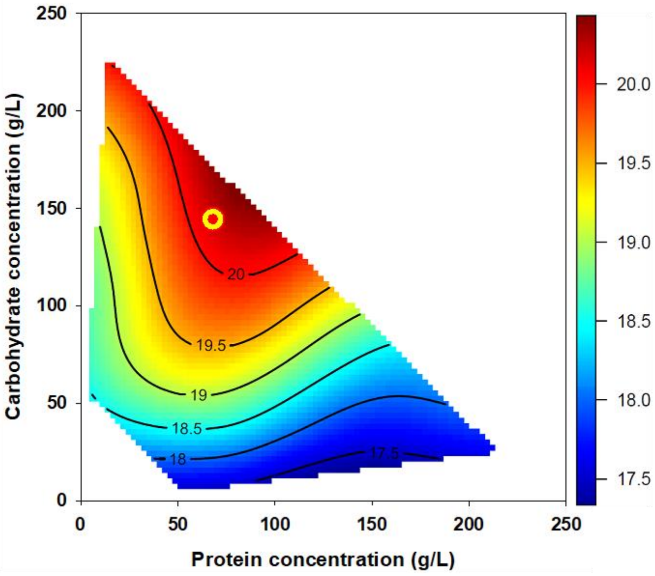
Two aspects of body composition measured from the individuals of BSF at adult emergence were lean body weight and lipid content. Females had ca. 34% heavier lean body (dry weight, male: 18.8 ± 0.27 mg; female: 25.2 ± 0.40 mg; t_{31}=20.13, $P<0.001$) and ca. 45% higher lipid content (male: 11.7 ± 0.41 mg; female: 16.9 ± 0.66 mg; t_{31}=12.48, $P<0.001$) than males across all diet treatments, but the overall shape of the nutritional landscapes constructed for these two traits was not significantly different between males and females (Table 2).

The lean body weight of both male and female adults increased linearly with increasing dietary C concentration, as indicated by a significant linear gradient for C concentration (Fig. 7A, B; Table 1). In addition to this linear effect, I detected a significant negative quadratic gradient for C concentration in females but not in males. Dietary P concentration did not exhibit any significant linear and nonlinear effect on lean body weight for both males and females. Lean body weight was maximized at the P:C ratio of 1:2.12 (P=68.0 g l^{-1}, C=144.4 g l^{-1}) for males and 1:1.70 (P=84.4 g l^{-1}, C=143.6 g l^{-1}) for females, with the distance and angle between these peaks being 16.4 g l^{-1} and 5.2 °, respectively.

Lipid content peaked at an extremely carbohydrate-biased P:C ratio of 1:14.1 (P=15.6 g l^{-1}, C=220.6 g l^{-1}) for males and 1:10.8 (P=12.3 g l^{-1}, C=133.1 g l^{-1}) for females. The distance and angle between these two peaks were 83.6 g l^{-1} and 1.1 °, respectively. From this peak, lipid content decreased with increasing P concentration and decreasing C concentration, as indicated by a significant negative linear gradient for P concentration and a positive linear and quadratic gradient for C concentration for both sexes (Fig. 8A, B; Table 1)

The protein and carbohydrate coordinates for the nutritional optima for all preadult life-history traits and body composition parameters are outlined in Fig. 9.

A) Male



B) Female

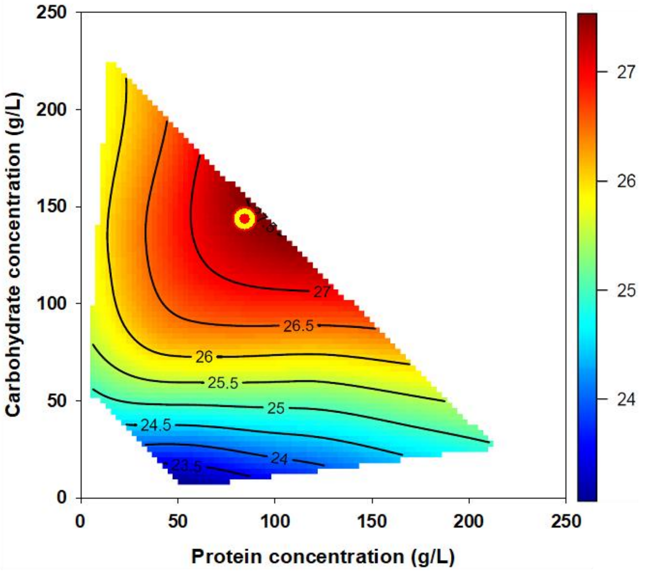


Figure 7. Nutritional landscapes for adult lean body weight in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

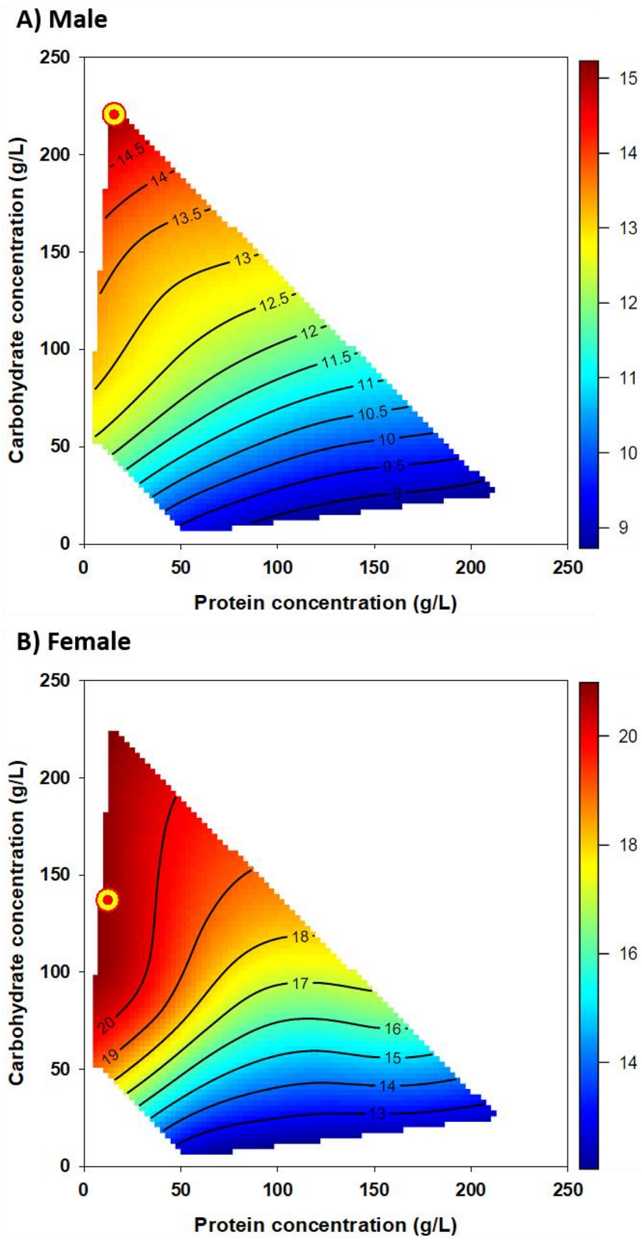


Figure 8. Nutritional landscapes for adult lipid content in (A) male and (B) female BSF larvae fed 32 chemically defined diets varying in the ratio and concentration of protein and carbohydrate. For each response surface, the regions where the traits were expressed at the highest and lowest level are represented by dark red and blue, respectively. The nutritional optima are represented by bull's-eyes.

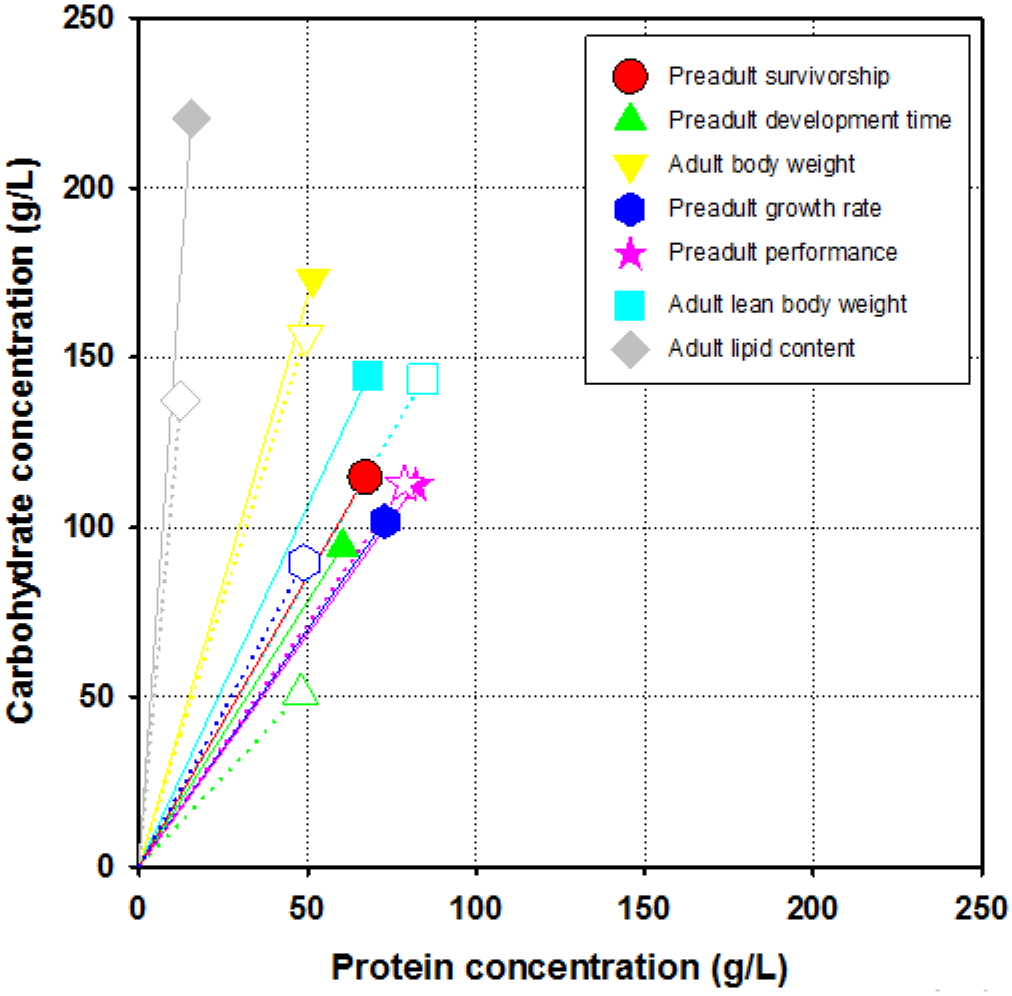


Figure 9. The plot summarizing the protein and carbohydrate coordinate for the nutritional optima for preadult life-history traits and body composition parameters. Closed and open symbols represent male and female BSF, respectively. The solid and dotted lines radiating from the origin to each nutritional optimum indicate the optimal P:C ratio for male and female BSF, respectively.

DISCUSSION

In this study, I have leveraged the integrative NG framework (Simpson and Raubenheimer, 2012; Simpson et al., 2015) to analyze the separate and combined effects of dietary protein and carbohydrate content on key life-history traits and body composition that are strongly linked to evolutionary fitness in BSF larvae. The nutritional landscapes for our focal traits were built using a total of 32 chemically defined diets in which the composition of protein and carbohydrate was experimentally manipulated. Through carefully inspecting the topography of these nutritional landscapes, I was able to identify the nutritional optima for these components of fitness and also the main nutritional factors contributing to their phenotypic expression with unprecedented detail and precision.

In this research, I measured various preadult traits from males and females separately and found that females were 29% heavier than males. Such sex difference in body weight or size has been previously described from a number of studies in BSF (e.g., Jones and Tomberlin, 2021). Female-biased sexual size dimorphism in insects is suggested to be the outcome of strong fecundity selection for large female body size (Honěk, 1993) and is proximately caused by either increased growth rate or extended development time in females or both (Fairbairn, 1990; Wiklund et al., 1991; Blanckenhorn et al., 2007; Esperk et al., 2007;

Stillwell et al., 2010). In accordance with this notion, I found that females had 5% higher preadult growth rate and 2.7days longer development time than males in BSF. Male BSF adults are known to display strong territorial or lekking behaviour to secure their mating success (Tomberlin and Sheppard, 2001). This suggests that earlier male emergence could offer significant mating advantages in BSF, thereby favoring selection for faster male development and protandry in this species. Despite such sex differences in the phenotypic expression of these key life-history traits, I did not find any major differences in the topography of the nutritional landscapes constructed for each trait between the two sexes, suggesting that males and females are qualitatively similar in their responses to dietary variation in protein and carbohydrate.

The larvae of BSF can consume a variety of organic matters, spanning vegetables to animal carrions and feces (Newton et al., 2005; St-Hilaire et al., 2007; Nguyen et al., 2013, 2015; Oonincx et al., 2015a, b; Spranghers et al., 2016; Barragan-Fonseca et al., 2017). This versatile feeding habit of BSF has led me to anticipate a broad nutritional niche for this extreme generalist feeder. The results in this study supported this prediction, showing that BSF larvae were able to maintain high preadult survivorship across a broad range of protein concentrations from near 0 to 120 g l^{-1}. A particularly striking aspect of results in this study was the remarkable tolerance of BSF larvae to the extreme shortage of protein available in the diet. In this study, I found that more than 60% of BSF larvae successfully survived to the adult stage despite being reared on a chemically defined diet with an extremely low P:C ratio of 1:16. This result contrasts with that of Jang and Lee (2018) who reported that the preadult survivorship of *D. melanogaster* larvae reared on exactly the same diet was close to 0%. Although the exact mechanism remains to be elucidated, it is possible that BSF larvae might have developed an improved capacity to compensate for extreme protein deficiency through increasing the post-ingestive protein utilization efficiency (Lee et al., 2002; Simpson and Raubenheimer, 2012) or obtaining essential amino acids produced by the gut microbiome, as has been described from other insects (Ayayee et al., 2016; Jing et al., 2020).

While being highly tolerable to protein shortage, BSF larvae were found to be more or less susceptible to protein surplus, suffering high mortality when restricted to consume diets containing excessively high concentrations of protein. This negative effect of consuming these protein-excess diets occurred independently of the concentration of carbohydrate in the diet and is closely aligned with the results of a number of previous studies which have demonstrated low preadult survivorship in BSF larvae reared on a variety of animal-derived and synthetic diets rich in protein content (Gobbi et al., 2013; Nguyen et al., 2013; Tschirner and Simon, 2015; Cammack and Tomberlin, 2017; Barragan-Fonseca et al., 2020). For example, Nguyen et al. (2013) showed that BSF larvae suffered significantly higher mortality when reared on pig liver and fish offal than on fruits/ vegetables and poultry feed. Similarly, Gobbi et al. (2013) reported that mortality during the larval stage reached as high as ca. 60% in BSF larvae fed meat meal, but was only ca. 7% in those fed hen feed. Using chemically defined diets, Barragan-Fonseca et al. (2020) found that the survivorship of BSF larvae decreased significantly in response to increasing protein concentration in the diet, validating the proposition that excessive protein intake is responsible for high preadult mortality on animal-derived foods in BSF. The detrimental consequence of excessive protein or nitrogen intake on preadult performance (e.g., survivorship, growth, body size) is widespread in insects (e.g., Fischer and Fiedler, 2000; Lee et al., 2003; Cease et al., 2012) and may arise from high energetic costs of catabolizing and excreting surplus protein and/or toxicity caused by increased emission of fecal ammonia (Schroeder, 1986; Lee et al., 2002; Green and Popa, 2012). The pattern of preadult survivorship was largely explained by that of survivorship during the larval stage, but the ingestion of diets containing excess protein also reduced the survivorship occurring during the non-feeding pupal stage in BSF. This result is consistent with the results of Gobbi et al. (2013) who observed high pupal mortality in BSF reared on meat meal. Since the process of pupal development and metamorphosis is energetically demanding (Merkey et al., 2011), high pupal mortality could be attributed to the limited energy storage of BSF pupae reared on high-protein, low-carbohydrate diets.

Body size achieved at sexual maturity has been shown to correlate positively with reproductive success in many insects (Honěk, 1993) and this can be particularly true for capital breeding insects that rely strictly on the internal resources accumulated during the larval development for manufacturing eggs (Leather, 1988). BSF adults are capital breeders (Sheppard et al., 2002; Tomberlin et al., 2002) and multiple studies have repeatedly demonstrated that the body weight or size of prepupa or adult is associated with increased female fecundity (Gobbi et al., 2013; Barragan-Fonseca et al., 2017, 2018b, 2020) and male mating success in BSF (Tomberlin and Sheppard, 2001). In this study, I found that maximal body weight at adult emergence occurred when BSF larvae were reared on a diet with carbohydrate-biased P:C ratios of 1:3.16–1:3.36 and with high total P+C concentrations of 205.9–224.6 g l^{-1}. Although adult reproductive success was not directly quantified in this study, such strong positive correlation known to exist between body size and reproductive success leads me to assume that the reproductive outcome of both male and female BSF will be maximized at this specific macronutrient composition.

In addition to body weight, development time is another important preadult trait that has strong bearing on fitness in immature insects (Nylin and Gotthard, 1998). Slow or extended larval development has been shown to incur fitness costs to individual insects through decreasing the likelihood of mating and reproductive success (Sibly and Calow, 1986) and increasing preadult mortality risks from predators (Benrey and Denno, 1997; Uesugi, 2015). I found that the duration of larval and pupal stage in BSF was the shortest at the intermediate protein concentration of 48 g l^{-1}, raising the possibility that any ecological costs associated with larval duration would be minimal for BSF reared at this protein concentration. As has been demonstrated from other insects (Lee et al., 2002; Raubenheimer and Simpson, 2003; Jang and Lee, 2018) and also in BSF (Cammack and Tomberlin, 2017; Barragan-Fonseca et al., 2020), the duration of preadult development was shown to be prolonged substantially in BSF larvae reared on diets deficit in protein (protein concentration, <35 g l^{-1}). Although the extent of extension was not as great as that seen in BSF larvae reared on protein-limiting diets, I additionally found that preadult development time was extended in BSF reared on diets with protein concentrations higher than 100 g l^{-1}, which could be explicable as the outcome of metabolic costs of coping with excess protein.

While an increased adult body size offers benefits in terms of improved reproductive success in insects, it is often attained through prolonged or longer juvenile period, which in turn entails increased juvenile mortality caused by predation (Benrey and Denno, 1997; Uesugi, 2015). Larval or immature insects are thus expected to face a fundamental trade-off between fast juvenile development and large body size at sexual maturity (Stearns, 1992; Nylin and Gotthard, 1998; Roff, 2002). The appearance and direction of life-history trade-offs can be genetically and environmentally determined (Stearns, 1992; Roff,

2002) and there is growing evidence showing that the balance of multiple macronutrients ingested from food can be a critical modulator of the trade-offs among various life-history traits (Lee et al., 2008). The nutrient-mediated or nutrient space-based life-history trade-offs are expected to occur when the nutritional optima for different life-history traits are located in significantly different regions in the nutrient space (Rapkin et al., 2018; Morimoto and Lihoreau, 2019). Theories also predict that the strength of this trade-off between the two traits in question increases as the distance between their nutritional optima increases (Rapkin et al., 2018; Morimoto and Lihoreau, 2019). Here I found that, among various life-history traits measured, the Euclidean distance was the farthest between the nutritional optimum identified for preadult development time and that for body weight at adult emergence in the nutrient space. This result indicates the occurrence of strong nutrient-mediated trade-off between these two life-history traits in BSF larvae. An interesting question to be addressed in future is whether BSF larvae will preferentially select dietary macronutrient composition that maximizes body weight, minimizes development time, or compromises between the two when given an opportunity to choose their preferred diet. Rodrigues et al. (2015) previously showed that the larvae of *Drosophila melanogaster* preferred the P:C intake ratio that minimized their development time, but whether this food choice holds for BSF remains an open question.

The evolutionary fitness of a larval insect is the combined outcome of preadult survivorship, development time, and body size at sexual maturity (Nylin and Gotthard, 1998). The proxy measure of fitness that takes into account these three parameters was calculated by multiplying preadult survivorship and growth rate (Simpson et al., 2004; Lee et al., 2006; Cease et al., 2012). Here I found that this fitness proxy was maximized at intermediate P:C ratios of 1:1.37–1:1.43 coupled with moderate P+C concentrations of 191.4–194.3 gl^{-1}. The finding I obtained here that the optimal P:C ratio for the fitness proxy was proximate to 1:1 in BSF larvae was largely consistent with the results of Cammack and Tomberlin (2017) who reported the highest larval survivorship and the fastest larval development on a diet with an equal ratio of protein to carbohydrate in BSF. The analysis on the pattern of isoclines fitted to the nutritional landscapes indicated that the fitness proxy measured in this study was predominantly influenced by the nonlinear effect of dietary protein content, showing a declining pattern of fitness as protein concentration in the diet either increased or decreased from the optimal protein content. This result is in line with the established notion that dietary protein is the most decisive nutritional factor determining the evolutionary fitness in immature insects (Mattson, 1980; Scriber and Slansky, 1981). The present data in this study also suggested that dietary carbohydrate content had a significant convex relationship with the fitness proxy although the size of this effect was not as strong as that described for dietary protein content. Taken together, despite the differences in magnitude, my results provide evidence for the presence of significant fitness costs incurred by both over- and under-ingestion of protein and carbohydrate, as theorized by Simpson et al. (2004). Further study is necessary to test whether the foraging decision of BSF larvae is geared towards balancing the intake of protein and carbohydrate to maximize their evolutionary fitness, as has been demonstrated in other insects (Simpson et al., 2004; Lee et al., 2008; Jensen et al., 2012).

Numerous studies have reported that the protein content in the body of BSF larvae and adults is stable across different dietary regimes (e.g., 40–43% dry matter; Spranghers et al., 2016) whereas their lipid content is highly variable (7–39% dry matter) (Barragan-Fonseca et al., 2017, 2018a, 2018b). Using three different meridic diets formulated by differentially mixing chicken feed and indigestible cellulose, Barragan-Fonseca et al. (2018a) found that BSF larvae maintained a similar body crude protein content (ca. 50% dry matter) regardless of the nutritional density of the diets, but showed highly variable body fat content (ca. 5–30%), which increased with increasing nutrient concentration in the diet. The results I obtained here were largely consistent with these previous reports, demonstrating ca. 2.5 times higher variability in body lipid content than in lean body weight across 32 diet treatments (e.g., coefficient of variation, lipid content: 3.90; lean body weight: 1.57). Such low variability in lean body weight across dietary treatments is likely to be the outcome of the strong homeostatic regulation for maintaining body protein growth through reducing post-ingestive protein utilization efficiencies when protein is ingested in excess (Zanotto et al., 1993; Lee et al., 2002). In marked contrast, the regulation against surplus carbohydrate intake has been shown to be not as strong as that against surplus protein intake in many insects (Lee et al., 2002; Raubenheimer and Simpson, 2003), leading insects to convert excessively ingested carbohydrate into body lipids via lipogenesis instead of completely dissipating it through wastage respiration. This propensity of insects to store excess carbohydrate intake in form of lipid may explain why lipid content was highly variable depending on the macronutrient, more specifically carbohydrate, composition in the diet. Apart from such difference in variability, these two aspects of body composition exhibited very different nutritional landscapes, with diverging nutritional optima. As generally anticipated based on previous studies (e.g., Jang and Lee, 2018), the maximal expression for lipid content occurred at the extremely carbohydrate-biased P:C ratios of 1:14.1–1:10.8 whereas that for lean body weight was located at higher P:C ratios of 1:2.11–1:1.70. Since lipids are the major form of energy storage in insects, the lipid-rich phenotype of BSF adults that had been reared on high-carbohydrate, low-protein diet is likely to live and remain active for longer than those on the other diets.

There are two previous studies that used NG paradigm to investigate the impacts of dietary protein and carbohydrate content on BSF performance (Beniers and Graham, 2019; Barragan-Fonseca et al., 2020). In particular, the results I obtained here can be compared with those of an extensive study carried out by Barragan-Fonseca et al. (2020) who used a total of 25 chemically defined diets that varied in protein and carbohydrate content to construct nutritional landscapes for various larval and adult life-history traits in BSF. Some life-history traits exhibited similar responses to dietary variation in protein and carbohydrate between the study of Barragan-Fonseca et al. (2020) and this study. For example, the shape of nutritional landscape mapped by both studies indicated a strong and positive linear effect of dietary carbohydrate on body weight measured at adult emergence. Nevertheless, there were several major disagreements between the two studies. The most contrasting difference between them was found in the way in which dietary protein and carbohydrate influenced body lipid content in BSF. Barragan-Fonseca et al. (2020) showed that the crude fat content of BSF larvae increased linearly with increasing protein concentration and also with increasing P+C concentration in the diet, which was visually represented in nutritional landscape as linear isoclines with a negative slope ascending from the origin (see Fig. 4B in Barragan-Fonseca et al., 2020). In marked contrast, the isoclines fitted for lipid content in this study was almost perpendicular to those described in Barragan-Fonseca et al. (2020), aligning linearly with a positive slope. This result suggests that the phenotypic variation of lipid content observed in this study is determined by dietary P:C ratio. Such contradictory results found between the study by Barragan-Fonseca et al. (2020) and this study could be due to differences in the experimental condition, BSF strains, gut microbiome, and the experimental diets used between the two studies.

The major caveat to this study was that the nutritional landscapes for all measured traits were mapped based on the concentration of protein and carbohydrate present in the experimental diet and not on the actual intake of these macronutrients by BSF individuals. Despite not being complete, many insects are known to exhibit a compensatory increase in food intake in response to dietary dilution (Yang and Joern, 1994; Lee et al., 2004; Berner et al., 2005). This raises the caution that the nutritional landscapes plotted based on macronutrient concentration in this study may not represent the most accurate description of the relationship between macronutrient intake and BSF performance. Future studies are thus required to measure the actual intake of these macronutrients to take into account the possible effect of compensatory feeding on BSF performance (see Lee et al., 2008). Another important reason to determine the actual intake of protein and carbohydrate in BSF is the necessity to characterize the nutritional regulatory responses of this species (Simpson and Raubenheimer, 2012). While this study has offered us a comprehensive understanding of the role played by dietary macronutrients in shaping BSF performance, I still need to have detailed and deep knowledge of behavioural and physiological regulatory mechanisms adopted by BSF to achieve the optimal amount and balance of these macronutrients. An interesting first step would be to test whether BSF larvae can actively and independently regulate the intake of protein and carbohydrate to a specific-specific amount and proportion when given an opportunity to choose between nutritionally complementary diets. I am also interested in examining how BSF larvae will balance their intake when forced to trade off the over-ingestion of surplus nutrient in the diet against the under-ingestion of deficit one, and how plastically they can redress imposed nutritional imbalances through adjusting their post-ingestive nutrient use efficiencies (Simpson and Raubenheimer, 2012).

In conclusion, this study has comprehensively demonstrated how dietary protein and carbohydrate concentrations act in concert to influence the phenotypes of various preadult life-history traits and body composition in BSF. The present data confirmed that a moderate increase in protein content in the diet was associated with improved performance, but also indicated that the supplementation of protein exceeding its optimal level would incur significant performance losses in BSF. Dietary protein was found to play a predominant role in shaping most of the key traits related to BSF performance and fitness, but the influence of dietary carbohydrate cannot be overlooked. The optimal P:C ratio for the index that closely represents the evolutionary fitness of BSF individuals during their juvenile stage was identified to be 1:1.4. These results provide not only a valuable addition to the literature on comparative insect nutrition but also insights for designing an optimal dietary formula for mass rearing this beneficial insect. A clear message from the present data in this thesis is that there is no single macronutrient composition that simultaneously satisfies the maximal phenotype of all components of fitness, thus leaving us only one option to maximize one trait at the expense of others. However, this situation may offer us a promising opportunity to customize the production of BSF phenotypes according to our needs by differentiating the mixtures of macronutrients in the food for mass culturing BSF larvae (Müller et al., 2017; Barragan-Fonseca et al., 2020). For instance, I can intentionally design larval rearing diets specially targeted to produce fat-rich BSF phenotypes that can be used to produce biodiesel and alternatives to fish oil (Surendra et al., 2016; Belghit et al., 2018) or lean BSF phenotypes that can replace or supplement traditional dietary protein source for poultry, swine, and aquaculture (Barragan-Fonseca et al., 2017; Dabbou et al., 2018). I believe that the outcome of this basic research on BSF nutrition will have broad implication for improving the process and efficacy of the mass production of this economically important species and for providing high-quality nutrient source for animal feed.

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ABSTRACT IN KOREAN

음식물의 거대영양소가 아메리카동애등에(*Hermetia illucens*)의 발육성적과 체성분 조성에 미치는 영향에 대한 연구

천두암

초록

동물성 단백질 생산에는 많은 양의 사료가 요구된다. 그러나, 곡물, 대두박, 어분과 같은 전통적인 사료 원료의 부족은 점차 심화되고 있으며, 이는 세계적인 식량위기로 이어질 것으로 예상된다. 최근 곤충은 이러한 식량위기를 해결할 유망하고 친환경적인 대안으로 부상하고 있다. 곤충은 다양한 종류의 유기 폐기물을 단백질로 효율적으로 전환할 수 있을 뿐 아니라, 사육이 용이하고, 온실가스도 적게 배출한다. 특히 아메리카동애등에는 음식물 쓰레기 및 농업 부산물을 처리하는 분해자이며, 전통적인 양돈, 양계, 양어 사료를 대체할 수 있는 경제적으로 가장 중요한 곤충 중 하나이다. 아메리카동애등에 유충은 광식성 섭식자로 다양한 식물성 및 동물성 유기물을

섭취할 수 있다. 이러한 경제적 중요성에도 불구하고 이들의 영양적 요구량을 정량적으로 분석한 연구는 부족한 실정이다.

영양은 곤충에서 거의 대부분의 생물학적 현상에 영향을 미친다. 특히 단백질과 탄수화물과 같은 거대영양소는 초식성 및 잡식성 곤충의 발육과 생존 그리고 번식에 대해 지대한 영향을 미치는 것으로 알려져 있다. 본 연구의 목적은 음식물의 단백질과 탄수화물의 함량이 아메리카동애등에 성충 이전 단계의 생활사 형질과 체성분 조성에 미치는 영향을 다차원 분석 기법인 영양기하학을 적용하여 규명하는 것이다. 이를 위해 유충기 섭식 기간 동안 단백질과 탄수화물의 8가지 비율(P:C= 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, 8:1)과 4가지 단백질과 탄수화물 함량(P+C= 60, 120, 180, 240 g l^{-1})의 총 32가지 비율 × 함량 먹이 조건에서 진화적 적응도와 연관된 아메리카동애등에 개별 유충의 다양한 생활사 형질들을 성별을 구분하여 측정하였다. 음식물의 단백질과 탄수화물 함량에 따른 개별 형질의 측정값은 3차원 영양경관도로 적합되었다.

아메리카동애등에 암컷은 수컷과 비교하여 느리게 자랐으며, 결국 상대적으로 더 큰 성충으로 성장하였다. 발육 시간과 몸의 크기를 제외하면 대부분의 형질에서 성별에 따른 영양경관도의 형태적 차이는 확인되지 않았다. 그러나 개별 형질의 영양경관도 사이에서는 전반적인 지형학적 차이가 발견되었는데, 영양공간에서 표현되는 개별 형질의 영양학적 최적점의 위치 또한 다르게 나타났다. 성충 이전 단계의 생존율은 음식물의 단백질 농도가

120 g l^{-1}보다 낮은 환경에서 모두 60% 이상으로 높게 유지되었고, 120 g l^{-1} 이상에서는 농도가 증가할수록 생존율이 낮아지는 경향을 보였다. 주목할만한 점은 아메리카동애등에 유충이 극한의 단백질 부족 상황에 대해 높은 저항성을 나타냈다는 점이다. 성충 이전 단계의 생존율은 P:C 1:1.7 비율에서 최대로 나타났다. 발달 시간은 P:C 1:1.56 – 1:1.11 비율에서 가장 짧았으며, 단백질 농도가 35 g l^{-1} 이하일 때 급격하게 증가했다. 성충의 몸무게는 탄수화물 쪽으로 치우친 P:C 1:3.16 – 1:3.36 비율에서 정점을 나타났으며, 음식물의 탄수화물 농도가 감소할수록 몸무게도 점차 감소하였다. 성장율의 최고점은 P:C 1:1.40 – 1:1.83 비율에서 나타났으며, 최고점으로부터 단백질 농도가 감소하거나, 증가할 경우 성장률도 감소하였다. 가장 중요한 지표인 진화적 적응도의 추정값은 생존율과 성장률의 곱으로 산출되었는데, 이는 P:C 1:1.37-1:1.43 비율에서 최대로 나타났다. 이와 같은 최대 지점에서의 단백질과 탄수화물 농도 조성은 아메리카동애등에의 대량 사육을 위한 최적 조건임을 의미한다. 적응도의 추정값은 최적 지점으로부터 단백질과 탄수화물 농도가 증가하거나 감소할 경우에 낮아지는 경향을 보였다. 성충의 탈지방 무게는 지방 무게와 비교하여 음식물의 단백질과 탄수화물 조성에 의한 차이가 상대적으로 크지 않은 반면, 지방 무게는 먹이의 탄수화물 농도에 따라 높은 다양성을 보였다.

이러한 결과를 종합하면 음식물의 단백질과 탄수화물의 가용성은 아메리카동애등에의 발육성적과 체성분 조성에 크게 영향을 미친다는 것을 알

수 있다. 특히 음식물의 단백질의 농도는 몸무게와 지방 무게를 제외한 대부분의 생활사 형질에서 가장 영향이 큰 인자로 확인되었다. 영양학적 최적점 간의 유클리드 거리(The Euclidean distance)가 가장 먼 형질은 몸무게와 발달시간이었는데, 이는 두 형질 사이에서 발생한 life-history trade-off가 가장 크게 나타났음을 의미한다.

본 연구에서 확인된 결과는 경제적으로 중요한 곤충인 아메리카동애등에의 대량 사육의 목적에 따라 최적 영양 조건을 설정함으로써 개별 형질의 발현 수준을 조절할 수 있는 가능성을 제시하고, 이를 통해 곤충 대량 생산의 효율을 향상시키기 위한 중요한 시사점을 제공한다.

표제어: 체성분 조성, 거대 영양소, 아메리카동애등에, 곤충 대량 생산, 영양 최적점, 유충 생활사 형질, 단백질

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