Journal of Insects as Food and Feed, 2017; 3(2): 121-130

Bioconversion of sorghum and cowpea by black soldier fly (*Hermetia illucens* (L.)) larvae for alternative protein production

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Received: 27 September 2016 / Accepted: 28 December 2016

RESEARCH ARTICLE

Abstract

The larvae and prepupae of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) have been explored as a means for alternate protein production for feed for animals that are raised for human food. However, processes for production of these and other insects must be refined if cultivation is to become widespread and efficient. In this study, black soldier fly larvae were fed one of six diets, consisting of the Gainesville house fly diet (control), and five diets of varying ratios of sorghum and cowpea. Effects on life-history traits and nutritional content of prepupae were observed. Flies were able to successfully complete larval development on all diets tested. There were significant differences in development rates based on diet, particularly the diets containing a higher percentage of sorghum. In general, larvae reared on the sorghum diets (which were lower in protein than that of the cowpea diets), developed slower (3-9 days longer from larval eclosion to the prepupal stage) than those on the cowpea diets. Diet treatment did not consistently influence weight or length of prepupae. Higher protein diets (7.73% protein) translated to higher protein content of prepupae (43.70-47.29% protein) and lower protein diets (3.51% protein) resulted in greater gross energy content of prepupae (5.22-6.21 Kcal/g). These differences suggest that macro-nutrient content of prepupae can be influenced by larval diet. This study provides further evidence of the viability of black soldier flies for protein production.

Keywords: entomophagy, food, gross energy, insect, nutrition, prepupae

1. Introduction

The human population is expected to reach between 8.1 and 10.6 billion by 2050 (Ezeh *et al.*, 2012). Population expansion of this size would normally require a proportionate expansion in land used for agriculture and food production, but there is limited land remaining that can be used for such production (Godfray *et al.*, 2010). Additionally, agricultural demands for water account for 92% of global water usage, which is also limited in some geographical areas (Gerbens-Leenes *et al.*, 2011). Food supply shortfall consequences are common in developing areas, such as certain parts of Africa, where protein malnutrition is a serious issue (Anyango *et al.*, 2011); alternate sources of protein for inhabitants of such regions could provide a solution for this nutritional deficit. Participants in a survey conducted in Niger consider sorghum (*Sorghum bicolor* L. Moench) or millet (*Pennisetum glaucum* (L.) R.Br.) porridge to be a staple food (Towns *et al.*, 2013). Anyango *et al.* (2011) advocated an increase of cowpea (*Vigna ungiculata* L. Walp), (aka black-eyed pea) consumption to increase the amount of protein in sorghum-based diets because cowpea grows well in the region and is more protein rich than sorghum, averaging approximately 23.5 vs 8.4-11% protein. Additionally, cowpeas are a common food source in Nigeria in the form of baked bean puddings, fried bean cakes, bean porridge, and boiled beans (Sanusi and Adebiyi, 2009).

Insects could serve as another viable option for protein production. Van Huis (2013) gives a broad account of the benefits of insects as food for humans and as feed for livestock, poultry and aquaculture industries. Such an

approach for alternate protein production could reduce the accumulation of organic waste, produce ingredients for animal feed, and reduce dependency on international fisheries for fishmeal to supply the aquaculture and livestock industries (Van Huis, 2013).

Black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) larvae primarily consume decaying plant matter such as fruits and vegetables, but are also known to consume other decaying organic matter such as animal flesh (Nguyen *et al.*, 2015) and manure (Myers *et al.*, 2008). This ability makes them ideal for transforming otherwise wasted organic material, such as food that is no longer safe for humans to consume, into larvae rich in protein and fat (Myers *et al.*, 2008; Nguyen *et al.*, 2015; Tomberlin *et al.*, 2005; Zhou *et al.*, 2013). These larvae, or some part of them, can then be used as feed for livestock, poultry, and fish (Van Huis, 2013).

The BSF has been explored as a partial substitute for fishmeal in aquaculture, and has been observed to have a nutritional value similar to that of soybean, meat, and bone meal (Kroeckel *et al.*, 2012; St-Hilaire *et al.*, 2007). Kroeckel *et al.* (2012) found that BSF meal can replace approximately 33% of the fish meal in the diet of turbot, *Psetta maxima*, (L.) with minimal adverse effects on protein, lipid, and gross energy content of the fish. The prepupal stage of BSF can be used as a partial replacement of the fish meal (~25%) and fish oil (~38%) in the diets of rainbow trout, *Oncorhynchus mykiss* (Walbaum), which cannot be fed plant-based protein (St-Hilaire *et al.*, 2007).

In developing nations where protein malnutrition is common, discovery of efficient alternate protein production methods are a high priority (Anyango *et al.*, 2011). The BSF is ideal for this application because no specialised equipment is necessary for production (Sheppard *et al.*, 1994), this species is widely distributed around the world (Tomberlin *et al.*, 2002), and it can subsist on many types of organic waste, including human food waste (Nguyen *et al.*, 2013).

The diet of BSF larvae has been shown to influence their life-history traits (Nguyen *et al.*, 2013; Tomberlin *et al.*, 2002). Laboratory colonies of BSF are frequently maintained on the Gainesville house fly, *Musca domestica* L. (Diptera: Muscidae), diet, which is composed of 30% alfalfa meal, 50% wheat bran, and 20% corn meal (dry mass), mixed with water to 60-70% moisture (Hogsette, 1992; Tomberlin *et al.*, 2002). This diet is appropriate for maintaining laboratory colonies, but wild BSF are significantly larger than laboratory-reared BSF (as much as double the size), suggesting that this diet may not meet all nutritional needs (Tomberlin *et al.*, 2002); optimisation of the larval diet could potentially increase the protein production by these insects. Therefore, the first objective of this study was to determine if the ratio of cooked sorghum to cowpeas impacts the life-history traits (larval daily weight, development time to the prepupal stage, prepupal weight, and survivorship) of BSF. The second objective was to determine the protein and gross energy content in BSF when larvae were fed different ratios of cooked sorghum and cowpeas (Table 1).

Table 1. Nutritional composition of dietary components in sorghum and cowpea fed to BSF in this study as found in the USDA National Nutrient Database (US Department of Agriculture, 2015).

Nutrient	Unit (per 100 g)	Diets		
		Sorghum	Cowpea	Gainesville house fly diet
Proximate				
Water	g	71.41	70.04	–
Energy	kcal	119	116	–
Protein	g	3.51	7.73	15.33
Total lipid	g	1.00	0.53	3.78
Carbohydrate	g	23.67	20.76	–
Fibre	g	1.3	6.5	12.6
Sugars	g	0.13	3.30	
Minerals				
Ca	mg	3	24	–
Fe	mg	0.63	2.31	–
Mg	mg	44	53	–
P	mg	100	156	–
K	mg	62	278	–
Na	mg	2	4	–
Zn	mg	0.91	1.29	–
Vitamins				
Vitamin C	mg	0.0	0.4	–
Thiamin	mg	0.106	0.202	–
Riboflavin	mg	0.082	0.055	–
Niacin	mg	1.33	0.495	–
Vitamin B6	mg	0.108	0.100	–
Folate, DFE	µg	19	208	–
Vitamin B12	µg	0	0	–
Vitamin A, RAE	µg	0	1	–
Vitamin A, IU	IU	3	15	–
Vitamin E	mg	0.02	0.28	–
Vitamin D (D3 + D3)	µg	0	0	–
Vitamin D	IU	0	0	–
Vitamin K	µg	0.3	1.7	–

2. Materials and methods

Acquisition of flies

This experiment was performed with BSF larvae reared from a colony maintained at the Forensic Laboratory for Investigative Entomological Sciences, or F.L.I.E.S. Facility at Texas A&M University, College Station, TX, USA. This colony was established in 2014 from eggs received from a laboratory colony at the Coastal Plains Experiment Station, University of Georgia, Tifton, GA, USA. This colony has been maintained since 1998 and periodically supplemented with wild-caught insects. The larvae of the colony were provided the Gainesville house fly diet (G), and maintained in a walk-in incubator at approximately 28.0±2.0 °C, 70% RH, and 14:10 L:D cycle. The adults of the colony were maintained in a mesh tent in a greenhouse at ambient temperature and humidity, with water provided several times per day. Eggs were collected from flutes of corrugated cardboard as described in Sheppard *et al.* (2002), and once hatched, the larvae were provided the G diet in a walk-in incubator under the conditions previously described; experiments were started with 4-day-old larvae. Larvae of this age are typically larger, more visible, and more likely to survive handling than younger larvae (Sheppard *et al.*, 2002).

Feed preparation

Dried sorghum of mixed varieties was obtained from the sorghum breeding and genetics programme at Texas A&M University, College Station, TX, USA. Dried cowpeas, or black-eyed peas (H-E-B brand, HEB Grocery Company, San Antonio, TX, USA), were purchased from a local grocery store. Sorghum and cowpeas were used to simulate wasted food. Sorghum and cowpeas were soaked separately from each other in stainless steel bowls for 5 h, in a one to four ratio of product to water then drained, followed by boiling for approximately 2 h. The sorghum grains and cowpeas were processed in a food processor and with a potato masher, respectively, until no whole grains or beans remained. These preparation methods were chosen based on bland versions of popular porridge recipes, to simulate wasted food.

The prepared sorghum and cowpeas were fed to BSF in the following ratios: (1) 100% sorghum, 0% cowpeas (100s); (2) 75% sorghum, 25% cowpeas (75s25c); (3) 50% sorghum, 50% cowpeas (50/50); (4) 25% sorghum, 75% cowpeas (25s75c); and (5) 0% sorghum, 100% cowpeas (100c). The G diet served as a control (Sheppard *et al.*, 2002; Tomberlin *et al.*, 2002). The diet treatments were prepared in four separate batches during the course of the experiment and stored in a refrigerator at 3 °C. Prior to preparing the treatments, the moisture content of the cooked sorghum and cowpea was determined gravimetrically by weighting out three 5 g replicates of each, drying at 60 °C, and weighing every 4 h until the weight stopped decreasing. The final (dry) weight was subtracted from initial weight to yield the weight of moisture evaporated during drying. The moisture content of the cooked sorghum and cowpea mixtures was 70.4-70.8%, consistent with that of the G diet (Tomberlin *et al.*, 2002).

Experiment design

Methods were adapted from those described in Sheppard *et al.* (2002), Tomberlin *et al.* (2002), and Myers *et al.* (2008). For each diet, 300 4-day-old larvae were placed in uncovered 0.53 l cups (n=3 per diet) with 10 g of diet. The cups with larvae were placed in a walk-in incubator set to the same conditions as described above. Larvae in each replicate were provisioned with 10 g of their respective diet (at approximately 70% moisture) daily once previously fed material was observed to be either digested or dry to prevent phorid infestation. If any one cup required feeding, all were fed. Each replicate (whole cup with remaining larvae and substrate) was weighed daily using an OHaus Scout™ Pro balance (OHaus Corporation, Parsippany, NJ, USA) prior to being fed to quantify daily moisture loss. Debris was not discarded, but each day's total cup weight was compared to the previous day's weight including any added feed weight. To prevent escape of prepupae and subsequent adults, the cups were covered with tulle fabric held in place with a rubber band after the first prepupae were observed. The entire experiment was repeated twice, referred to as trials A and B throughout the remainder of this manuscript.

Life-history traits

Daily larval length and weight, development time to the prepupal stage, prepupal length and weight, and survivorship to pre-pupal stage were determined and recorded. Every second day beginning at larval age of 10 d, for all three of the replicates of each treatment, three larvae were selected, killed with boiling water, then measured in millimetres and weighed using an OHaus Adventurer™ Pro balance (OHaus Corporation). These replicates were also examined daily for the presence of prepupae, which can be identified by their much darker colour than individuals still in the larval stage (Sheppard *et al.*, 2002). Larvae were fed until 40% of the starting number had reached the prepupal stage or less than ten larvae remained, whichever occurred first (Tomberlin *et al.*, 2002). Differences in the amount of time necessary to reach this stage led to different total feed amounts. Each container was examined daily for prepupae by sorting through the material until all individuals had become pre-pupae or died.

All prepupae were removed from the replicates and individual weight recorded. The first prepupa collected, and one for every additional 10 prepupae from each container,

was placed in a 59 ml lidded condiment cup with a damp cotton ball, which was checked daily to ensure that it stayed damp, in the same incubator and were monitored daily for adult emergence (reported as time from prepupation to emergence) and longevity (reported as time from emergence to death). All additional prepupae collected daily from each replicate were stored in a self-sealing plastic bag, and placed in a 0 °C freezer for subsequent nutritional analysis (see below).

Nutritional analysis

Frozen prepupae were thawed and dried at 60 °C, and weighed every 4 h until the weight stopped decreasing (Tomberlin *et al.*, 2002). Measurement of protein content was conducted using Dumas total combustion methods in an Elementar rapid N cube (Elementar Analysensysteme, Langenselbold, Germany) analyser for total nitrogen content, which was subsequently converted to crude protein using the conversion factor of 6.25 (Etheridge *et al.*, 1998; Lam *et al.*, 2009). Caloric content (gross energy) was measured using a bomb calorimeter and methods similar to those used by Doyle *et al.* (2007).

Statistical analysis

Statistical methods were adapted from those used by Nguyen *et al.* (2013). Regression analysis (general linear model) in SAS® (SAS® 9.4 for Windows; SAS Institute Inc., Cary, NC, USA) was used to test for treatment differences in larval growth measurements across time. Survival to the prepupual stage was assessed using a generalised linear model with a binomial probability distribution in SAS 9.4. Analysis of variance (ANOVA) and Tukey's honest significant difference (P<0.05) in SAS (SAS 9.4 for Windows) were used to test for treatment differences in nutritional value measurement, and analyses (ANOVA followed by Tukey's HSD) for life history parameters (dry weight, time to pupation, time to emergence, adult longevity, and total lifespan) were performed using JMP® Pro 12.0 (JMP® Pro 12.0; SAS Institute Inc.). Data were checked with regards to basic assumptions of an ANOVA prior to implementing the analysis.

3. Results

Life-history traits

Diet had a significant effect (P<0.01) on larval weight and length over time. Insects reared on the G diet and the higher percentages of sorghum generally took longer to reach the same sizes as those reared on other diets (Table 2, Figure 1 and 2). However, trial also had a significant effect (P<0.01) on length and weight over time with insects in trial B taking longer to reach the same sizes as those of trial A. Notably, larval and prepupal measurements were conducted as long as specimens were available. This limitation led to differences in time-sensitive data between larval and prepupal measurements, as average time from hatching to the prepupal stage did not necessarily indicate how long late-prepupating larvae would be present. Diet had a significant effect on prepupal weight (P<0.01) and length (P<0.01) in trial A, with individuals fed diets 75s25c, 50/50, and 25s75c being larger than those fed the other diets. However, diet did not have a significant effect on either prepupal weight (P=0.73) or length (P=0.07) in trial B (Table 3). Again, trial had a significant effect (P<0.01) on both weight and length, with individuals from trial B being smaller than those from trial A. The percentage of larvae that successfully entered the prepupal stage was significantly different between trials (P=0.013), but diet did not have a significant effect (P=0.77) nor was there a significant interaction between trial and diet (P=0.90) (Table 3). Mean total feed provided between diets differed due to differences in development time rather than feed rate, as all diet treatments were fed at the same rate (Table 3). Diet had a significant effect on time to prepupation (P<0.01), time to emergence (P=0.01), adult longevity (P<0.01) and

Table 2. Table of statistical values from regression output for black soldier fly larval measurements over time when fed different diets[1] during two trials (A and B)[2].

Variable	R^2	DF[3]	*F*-value	Prob.>*F*
Larval weight over time trial A	0.797423	108	25.22	<0.05
Larval weight over time trial B	0.618408	101	10.72	<0.05
Larval length over time trial A	0.859556	108	39.22	<0.05
Larval length over time trial B	0.584865	101	9.32	<0.05

[1] Diets were 100% cowpea, 100% sorghum, 25% sorghum and 75% cowpea, 50% cowpea and 50% sorghum, 75% sorghum and 25% cowpea, and the Gainesville house fly diet (control).

[2] Both trials took place in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D.

[3] DF varies between trials A and B due to differing development rates leading to different numbers of measurements as treatments were sampled every second day as long as larvae remained.

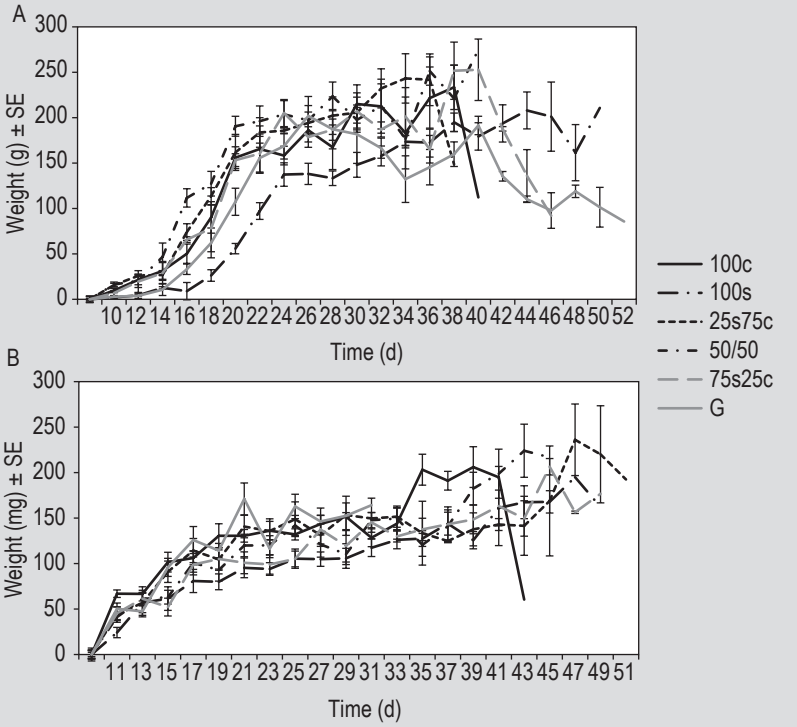

Figure 1. Mean weight (mg) ± standard error (SE) of black soldier fly larvae (n=3) over time (days) when fed different diets during two trials (A and B) in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D. Diets were: 100c = 100% cowpea; 100s = 100% sorghum; 25s75c = 25% sorghum and 75% cowpea; 50/50 = 50% cowpea and 50% sorghum; 75s25c = 75% sorghum and 25% cowpea; and G = Gainesville house fly diet (control).

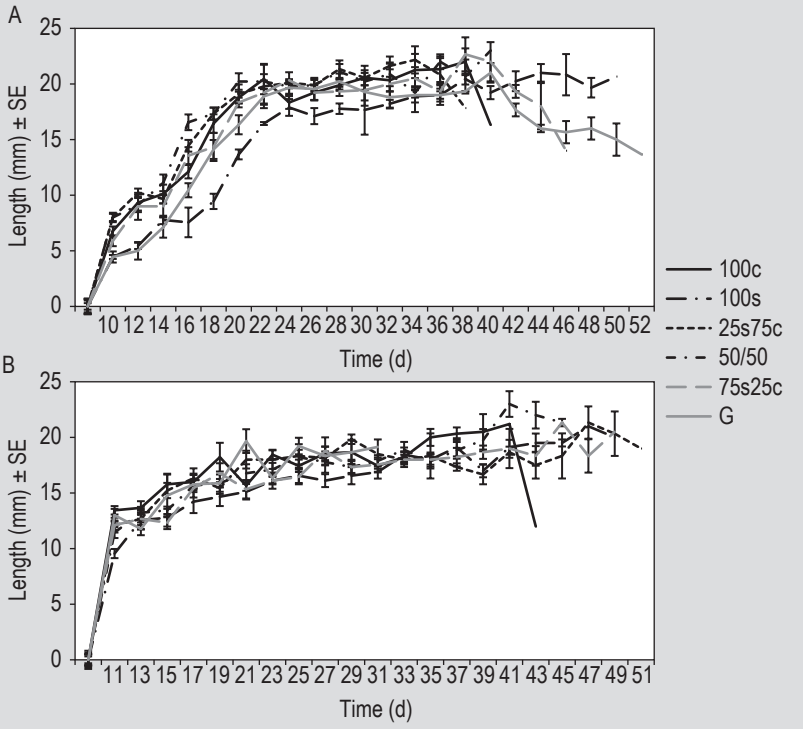

Figure 2. Mean length (mm) ± standard error (SE) of black soldier fly larvae (n=3) over time (days) when fed different diets during two trials (A and B) in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D. Diets were: 100c = 100% cowpea; 100s = 100% sorghum; 25s75c = 25% sorghum and 75% cowpea; 50/50 = 50% cowpea and 50% sorghum; 75s25c = 75% sorghum and 25% cowpea; and G = Gainesville house fly diet (control).

Table 3. Mean values ± standard error (SE) for black soldier fly prepupal life-history data when fed different diets during two trials (A and B)[1,2].

Diet[3]	Mean prepupal weight (mg) ± SE	Mean prepupal length (mm) ± SE	Mean percentage of prepupae produced ± SE	Mean total (wet) feed provided (g) ± SE
Trial A (n=3/diet)[4]				
G	106.29±2.01 A	16.35±0.19 A	42.84±12.00	220.00±15.28
100s	144.96±8.39 B	17.44±0.30 AB	50.82±12.48	296.66±3.33
75s25c	151.80±2.97 B	17.43±0.11 BC	41.50±11.97	226.66±14.53
50/50	156.67±7.14 B	17.86±0.24 BC	43.13±12.02	230.00±10.00
25s75c	154.34±7.15 B	17.96±0.21 C	40.26±11.88	230.00±15.28
100c	137.91±5.38 B	16.72±0.21 A	44.29±12.00	213.33±3.33
Trial B (n=3/diet)				
G	100.15±4.37	16.47±0.16	9.83±7.09	93.33±3.33
100s	95.29±3.59	15.59±0.07	33.88±11.75	140.00±5.77
75s25c	91.55±6.79	15.18±0.21	32.04±11.52	130.00±5.77
50/50	100.96±1.36	15.40±0.06	23.11±10.27	120.00±11.55
25s75c	92.10±11.07	15.38±0.60	25.00±10.48	120.00±20.82
100c	100.95±3.15	15.39±0.13	32.68±11.46	130.00±5.77

[1] Both trials took place in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D.

[2] Means within a column and trial followed by the same letter are not significantly different. Different letters represent a significance of at least $P<0.05$.

[3] 100c = 100% cowpea; 100s = 100% sorghum; 25s75c = 25% sorghum and 75% cowpea; 50/50 = 50% cowpea and 50% sorghum; 75s25c = 75% sorghum and 25% cowpea; and G = the Gainesville house fly diet (control).

[4] n = replicates.

total survival time ($P<0.01$) of insects in trial A, and time to prepupation ($P<0.01$), time to emergence ($P=0.01$), and total survival time ($P=0.02$) of insects in trial B (Table 4). Diet did not have a significant effect on time to emergence ($P=0.65$) or adult longevity ($P=0.63$) in Trial B. For all three measurements (time to prepupation, time to emergence, and total survival time) the insects reared on the G diet and the higher percentages of cowpea had shorter times, while those reared on diets containing higher percentages of sorghum had longer times. Trial also had a significant effect on all four measurements: time to prepupation ($P<0.01$), time to emergence ($P<0.01$), adult longevity ($P<0.01$) and total survival time ($P<0.01$), with trial B in general having longer times than trial A.

Nutritional analysis

Diet had a significant effect on protein content ($P<0.01$) and gross energy in calories ($P<0.01$), but trial did not ($P=0.76$ for protein and $P=0.15$ for gross energy) (Table 5). Protein content was lowest in insects reared on the G diet, then the 100s diet, then increased as percentage cowpea increased in diet. Gross energy was lowest in the G diet, then the 100c diet, then increased as percentage sorghum increased in diet. Protein per gram of diet (g/g) was a calculated value which, because of the differing amount of feed due to differing development times between trials, varied widely between trials in the same diets.

4. Discussion

BSF were able to successfully complete larval development on all tested diets. Significant differences in development rates based on diet were observed, particularly in those containing a higher percentage of sorghum. In general, larvae on the sorghum diets developed slower (3-9 days longer from hatching to prepupation) than those on the higher protein cowpea diets (Table 1).

These differences are similar to those demonstrated by Nguyen *et al.* (2013), who observed faster development (19-27 days difference during prepupal stage) in higher-protein (4-18 g more protein per 100 g) diets (kitchen waste, liver) and slower development in lower-protein diets (fruits and vegetables, manure). Additionally, Tomberlin *et al.* (2002) observed that wild BSF prepupae with different access to nutritional resources, at an average weight of 0.220 g, were larger than lab-reared BSF at 0.104 to 0.111 g. In contrast, the largest average prepupal weight of lab-reared BSF measured in this experiment was 0.157 g, and the smallest was 0.092 g (Table 3). Diet did not have a significant impact on survivorship, indicating that any of the tested diets could potentially sustain a population of BSF. When compared with the original 300 larvae, survival numbers appear very low, but when destructive sampling is considered the survival rate in trial A falls within the range of 40-80% reported in similar studies (Myers *et al.*,

Table 4. Mean values ± standard error (SE) for black soldier fly adult life-history traits when fed different diets during two trials (A and B)[1,2].

Diet[3]	Time to prepupation (day) ± SE	Time to emergence (day) ± SE	Adult longevity (day) ± SE	Total lifespan (day) ± SE
Trial A (n=3/diet)[4]				
G	33.42±0.86 B	20.40±0.48 A	11.56±0.78 AB	65.37±1.16 BC
100s	38.11±0.82 A	22.79±0.46 B	10.57±0.74 B	71.47±1.11 A
75s25c	32.19±0.87 BC	22.21±0.48 AB	14.40±0.79 A	68.81±1.18 AB
50/50	28.23±0.85 CD	22.00±0.47 AB	14.66±0.77 A	65.89±1.15 BC
25s75c	28.35±0.83 D	21.96±0.46 AB	12.67±0.75 AB	62.98±1.12 C
100c	29.69±0.90 CD	21.28±0.50 AB	10.49±0.82 B	61.46±1.22 C
Trial B (n=3/diet)				
G	26.13±2.24 A	24.38±2.04 A	10.13±1.53 A	60.63±3.28 B
100s	38.08±1.30 B	23.79±1.18 A	12.04±0.88 A	73.92±1.89 A
75s25c	35.29±1.30 B	25.21±1.18 A	11.04±0.88 A	71.54±1.89 AB
50/50	35.12±1.54 B	24.82±1.40 A	10.47±1.05 A	70.41±2.25 AB
25s75c	35.83±1.50 B	23.89±1.36 A	9.72±1.02 A	69.44±2.19 AB
100c	34.80±1.42 B	22.25±1.29 A	10.75±0.97 A	67.80±2.07 AB

[1] Both trials took place in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D.

[2] Means within a column and trial followed by the same letter are not significantly different. Different letters represent a significance of at least $P<0.05$.

[3] 100c = 100% cowpea; 100s = 100% sorghum; 25s75c = 25% sorghum and 75% cowpea; 50/50 = 50% cowpea and 50% sorghum; 75s25c = 75% sorghum and 25% cowpea; and G = the Gainesville house fly diet (control).

[4] n = replicates.

Table 5. Mean values ± standard error (SE) for nutritional data of black soldier fly prepupae when reared on different diets during two trials (A and B), or both trials combined where one is not indicated, in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D.[1]

Diet[2]	Mean percentage protein (n=6/diet)[3] ± SE	Mean gross energy (kcal/g) (n=6/diet) ± SE	Dry matter (g) trial A ± SE	Dry matter (g) trial B ± SE
G	43.70±1.44 A	5.22±0.10 A	49.42±7.71 BC	40.17±5.55 A
100s	44.05±1.38 AB	6.20±0.06 B	48.36±7.74 C	40.73±7.01 A
75s25c	44.92±0.87 AB	6.21±0.04 B	62.03±8.94 ABC	37.80±4.80 A
50/50	45.40±0.58 ABC	6.06±0.06 C	66.92±5.43 A	45.53±3.71 A
25s75c	46.11±0.79 BC	5.95±0.07 CD	64.38±9.20 AB	37.67±13.72 A
100c	47.29±1.89 C	5.89±0.07 D	56.28±9.13 ABC	36.77±2.44 A
	Total weight of prepupae per gram fed (g/g)[4] trial A ± SE	**Total weight of prepupae per gram fed (g/g) trial B ± SE**	**Protein produced per g diet (g/g) trial A**	**Protein produced per g diet (g/g) trial B**
G	0.050±0.024	0.028±0.006	0.022	0.012
100s	0.061±0.016	0.057±0.015	0.027	0.025
75s25c	0.069±0.017	0.056±0.015	0.031	0.025
50/50	0.075±0.015	0.048±0.010	0.035	0.022
25s75c	0.069±0.005	0.044±0.017	0.032	0.020
100c	0.073±0.015	0.063±0.014	0.035	0.030

[1] Means within a column followed by the same letter are not significantly different. Different letters represent a significance of at least $P<0.05$.

[2] 100c = 100% cowpea; 100s = 100% sorghum; 25s75c = 25% sorghum and 75% cowpea; 50/50 = 50% cowpea and 50% sorghum; 75s25c = 75% sorghum and 25% cowpea; and G = the Gainesville diet (control).

[3] n = replicates.

[4] Weights of both prepupae and feed were fresh weight, not dry weight.

2008; Nguyen *et al.*, 2013). Survival to prepupation in trial B; however, especially in G diet (9.83%), remains very low, even when accounting for destructive sampling (Table 6).

This variation in life-history traits could be due to differences in dietary protein or energy. For example, Nguyen *et al.* (2013) fed BSF six diets of different nutritional values and observed variation based on nutritional quality (i.e. availability of balanced calories, fat, and protein) of diet, indicating that too much or too little of any nutrient could be detrimental to larval performance. They observed longer development times in diets lower in protein and energy and speculated that too much fat might be detrimental to BSF development, as the higher fat diets in their study resulted in higher larval mortality rates than lower fat diets.

Higher protein diets translated to higher protein prepupae in both trials. This could be because of the protein content of the diet, but could also be due to vitamin or mineral contents, which differ between the diets as well (Table 1) (Anyango *et al.*, 2011). Lower protein diets led to higher gross energy prepupae in both trials, which may be due to higher lipid or carbohydrate content of the lower protein diets, or due to other differences between diets such as vitamins or minerals (Anyango *et al.*, 2011). The control (Gainesville) diet may have been lower in both protein and gross energy than other diets because the other diets were cooked while the Gainesville diet was only moistened. Cooking may have made nutrients in the diets more available for digestion by the BSF larvae (Kon *et al.*, 1971).

Trial had a significant effect on all of the life-history measurements, which suggests other sources of variation may have been involved. This variation between trials means that the variation observed between diets may be less significant in these traits. On the 12th day of trial A, the walk-in incubator malfunctioned and larvae were immediately moved to a different walk-in incubator with the same settings for the duration of trial A. The entirety of trial B took place in the original walk-in incubator after the malfunction was fixed. Because of this use of different incubators, there may have been minute variation in temperature and humidity, both of which could influence life-history traits of BSF (Holmes *et al.*, 2012; Tomberlin *et al.*, 2009). Containers were removed from incubators when measurements were taken, and since trial A took place in the fall and trial B took place in the summer, there may have been variation in ambient temperature and humidity in the laboratory, both of which could also influence BSF life-history traits, and seasonal differences in the greenhouse where the adults of the colony were kept may have had an impact on egg development (Holmes *et al.*, 2012; Tomberlin *et al.*, 2009). Likewise, separate batches of diet were prepared for the two trials, which could have provided an additional source of variation.

Table 6. Mean values ± standard error (SE) for black soldier fly prepupal survival data when fed different diets during two trials (A and B).[1,2]

Diet[3]	Mean number of larvae lost to destructive sampling ± SE	Mean total number of prepupae ± SE	Percentage survival to prepupation
Trial A (n=3/diet)[4]			
G	44.00±11.06	109.67±57.51	42.84±12.00
100s	56.67±3.76	123.67±28.71	50.82±12.48
75s25c	43.00±7.55	106.67±34.67	41.50±11.97
50/50	42.67±2.73	111.00±23.44	43.13±12.02
25s75c	41.67±3.33	104.00±15.50	40.26±11.88
100c	40.33±3.84	115.00±27.57	44.29±12.00
Trial B (n=3/diet)			
G	32.33±0.67	26.33±6.11	9.83±7.09
100s	54.00±3.06	83.33±21.93	33.88±11.75
75s25c	51.33±3.84	79.67±19.06	32.04±11.52
50/50	44.67±4.98	59.00±15.10	23.11±10.27
25s75c	41.33±11.29	63.67±28.99	25.00±10.48
100c	46.00±2.08	83.00±21.36	32.68±11.46

[1] Both trials took place in a walk-in incubator set at 28.0±2.0 °C, approximately 70% RH, and 14:10 L:D.

[2] Means within a column and trial followed by the same letter are not significantly different. Different letters represent a significance of at least $P<0.05$.

[3] 100c = 100% cowpea, 100s = 100% sorghum, 25s75c = 25% sorghum and 75% cowpea, 50/50 = 50% cowpea and 50% sorghum, 75s25c = 75% sorghum and 25% cowpea, and G = the Gainesville house fly diet (control).

[4] n = replicates.

Crude protein content was also estimated from gross nitrogen measurement, rather than measured directly in an amino acid profile. This means that indigestible nitrogen such as chitin could also have been measured as protein (Diener *et al.*, 2009). Crude protein and gross energy were measured on a dry matter basis, so dry matter data has also been included in Table 5 to aid in interpretation. Carbohydrates, vitamins, and minerals of BSF were not measured at all in this study, though there may be differences by diet.

This work is a piece in the puzzle of refining insects' diets to improve them as a food source themselves, much as diets were studied nearly 100 years ago in traditional livestock (Maynard *et al.*, 1979). If these or other insects are to become a more mainstream food or feed source, diets must be optimised to allow for more efficient mass production (Van Huis, 2013). Data collected here suggest the quality of BSF prepupae can be influenced drastically by diet, as was expected. These data represent further evidence of the viability of BSF for protein production, as they rank high in crude protein percentage of dry weight as compared with common food insects listed by Bukkens (1997). Of the insects listed by Bukkens (1997), the highest in protein was a caterpillar in the family Limacodidae (species unknown) at 69.6%, and the lowest were female flying ants (*Carebara* sp., Hymenoptera: Formicidae) at 3.0%. Only 14 of the 51 insects listed have a protein content above 50% (Bukkens, 1997). The BSF measured here contained an average of 43.70-47.29% crude protein depending on the diet.

Insects are already used in novelty and animal foods, but this is just the beginning of acceptance of entomophagy. Moving forward, insects will only become mainstream food if more studies like this one sway consumer opinions by contributing to efficiency and practicality of mass-rearing efforts. This work serves as a part of that process by identifying options for improving insect diets for more viable protein production.

Acknowledgements

This work was done in partial fulfilment of a MSc degree for Amanda C. Tinder. Thanks to Dr William Rooney and Mr Stephen Labar for the donated sorghum, to Dr Tryon Wickersham and Ms Jessica Baber for sharing their time and equipment, Mr Le Zheng for assistance with statistical analyses, and to current and former researchers and students in both the FLIES Facility and Urban and Structural Entomology Lab.

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