BIOCONVERSION OF SORGHUM AND COWPEA BY BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE FOR ALTERNATE PROTEIN PRODUCTION

A Thesis

by

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ABSTRACT

Black soldier flies, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), typically feed on decaying organic matter but have been explored as a possible means of alternate protein production, either for food for humans directly or as feed for animals that are raised for human food. If cultivation of these animals is to become as widespread and efficient as traditional livestock, processes for production of these and other insects must be refined. In this study, black soldier fly larvae were fed six different diets including the Gainesville diet (control), and five different mixtures of sorghum and cowpea. Effects on life history traits of the black soldier fly and nutritional content of prepupae were observed. Black soldier flies were able to successfully complete larval development on all tested diets. There were subtle but discernable 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, developed slower (3-9 days longer from hatching to prepupal stage) than those on the cowpea diets, which were higher in protein. Diet treatment did not consistently influence size (weight or length) of prepupae. Diets did not influence lipid content in prepupae. Higher protein diets translated to higher protein content of prepupae in both trials. Lower protein diets resulted in higher gross energy content of prepupae in both trials. However, in addition to protein, lipid, vitamins, and minerals also differed between diet treatments. This study provides further evidence of the viability of black soldier flies for protein production.

DEDICATION

This thesis is dedicated to Grandpa Leo. I never met him, but my mom is pretty sure he sent me to scare her with creepy crawly things as a punishment from beyond the grave.

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I would like to thank my Dad, who showed me how to appreciate agriculture, nature, and science. And my Mom, who taught me to be strong and learn from every situation, even (especially) the ones that do not go as expected. I couldn't ask for better parents, thank you for everything. I love you both.

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NOMENCLATURE

BSF	Black Soldier Fly
G	Gainesville Housefly Diet
100s	100% Sorghum Diet
75s25c	75% Sorghum, 25% Cowpea Diet
50/50	50% Sorghum, 50% Cowpea Diet
25s75c	25% Sorghum, 75% Cowpea Diet
100c	100% Cowpea Diet

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Global Food Security

With a growing global human population, estimated at over 7 billion in 2011, and projected to increase to between 8.1 and 10.6 billion by 2050, many concerns have arisen for providing the necessary food resources to support this projected global human population (Ezeh et al. 2012). In order to ensure that enough food is produced to feed everyone, food production would need to increase 70-100% by 2050 (Godfray et al. 2010). In the past, this type of shortage would be remedied by using more land for agriculture, but only a finite area of arable land exists that can be used for this purpose (Godfray et al. 2010). This limitation, particularly in conjunction with other human uses like urbanization, makes expansion of agriculturally cultivated areas an unlikely solution (Godfray et al. 2010).

Unfortunately, land is not the only limiting factor in food production; humanity's water footprint can also limit agricultural productivity. Agriculture demands account for 92% of global water usage, 29% of which is used for growing animal feed (Gerbens-Leenes et al. 2011). Two major factors contribute to this large water footprint: feed conversion efficiency and composition of feed, both of which vary depending on the type of livestock (Gerbens-Leenes et al. 2011). Livestock production also contributes significantly to greenhouse gas (GHG) production, various sources estimating it from

18% to 51% of global GHG emissions (Goodland and Anhang 2009). Godfray et al. (2010) call for a new revolution as massive as the industrial revolution and the agricultural revolution of the 18th and 19th centuries as the only way to produce enough food to feed our growing population with our limited resources. Such a revolution would involve drastic increases in efficiency of existing agricultural and food storage processes through the use of genetically modified crops, expansion of aquaculture, and educational tactics for waste reduction and diet change (Godfray et al. 2010). These concerns have sparked a search for alternate sources of food, not only for direct human consumption, but also as a means to support livestock, poultry, and aquaculture (van Huis 2013).

Concern over the food supply has already manifested itself in developing countries. Globally, meat consumption per capita per year is expected to increase by approximately 10 kg between the years 2000 and 2030, an increase of approximately 25% over the course of 30 years (Msangi and Rosegrant 2011). People in developing regions, such as the Middle East and North Africa, who consumed approximately 20 kg of meat per capita per year in 2000, are expected to almost double that level by 2030. This increase means that cereal grains, which offer an important source of nutrients to people, may well be fed to livestock instead. Thus, Msangi and Rosegrant (2011) urged people to consume less meat and more plant protein in an effort to optimize human health within the bounds of the environment and economy. Taylor (2012) also urged people to consume less meat because of the significant impact livestock animals have on the environment in arenas such as limited resource use and greenhouse gas emissions. Such a shift toward less meat consumption could also lead to better food security by

"releasing" grain for food use; however, this approach would cause little impact on prices of cereals, the main staple crops in most developing countries (Msangi and Rosegrant 2011).

In Africa, protein malnutrition is a serious problem (Anyango et al. 2011). Participants in a survey conducted in Niger consider sorghum (*Sorghum bicolor* L. Moench) or millet (*Pennisetum glaucum*) porridge to be a staple food (Towns et al. 2013). Anyango et al. (2011) advocate 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 about 23.5% protein where sorghum averages only about 8.4-11% protein. Additionally, black-eyed peas are known to be 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).

Influenced by the above-described concerns regarding food security, many alternative sources of food and feed are being explored. For example, algae have been examined as a possible feed for fish as a partial substitute for fishmeal (Patterson and Gatlin III 2013). Insects are also viable sources of protein that could potentially be cultured for protein production, just as insects have been farmed for at least 7,000 years to produce human-valued goods such as silk, shellac, and honey (Rumpold and Schlüter 2013).

Food Waste

Food waste represents a significant source of food production inefficiency. Cuéllar and Webber (2010) cite a 1995 study suggesting that 27% of all edible food is wasted, but point out several discrepancies that might drive that percentage even higher. In fact, Parfitt et al. (2010) claim that the most common estimate is that as much as half of all food grown is lost or wasted, but this claim is tempered with clearer definitions of what constitutes food waste in three parts. First, it is defined as waste from simply discarded edible material. Second, it is defined adding waste which is intentionally fed to animals or is lost through processing. Finally, it is defined to include the food that makes up the gap between energy consumed and energy needed per capita (Parfitt et al. 2010). A more recent study by Martínez et al. (2014), estimates that 35% of all food is wasted. Furthermore, Levis et al. (2010) estimate that over 97% of wasted food in the United States is buried in landfills. While Levis et al. (2010) suggest a composting approach, this food waste could also be used as feed, providing a source of alternate protein production that does not remove food upstream of humans in the food chain, but rather recovers nutrients through conversion of waste by certain decomposer insects (Diener et al. 2009). Other studies suggest using food waste directly for fish food (Cheng et al. 2014), or using food waste to feed algae (Lau et al. 2014).

Insects for Alternate Protein Production

Insects could serve as a viable option for protein production. van Huis (2013) gives a broad account of the benefits of insects as food and feed. Such an approach for alternate protein production could reduce the production of organic waste, produce feed ingredients for animal feed, and reduce dependency on international fisheries for fishmeal to supply the aquaculture industry (van Huis 2013).

Several insect species produce as little as half as much CO_2 emissions as beef cattle in g/kg mass gain (Oonincx et al. 2010). Measured by comparing the edible weight to dietary intake, crickets *Acheta domesticus* (Orthoptera: Gryllidae) are twice as efficient as chickens, four times as efficient as pigs, and twelve times as efficient as cattle at converting feed to meat (van Huis 2013). Insects do not carry livestock pathogens among themselves like traditional livestock can, such as swine flu or bovine spongiform encephalopathy (which could spread to humans by proximity to, or by eating traditional livestock), because insects are very taxonomically distant from humans and other mammals (van Huis 2013). Some studies have even indicated that certain insects such as black soldier flies and house flies demonstrate innate antibacterial qualities (Choi et al. 2012). Insects are also hypothesized to use less water than traditional livestock because many edible insects are drought tolerant, although studies confirming this have not yet been completed (van Huis 2013).

Certain insects can reduce dry mass of organic waste by up to 58% (van Huis 2013). Diener et al. (2009) demonstrate a capacity of certain insects to reduce chicken

feed up to 43.2% at an optimal feeding rate of 50 mg per day per larvae. This ability makes insects ideal for use in recycling waste food into a food or feed source, and allows the production of a nutritious soil amendment with increased ammonia content compared to that of the waste food alone (Diener et al. 2009, Green and Popa 2012). This process, combined with the antibacterial properties of certain insects, could revolutionize composting culture by further reducing the possibility of pathogenic microbes present in waste reaching the consumer (Jones and Martin 2003, Erickson et al. 2004).

History of Insects as a Food Source

Insects have been used as a food across the world since prehistoric times (Ramos-Elorduy 2009, Van Itterbeeck and van Huis 2012). In Mexico, for instance, insect consumption is such an important cultural practice that overexploitation of several species of insects has caused a significant decrease in their populations (Ramos-Elorduy 2006). Sutton (1995) explains that archaeological evidence suggests insects have been used for human and veterinary medicine, ritual practice, food, oral tradition, and in sandpaintings. However, entomophagy became less common as countries industrialized. Rumpold and Schlüter (2013) suggest that, while little eating of insects occurs in Europe today, it is reported to have been common until about 30 years ago. Zhi-Yi (1997) explains that, while evidence of insects' use as a food source exists from as early as 1200 B.C. in China and 500 B.C. in Liberia, it has mostly been isolated and infrequent, and

has only become more popular relatively recently. According to Van Itterbeeck and van Huis (2012) there is evidence to suggest human consumption of insects as long ago as 9,500 years, and that entomophagy practices in modern cultures have been decreasing for a few hundred years.

Commonly Consumed Insects

There are many examples of insects that could be cultured for alternate protein production. These include, but are not limited to, termites, silkworms, grasshoppers, mealworms, house fly larvae, and black soldier flies (Rumpold and Schlüter 2013).

In fact, many of these insects are already cultured on a relatively large scale for use by hobbyists and zoos as feed for pets and other insectivorous animals (Finke 2002). Some examples of this include house crickets, superworms, giant mealworms, mealworms, waxworms, and silkworms (Finke 2002). However, use of this resource need not be limited to animal feed, but could contribute directly to human diets as well (Bukkens 1997).

Termites (Isoptera) are the second most frequently eaten insect in the world and have high protein content and higher fat content than many other insects (Bukkens 1997, Rumpold and Schlüter 2013). While termites have the potential to destroy wood, which could be beneficial in the case of waste wood, their capacity to destroy wooden structures could cause problems in cultivation as prevention of escape is a high priority (Becker 1969, Rumpold and Schlüter 2013).

Several Lepidoptera species are also widely eaten, such as the silkworm pupae *Bombyx mori* L. (Lepidoptera: Bombycidae), although they are cultured primarily for the production of silk (Rumpold and Schlüter 2013). Silkworms have also been shown to serve as a suitable replacement for fish meal in poultry diets (van Huis 2013).

Grasshoppers (Orthoptera: Acrididae) are another potential high-protein feed ingredient (Rumpold and Schlüter 2013). Several species of grasshopper have been explored as food sources. The Chinese grasshopper (*Acrida cinerea*), for example, was able to replace 15% of chicken diets with no noticeable adverse effects (van Huis 2013).

Larvae of the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) have potential as a feed resource and waste reduction agent for use as a supplement to chicken feed (Rumpold and Schlüter 2013). The yellow mealworm (*Tenebrio molitor*) (Coleoptera: Tenebrionidae) larvae has also been studied as an alternative protein source for chicken feed as well as African catfish (van Huis 2013). Mealworms have historically been cultured as feed for captive animals. This is because they, like many insects, are inexpensive sources of protein and other nutrients, and are widely available (Martin et al. 1976). Unfortunately, the mealworm can be an important pest of stored products (Fraenkel 1950). This is a problem because escaped individuals from a colony of mealworms could potentially lead to an outbreak such as the one described by Harding and Bissell (1958), which describes a heavy infestation of a chicken brooder house, including the corn cob litter floor covering, and dead or dying chicks.

Dried house fly *Musca domestica* L. (Diptera: Muscidae) larvae, pupae, and adults grown on chicken manure could replace soybean meal as a protein source for

chicken feed (Calvert 1979). However, house flies are more commonly associated with food contamination than considered as a food source themselves, and are thought to transmit bacteria and other disease factors (Ostrolenk and Welch 1942).

The black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae) also has waste reduction potential, but unlike the house fly, the black soldier fly (BSF) is considered to be at best a beneficial species because they colonize and reduce animal waste, reduce pathogenic bacteria, and inhibit house fly colonization of resources both species can utilize, and at worst not considered a pest species because they are not synanthromorphic (Furman et al. 1959, Erickson et al. 2004, Myers et al. 2008, Rumpold and Schlüter 2013, Zhou et al. 2013).

Black Soldier Fly, *Hermetia illucens*

The BSF can develop from egg to adult in approximately 40 to 43 d, eggs hatching in 4-6 d, spending at least 22 to 24 d as larvae, and 14 d or more as pupae depending on temperature (Tomberlin et al. 2009), humidity (Holmes et al. 2012), bacterial community present (Yu et al. 2011), pupation substrate (Holmes et al. 2013), and location of origin (Tomberlin et al. 2009, Yu et al. 2011, Holmes et al. 2012, 2013, Zhou et al. 2013). Bacteria, light intensity, temperature, and humidity have also been shown to influence oviposition preference and clutch size (Tomberlin and Sheppard 2002, Zheng et al. 2013). The BSF has also been shown to be useful in the process of converting manure to biofuel (Li et al. 2011), reduce *Escherichia coli* in dairy manure

(Liu et al. 2008), and have an antibacterial effect against gram-negative bacteria (Choi et al. 2012). Not only has the BSF been shown to reduce dangerous bacteria associated with various types of manure, it also provides a hygienic method for disposal of human waste (Banks et al. 2014). House fly oviposition is inhibited by the presence of BSF larvae (Bradley and Sheppard 1984, Tomberlin and Sheppard 2002). This is important because, unlike the house fly, the BSF reduces pathogenic bacteria in substrates (Erickson et al. 2004). BSF larvae primarily consume decaying plant matter, but are also known to consume decaying animal flesh such as outdoor carrion (Tomberlin et al. 2005). BSF, and other insects that are capable of utilizing waste food, open the door to utilizing food supply chain waste as a renewable resource that would otherwise go to waste (Pfaltzgraff et al. 2013). While BSF adults do not require food, they do have somewhat complex mating behavior, requiring sunlight and space to fly (Tomberlin and Sheppard 2001, Zhang et al. 2010). BSF prepupae can be self-harvested under the correct conditions with little extra effort (Tomberlin and Sheppard 2001). A successful self-harvesting system was built by Sheppard et al. (1994) using strategically placed openings and 40 degree inclined ramps to allow dispersing BSF prepupae to exit the area underneath layer hen cages where manure accumulated and was eaten by BSF larvae. According to Booth and Sheppard (1984), using strips of corrugated cardboard and oviposition substrate, a single worker could conceivably collect one million eggs per day, making the BSF a very efficient system for potential waste reduction and alternative food production.

The BSF has been explored as a possible partial substitute for fishmeal in aquaculture (Kroeckel et al. 2012). It has nutritional value similar to that of soybean, meat, and bone meal in that all have high protein content (Zhang et al. 2010). Barroso et al. (2014) indicate that insects in general contain adequate nutritional components for inclusion in fish diets. In fact, Bell et al. (1994) analyzed the nutritional content of invertebrates (mainly insects) that are consumed by Atlantic salmon (*Salmo salar*) (L), finding that the insects studied were better sources of required fatty acids than commercially produced fish food. Other insects, such as silkworm pupae, have also been explored as part of carp (Cyprinidae) diet, and light-trapped insects used to supplement diets for bluegill sunfish *Leopomis macrochirus* (Rafinesque) production (Bondari and Sheppard 1981). Kroeckel et al. (2012) found that BSF can be a partial substitute as a protein source for aquaculture fish. BSF meal can replace approximately 33% of the fish meal and fish oil diet of turbot, *Psetta maxima*, (L.) with minimal adverse effect (Kroeckel et al. 2012). Another trial showed that BSF prepupae could replace 25% of the fish meal and 38% of the fish oil in the diet of the rainbow trout, *Oncorhynchus mykiss* (Walbaum) (St-Hilaire et al. 2007a). Similar studies have shown that BSF can also partially substitute for fish meal and fish oil in blue tilapia, *Oreochromis aureus* (Steindachner), and channel catfish, *Ictalurus puncatus* (Rafinesque) without significant growth or flavor differences (Bondari and Sheppard 1987). In addition, BSF can be used to recycle fish offal (organ meat), which is a waste product generated from the aquaculture industry and currently has limited uses. Some studies have shown that such recycling of fish offal can enrich BSF, increasing content of long chain-polyunsaturated

fatty acids, which fish need in their diets (Sealey et al. 2011). The prepupal stage of BSF can be used as a partial replacement of the fish meal and fish oil in the diets of carnivorous fish which cannot be fed plant-based protein (St-Hilaire et al. 2007a). The amino acid content of BSF as compared to house flies is known: a total of 38.85% in BSF and 47.06% in house flies (St-Hilaire et al. 2007b).

In developing where protein malnutrition is a serious struggle, efficient alternate protein production methods are a high priority (Anyango et al. 2011). BSF are ideal for this application because no specialized 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 a variety of wastes (Nguyen et al. 2015).

The goal of my research on the BSF is to determine if this insect could be improved for use as a source of alternate protein for use as food for humans or feed for aquaculture, livestock, or poultry. The global distribution of the BSF and ease with which it can be mass-produced suggests that the BSF could be ideal for use as an alternate protein source.

Objectives and Hypothesis

1.) To examine the ability of the BSF to develop on different ratios of cooked sorghum and black-eye peas.

H_0: The ratio of cooked sorghum and black-eye peas will not impact the life-history traits (larval daily weight, development time to the prepupal stage, prepupal weight, and survivorship) of BSF.

H_1: The ratio of cooked sorghum and black-eye peas will impact the life history traits of the BSF.

Rationale: Black-eye peas have more protein (23.5%) per gram than does sorghum (8.4-11%) (Anyango et al. 2011). I expect this difference in nutritional quality of diet to play an important role in healthy insect growth, in which case there will be a change in some or all of the measured life history traits across diets. I also expect that larvae on mixed diets rather than diets consisting of only black-eye pea or sorghum respectively will perform the best due to diversity of available resources. This is because generalists typically regulate their food intake for the nutrients they need to develop properly (Nersesian et al. 2012).

2.) To determine the protein and lipid ratios in BSF larvae fed different ratios of cooked sorghum and black-eye peas.

H_0: The protein and lipid ratios in BSF larvae provided different ratios of cooked sorghum and black-eye peas will not vary.

H_1: The protein and lipid ratios in BSF larvae provided different ratios of cooked sorghum and black-eye peas will vary.

Rationale: Black-eye peas have more protein (23.5%) per gram than does sorghum (8.4-11%) (Anyango et al. 2011). I expect this difference in nutritional quality of feed to cause an observable difference in development of the BSF, causing a change in nutritional value of the BSF as a protein source.

CHAPTER II

RESEARCH, RESULTS, AND DISCUSSION

Introduction

The human population is expected to reach between 8.1 and 10.6 billion by 2050 (Ezeh et al. 2012). This expansion in population would normally require a proportionate expansion in land used for agriculture and food production, but there is only very limited land remaining that could be used (Godfray et al. 2010). Agricultural demands for water account for 92% of global water usage (Gerbens-Leenes et al. 2011). Concern over the food supply has already manifested itself in developing areas, such as certain parts of Africa, where protein malnutrition is already an issue (Anyango et al. 2011). In these areas, alternate sources of protein are desperately needed.

Insects could serve as a viable option for protein production. van Huis (2013) gives a broad account of the benefits of insects as food and feed for the livestock, poultry and aquaculture industries. Such an approach for alternate protein production could reduce the production of organic waste, produce feed ingredients for animal feed, and reduce dependency on international fisheries for fishmeal to supply the aquaculture industry (van Huis 2013). Black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) larvae primarily consume decaying plant matter, but are also known to consume decaying animal flesh, making them ideal for utilizing otherwise wasted organic material (Zhou et al. 2013). Once this organic waste has been converted into

high protein animal material, it can be used as feed for livestock, poultry, and fish (van Huis 2013).

There are many examples of insects that could be cultured for alternate protein production (Rumpold and Schlüter 2013). Termites (Isoptera) are the second most eaten insect in the world (by humans) and have high protein and fat content. However, termites degrade wood, which could be beneficial in the case of waste wood, but could be negative due to their being an urban pest. Several Lepidoptera species are also widely eaten, such as the silkworm pupae *Bombyx mori* L. (Lepidoptera: Bombycidae) (Rumpold and Schlüter 2013). Grasshoppers (Orthoptera: Acrididae) are another example of an arthropod that could be used to produce a high-protein feed ingredient. Larvae of the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) have potential as a feed resource and waste reduction agent for use as a supplement to chicken feed. House fly *Musca domestica* L., (Diptera: Muscidae) pupae and dried adults grown on chicken manure could replace soybean meal as a protein source for chicken feed (Rumpold and Schlüter 2013). The BSF also has waste reduction potential, but unlike the house fly, the BSF is a beneficial species reduce *Escherichia coli* in dairy manure (Liu et al. 2008), as well as other Gram-negative bacteria (Choi et al. 2012). Furthermore, unlike the house fly, BSF adults are not synanthropic (attracted to people and their dwellings) and can suppress house fly production in wastes (Bradley and Sheppard 1984).

The BSF has been explored as a possible partial substitute for fishmeal in aquaculture (Kroeckel et al. 2012). Kroeckel et al. (2012) found that BSF cannot be a

complete substitute for fishmeal, but it can be a partial substitute as a protein source for aquaculture. The pre-pupal stage of BSF can be used as a partial replacement of the fish meal (about 25%) and fish oil (about 38%) in the diets of carnivorous fish which cannot be fed plant-based protein (St-Hilaire et al. 2007a). BSF meal can replace approximately 33% of the fish meal and fish oil diet of turbot, *Psetta maxima*, with minimal adverse effect (Kroeckel et al. 2012). Such protein produced from BSF could potentially be used for human consumption.

In third world nations where protein malnutrition is a serious struggle, efficient alternate protein production methods are a high priority (Anyango et al. 2011). BSF is ideal for this application because no specialized 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 human food waste (Nguyen et al. 2013).

BSF larval diet has been shown to influence their life history traits (Tomberlin et al. 2002, Nguyen et al. 2013). Laboratory colonies of BSF are typically maintained on the Gainesville Housefly 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 successful for maintaining laboratory colonies, but wild BSF are significantly larger than laboratory-reared BSF, as much as double the size (Tomberlin et al. 2002). This suggests that this diet may not meet all of the BSF's needs (Tomberlin et al. 2002). This means that optimization of the rearing process could potentially double the protein production by these insects. The first objective of this research was to determine if the ratio of cooked sorghum and black-eye peas will impact

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 lipid ratios in BSF larvae fed different ratios of cooked sorghum and black-eye peas.

Materials and Methods

Acquisition of Flies. This experiment was performed with BSF larvae resulting from a colony maintained at the Forensic Laboratory for Investigative Entomological Sciences, or F.L.I.E.S. Facility at Texas A&M University. The BSF eggs were collected from flutes of corrugated cardboard as described in Sheppard et al. (2002). Eggs were placed in the Gainesville diet, a standard laboratory diet of 50% wheat bran, 30% alfalfa meal, and 20% corn meal (Hogsette 1992), for a period of four days in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D.

Feed Preparation. Dried cowpea, or black-eyed peas (HEB® brand) were purchased from HEB Grocery Company (San Antonio, TX). Mixed dried sorghum was obtained from the sorghum breeding and genetics program at Texas A&M University. Cowpea was prepared using methods described on the package, and sorghum was prepared similarly. Essentially, dried sorghum and cowpea were soaked separately from each other in a 1.9 L stainless steel bowls for 5 hr in a one to four ratio of product to water then drained. Both were then boiled approximately 2 hr. Cowpeas were mashed with a potato masher, while the smaller sorghum grains were mashed using a blender as

the grains were too small to be caught by a potato masher. Both sorghum and cowpea were mashed until no whole grains or beans (respectively) remained.

The prepared sorghum and cowpeas were fed to BSF in the following treatments: 1) 100% sorghum, 0% cowpeas; 2) 75% sorghum, 25% cowpeas; 3) 50% sorghum, 50% cowpeas; 4) 25% sorghum, 75% cowpeas; 5) 0% sorghum, 100% cowpeas. A sixth diet (Gainesville Housefly Diet) served as a control as it has been used in previous studies (Sheppard et al. 2002, Tomberlin et al. 2002). The treatments were prepared and stored in a refrigerator at 3.33° Celsius in four separate batches during the course of the experiment. Prior to preparing the treatments, the moisture content of the cooked sorghum and cowpea was determined gravimetrically by weighting out 5g of each, three times each, and placing them in a drying oven in intervals of about 4 hours until the weight stopped decreasing. The final weight (dry weight) was then compared to the initial weight to determine moisture content. The moisture content of the cooked sorghum and cowpea mixtures was approximately 70%, consistent with that of the Gainesville 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 replicate, 300 4-d-old (Tomberlin et al. 2002, Myers et al. 2008) larvae were placed in uncovered 18 oz Hefty brand disposable cups (Reynolds Consumer Products LLC, IL, USA), one for each of four replications of each of the five treatments and the control diet. Larvae of this age are typically larger, more visible, and more likely to survive handling than younger larvae (Sheppard et al. 2002). Replicates were placed in a walk-in incubator set to the

same conditions as described above. Larvae in each replicate were fed 10 g (at approximately 70% moisture) of their respective diet once previously fed material was either digested or dry. Each replicate (whole cup with remaining larvae and substrate) was weighed daily using an OHaus Scout™ Pro balance (OHaus Corporation, NJ, USA) prior to being fed. To prevent escape of preupae and subsequent adults, the cups were covered with tulle fabric held in place with a rubber band after the first prepupae were observed. During 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.

Life-History Traits. Daily larval weight, development time to the prepupal stage, prepupal weight, and survivorship were determined and recorded. Every second day beginning on day 10, for three of the replicates of each treatment, three larvae were selected, killed with boiling water, measured in millimeters with a ruler, and weighed using an OHaus Adventurer™ Pro balance (OHaus Corporation, NJ, USA). These three replicates were also examined daily for the presence of prepupae which can be identified by their much darker color than individuals still in the larval stage (Sheppard et al. 2002). Larvae were fed until 40% of the original larvae had reached the prepupal stage or less than ten larvae remained, whichever occurred first (Tomberlin et al. 2002). Each container continued to be examined daily for prepupae by sorting through the material until all individuals had become pre-pupae or died. All prepupae were removed and individual weight recorded. The first prepupa collected, and one for every additional 10

prepupae from each container, was placed in a 59mL condiment cup (Jarden Home Brands, IN, USA) with a damp cotton ball (Fig. 1) in the same incubator described above to be monitored for adult emergence and longevity. Additional prepupae, collected daily from each replicate, were stored in a Ziploc (SC Johnson, WI, USA) bag, and placed in a 0°C freezer for subsequent nutritional analysis (see below). The fourth replicate was fed as the other replicates; however, it was not disturbed by examination for prepupae as daily handling could impact development and survivorship (Nguyen et al. 2013). In this replicate, adult emergence was monitored daily.

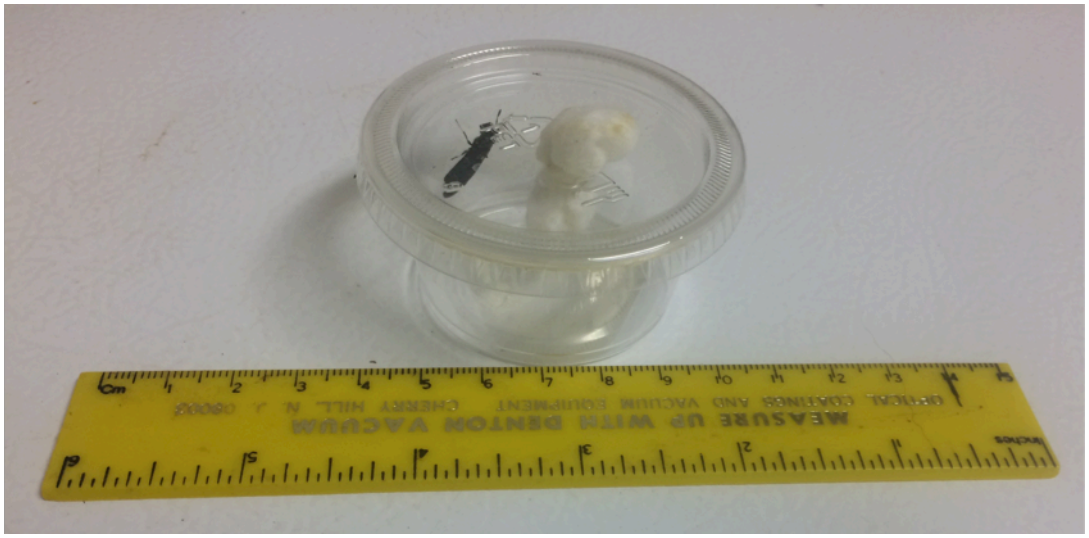

Fig. 1: Condiment cup used to monitor BSF adult emergence and longevity.

Nutritional Analysis. Measurement of protein content was conducted, using Dumas total combustion methods in an Elementar rapid N cube (Elementar

Analysensysteme, Germany) analyzer for total nitrogen content, which was subsequently converted to crude protein using the conversion factor of 6.25 (Etheridge et al. 1998, Chung Chun Lam et al. 2009). Samples of 250 mg dried, homogenized ground BSF prepupae were compressed into a tin foil (50x50mm squares, Elementar Analysensysteme, Germany) wrapped tablet with a mechanical tablet maker and introduced into the combustion chamber via the top carousel of the machine (Etheridge et al. 1998). The combustion process converts covalently bonded nitrogen into gaseous nitrogen, which can be detected when passed through the conductivity cell (Etheridge et al. 1998). Caloric content (gross energy) was measured, using a bomb calorimeter, and methods similar to those used by Doyle et al. (2007). One gram of dried, homogenized ground BSF prepupae was placed in a non-combustible sample holder, placed on the bomb head, and inserted into the bomb chamber, which then processed the sample and determined the gross heat of combustion (calories/gram).

Lipid measurement was conducted gravimetrically using a series of three chloroform washes to remove the lipid from dried BSF prepupae following the methods of Loveridge (1973). Dried prepupae were weighed using a Mettler Toledo Excellence Plus balance (Mettler-Toledo, OH, USA). They were then soaked in chloroform for 24 h, this chloroform was removed and replaced with new chloroform, soaked another 24 h, removed and replaced, soaked another 24 h, then removed and left to air dry for 24 h more. As described by Loveridge (1973), the first two changes of chloroform appeared yellowish, while the last remained colorless, indicating complete lipid extraction. When these prepupae proved not to be entirely dry (weight had not decreased below original

weight, and continued to decrease in weight after additional time had passed), they were returned to the drying oven until they were satisfactorily dried, meaning that the weight no longer decreased after additional time in the drying oven. Once prepupae were completely dry again, they were weighed again using the same balance. This final weight was compared to the original dry weight to determine lipid content. In an effort to interpret measured protein and gross energy data that cannot correspond with measured lipid data, values for estimated carbohydrate and lipid percentages were calculated using measured protein and gross energy values and known gross energy of each macronutrient, with carbohydrates typically at 4Kcal/g, protein at 4Kcal/g, and lipids at 9Kcal/g (Maynard et al. 1979).

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, Cary, NC, USA) was used to test for treatment differences across time in the case of the larval growth measurements. Analysis of variance (ANOVA) in SAS (SAS 9.4 for Windows, Cary, NC, USA) was used to test for treatment differences in each life history trait (other than larval growth over time) and nutritional value measurement. A least significant difference (LSD) test was then used to determine significance ($P < 0.05$) between means.

Results

Diet had a significant effect ($P < 0.0001$) on larval weight and length over time with G diet and the higher percentages of sorghum generally taking longer to reach the same sizes as other diets (Table 1, Figs. 2 and 3). However, trial also had a significant effect ($P < 0.0001$) on both growth rates, with the second trial (B) taking slightly longer to reach the same sizes as the first trial (A). Diet had a significant effect on prepupal weight ($P < 0.0001$) and length ($P = 0.0003$) with individuals fed diets 75s25c, 50/50, and 25s75c consistently larger than the other diets in trial A, but diet did not have a significant effect on either prepupal weight ($P = 0.7347$) or length ($P = 0.0703$) in trial B (Table 2, Figs. 4 and 5). Again, trial had a significant effect ($P < 0.0001$) on both measurements, with individuals from the second trial (B) tending to be smaller than those from the first trial (A). Diet did not have a significant effect ($P = 0.6712$, 0.3673 in trials A and B respectively) on the total number of individuals reaching pupation from the original three hundred in either trial (Table 2, Fig. 6). Diet had a significant effect on time to pupation ($P = 0.0297$), time to emergence ($P = 0.0085$), and total survival time ($P = 0.0047$) in trial one, and time to pupation ($P = 0.0205$), time to emergence ($P = 0.0275$), and total survival time ($P = 0.0127$) in trial two (Table 3, Figs. 7-9). In all three measurements, time to pupation, time to emergence, and total survival time, the G diet and the higher percentages of cowpea had shorter times, while higher percentages of sorghum had longer times. Trial also had a significant effect on all three measurements, time to pupation ($P = 0.0397$), time to emergence ($P = 0.0013$), and total survival time

(P = 0.0322), with the second trial (B) in general having slightly longer times than the first trial (A).

Diet did not have a significant (P = 0.8046, 0.0789) effect on lipid content in either trial (Table 4, Fig. 10). However, trial had a significant effect (P = 0.0003) on lipid content, with those from the second trial (B) generally lower in lipid content than the first trial (A). In contrast, diet had a significant effect on protein content (P = 0.0002) and gross energy in calories (P < 0.0001), but trial did not have a significant effect (P = 0.7571 in protein and P < 0.1469 in gross energy) on either (Table 4, Figs. 11 and 12). Protein content was lowest in the G diet, then the 100s diet, then increasing as percent cowpea increased in diet. Gross energy was lowest in G diet, then 100c diet, then increasing as percent sorghum increased in diet.

In an effort to interpret measured protein and gross energy data that cannot correspond with measured lipid data, values for estimated carbohydrate and lipid percentages were calculated using measured protein and gross energy values and known gross energy of each macronutrient (Table 4, Figs. 13 and 14).These estimates show the G diet highest in carbohydrates and lowest in lipids, with carbohydrates increasing and lipids decreasing from 100s to 100c as amount of cowpea in diet increases.

Table 1. Table of statistical values for black soldier fly larval growth rates when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D.

Variable	R^2	DF	*F* value	Prob. > *F*
Larval Growth Weight A	0.797423	108	25.22	<0.05
Larval Growth Weight B	0.618408	101	10.72	<0.05
Larval Growth Length A	0.859556	108	39.22	<0.05
Larval Growth Length B	0.584865	101	9.32	<0.05

Diets were 100% Cowpea, 100% Sorghum, 25% sorghum and 75% cowpea, 50% cowpea and 50% sorghum, 75% sorghum and 25% cowpea, and the Gainesville diet (control).

Table 2. Mean values for black soldier fly prepupal life history data when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D.

Trial A (n = 3)[a]					
Diet[b]	Mean Prepupal Weight (mg) ± SE	Mean Prepupal Length (mm) ± SE	Mean Total Number Prepupae ± SE	Mean Total Feed Provided (g) ± SE	Total Weight of Prepupae per gram fed (g/g) ± SE
G	106.29 ± 2.01A	16.35 ± 0.19A	109.67 ± 57.51	220.00 ± 15.28	0.050 ± 0.024
100s	144.96 ± 8.39B	17.44 ± 0.30AB	123.67 ± 28.71	296.66 ± 3.33	0.061 ± 0.016
75s25c	151.80 ± 2.97B	17.43 ± 0.11BC	106.67 ± 34.67	226.66 ± 14.53	0.069 ± 0.017
50/50	156.67 ± 7.14B	17.86 ± 0,24BC	111.00 ± 23.44	230.00 ± 10.00	0.075 ± 0.015
25s75c	154.34 ± 7.15B	17.96 ± 0.21C	104.00 ± 15.50	230.00 ± 15.28	0.069 ± 0.005
100c	137.91 ± 5.38B	16.72 ± 0.21A	115.00 ± 27.57	213.33 ± 3.33	0.073 ± 0.015
Trial B (n = 3)					
G	100.15 ± 4.37	16.47 ± 0.16	26.33 ± 6.11	93.33 ± 3.33	0.028 ± 0.006
100s	95.29 ± 3.59	15.59 ± 0.07	83.33 ± 21.93	140.00 ± 5.77	0.057 ± 0.015
75s25c	91.55 ± 6.79	15.18 ± 0.21	79.67 ± 19.06	130.00 ± 5.77	0.056 ± 0.015
50/50	100.96 ± 1.36	15.40 ± 0.06	59.00 ± 15.10	120.00 ± 11.55	0.048 ± 0.010
25s75c	92.10 ± 11.07	15.38 ± 0.60	63.67 ± 28.99	120.00 ± 20.82	0.044 ± 0.017
100c	100.95 ± 3.15	15.39 ± 0.13	83.00 ± 21.36	130.00 ± 5.77	0.063 ± 0.014

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

[a]n = replicates

[b]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).

Table 3. Mean values of black soldier fly adult life history when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D.

Trial A (n = 3)[a]			
Diet[b]	Time to Pupation (d) ± SD	Time to Emergence (d) ± SD	Total Survival Time (d) ± SD
G	30.52 ± 2.83AB	51.08 ± 2.67A	65.20 ± 2.12A
100s	37.83 ± 0.92A	60.67 ± 0.95B	71.05 ± 1.49B
75s25c	30.72 ± 2.83AB	52.97 ± 2.55AB	67.72 ± 1.96AB
50/50	28.62 ± 1.41B	50.70 ± 1.22A	65.56 ± 0.96AB
25s75c	28.12 ± 0.08B	50.29 ± 0.08A	62.83 ± 1.05A
100c	29.26 ± 0.97AB	51.18 ± 1.30A	61.39 ± 0.17A
Trial B (n = 3)			
G	25.33 ± 1.20A	49.28 ± 1.88A	58.64 ± 3.39A
100s	39.11 ± 1.65B	62.81 ± 2.09B	74.74 ± 1.81B
75s25c	34.96 ± 1.42AB	60.50 ± 0.22AB	71.37 ± 0.80B
50/50	34.46 ± 1.81AB	59.00 ± 3.18AB	70.27 ± 2.90AB
25s75c	31.82 ± 4.40AB	56.29 ± 3.68AB	65.88 ± 3.73AB
100c	35.03 ± 1.41AB	55.65 ± 1.96AB	67.21 ± 1.15AB

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

[a] n = replicates

[b] 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).

Table 4. Mean values of nutritional data of black soldier fly prepupae when reared on different diets during 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.

Diet[a]	Mean Percent Protein (n = 6)[b] ± SD	Mean Gross Energy (Kcal/g) (n = 6) ± SD	Mean Percent Lipid Trial A (n = 3) ± SD	Mean Percent Lipid Trial B (n = 3) ± SD
G	43.70 ± 1.44A	5.22 ± 0.10A	19.03 ± 7.10	19.76 ± 1.61
100s	44.05 ± 1.38AB	6.20 ± 0.06B	19.82 ± 7.23	11.27 ± 3.09
75s25c	44.92 ± 0.87AB	6.21 ± 0.04B	22.21 ± 8.70	6.41 ± 5.14
50/50	45.40 ± 0.58ABC	6.06 ± 0.06C	22.91 ± 9.04	8.24 ± 2.06
25s75c	46.11 ± 0.79BC	5.95 ± 0.07CD	16.37 ± 1.04	11.33 ± 11.39
100c	47.29 ± 1.89C	5.89 ± 0.07D	16.53 ± 6.42	5.76 ± 2.71
Diet[a]	Dry Matter (g) Trial A ± SD	Dry Matter (g) Trial B ± SD	Estimated Percent Lipid[c]	Estimated Percent Carbohydrate[c]
G	49.42 ± 7.71	40.17 ± 5.55	24.40	31.90
100s	48.36 ± 7.74	40.73 ± 7.01	44.00	11.95
75s25c	62.03 ± 8.94	37.80 ± 4.80	44.40	10.68
50/50	66.92 ± 5.43	45.53 ± 3.71	41.20	13.41
25s75c	64.38 ± 9.20	37.67 ± 13.72	39.00	14.89
100c	56.28 ± 9.13	36.77 ± 2.44	36.20	16.51

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

[a]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).

[b]n = replicates

[c]Estimated percentages of lipid and carbohydrate were calculated from average measured values of percent protein and gross energy.

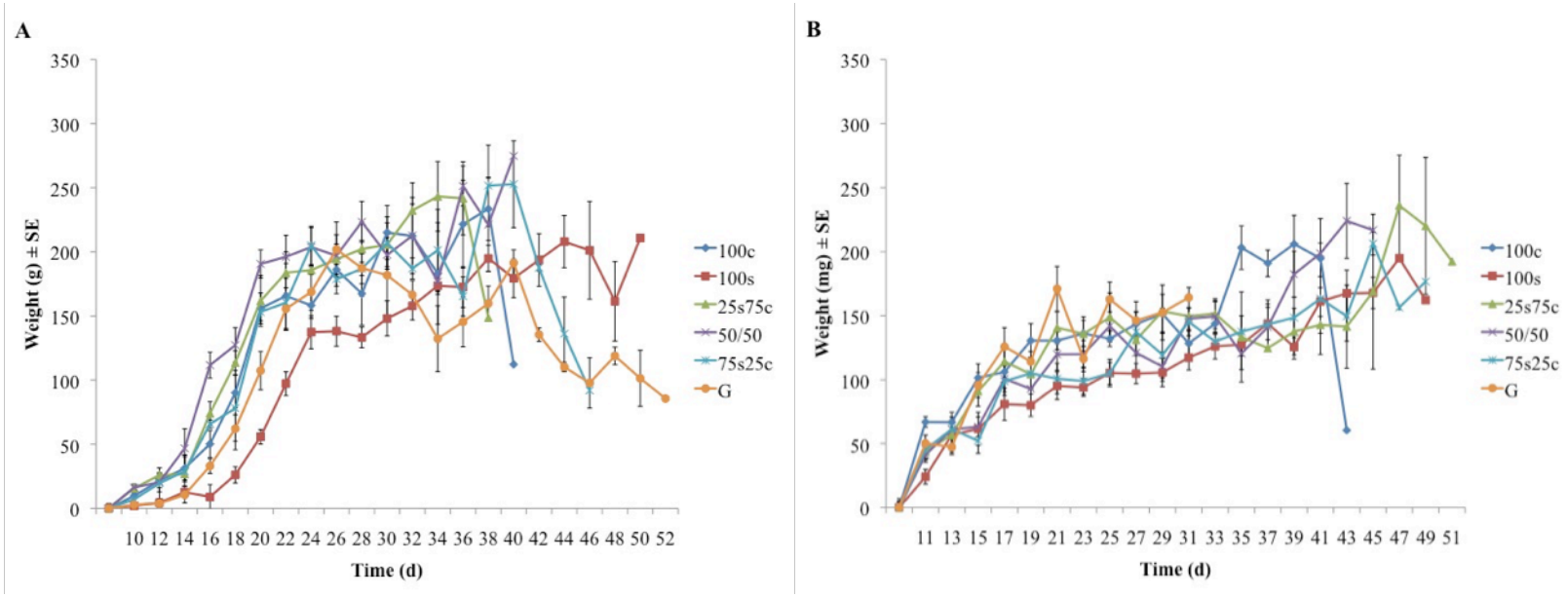

Fig. 2: Average weight (mg) ± SE of black soldier fly larvae (n = 3) over time (d) when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Diets were 100c = 100% Cowpea, 100s = 100% Sorghum, 25s75c = 25% sorghum and 75% cowpea, 50/50 = 50% cowpea and 50% sorghum, 75s25c = 75% sorghum and 25% cowpea, and G = Gainesville diet (control).

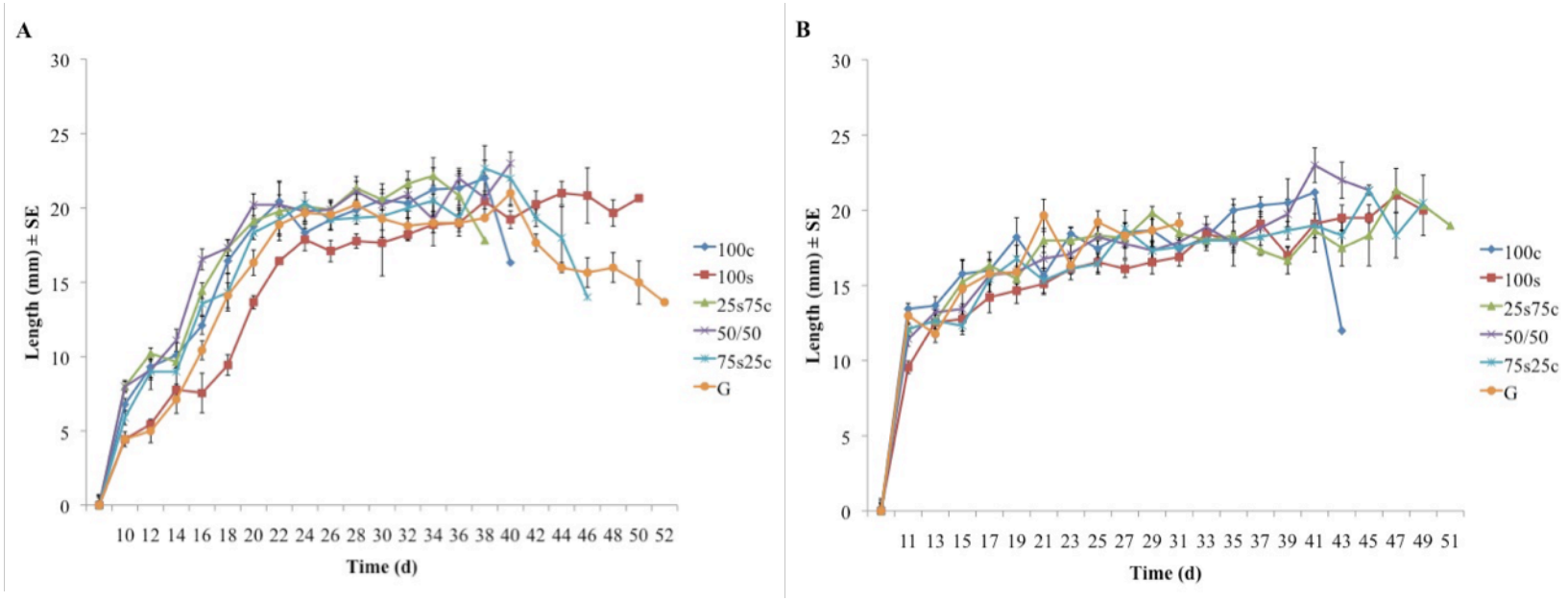

Fig. 3: Average length (mm) ± SE of black soldier fly larvae (n = 3) over time (d) when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Diets were 100c = 100% Cowpea, 100s = 100% Sorghum, 25s75c = 25% sorghum and 75% cowpea,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

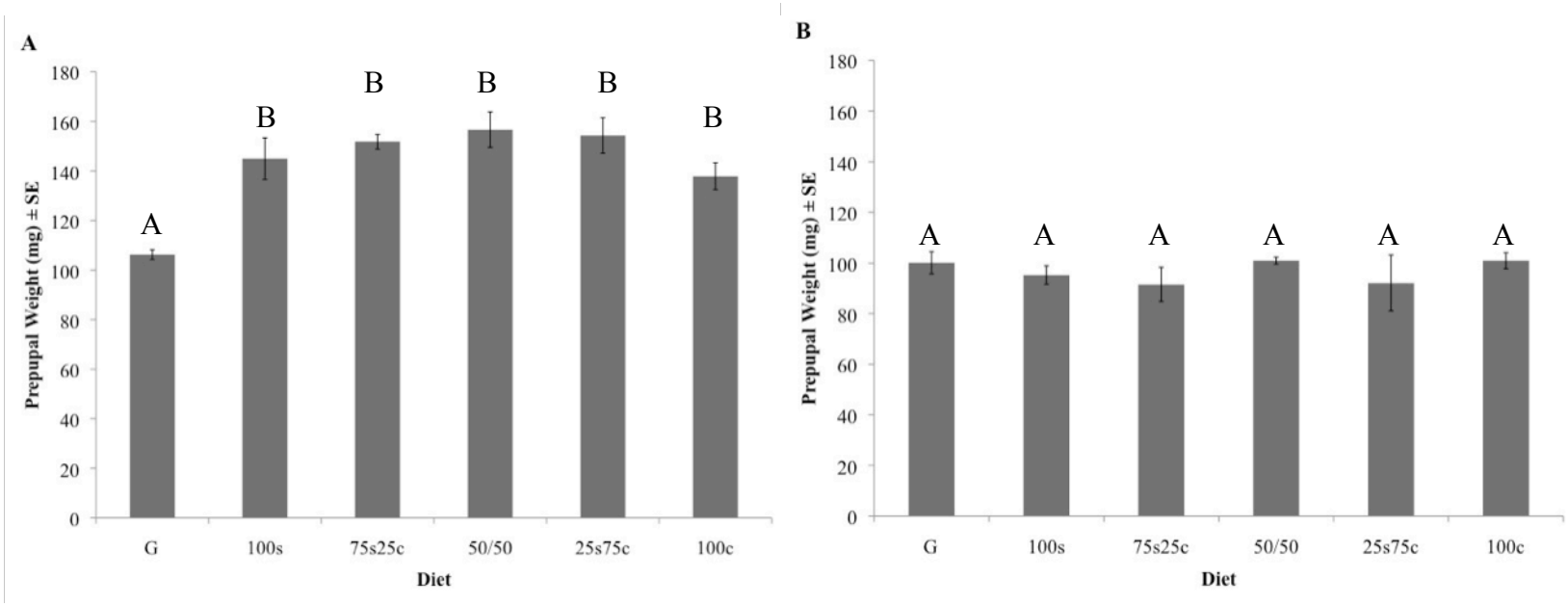

Fig. 4: Average weight (mg) of black soldier fly prepupae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). Diets were 100c = 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 diet (control).

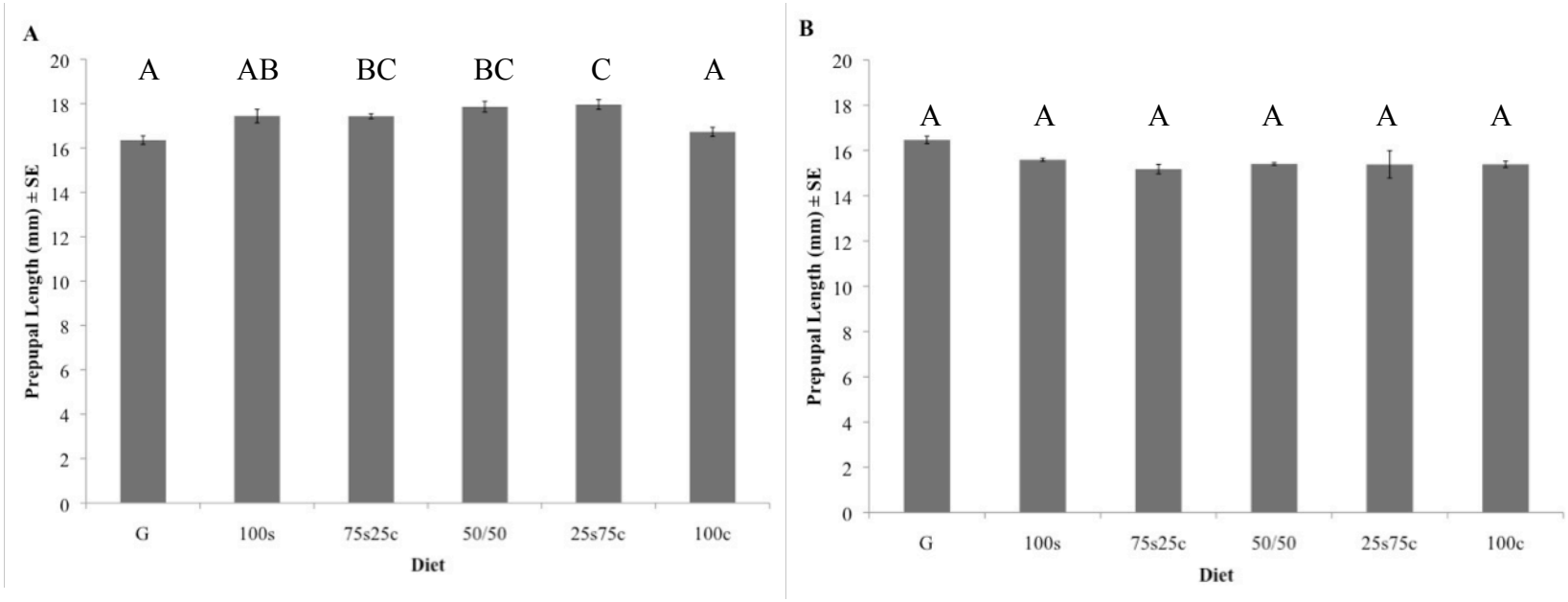

Fig. 5: Average length (mm) of black soldier fly prepupae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). Diets were 100c = 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 diet (control).

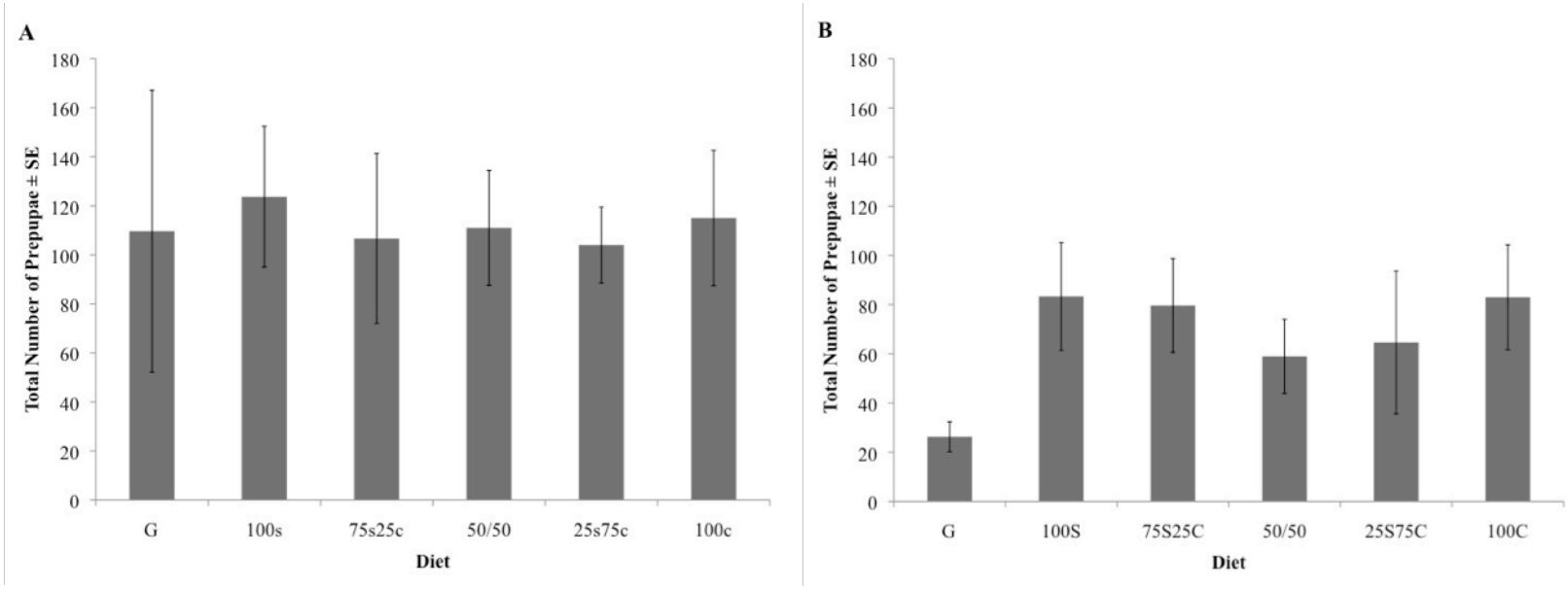

Fig. 6: Average number of individual black soldier flies surviving to pupation out of the original 300 larvae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Significant differences were not observed. Diets were 100c = 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 diet (control).

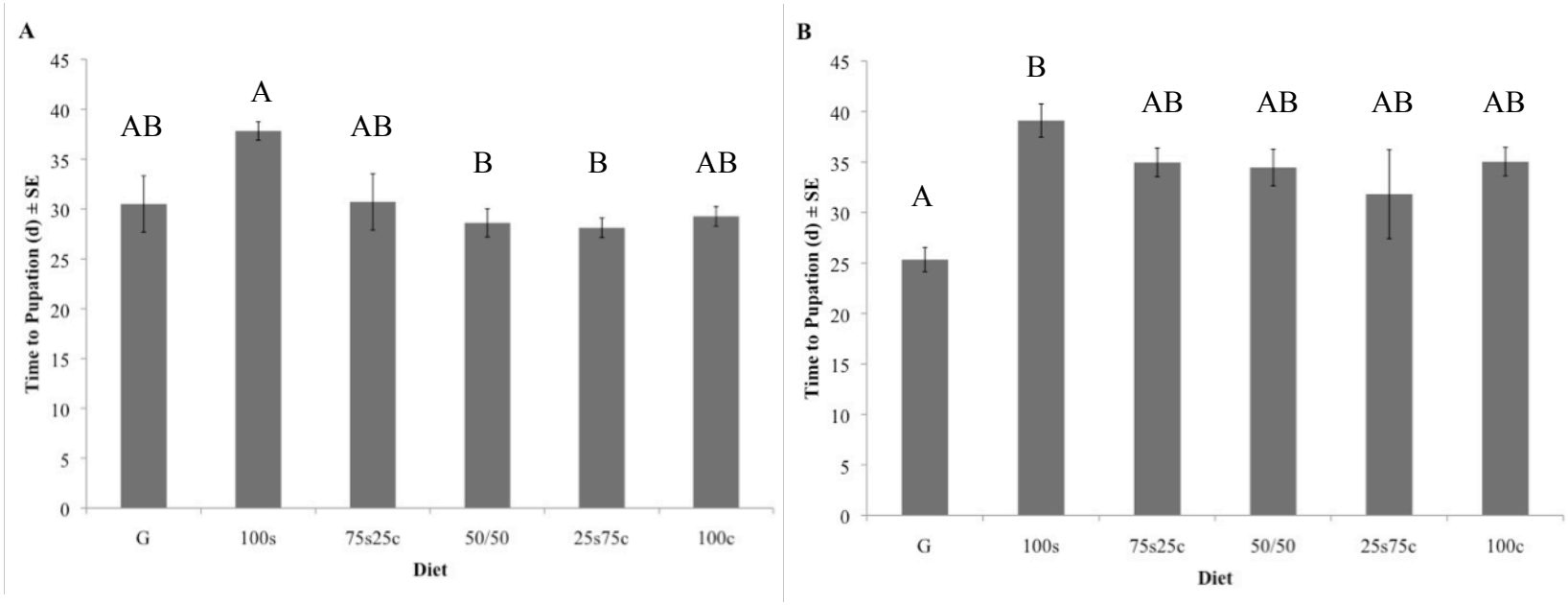

Fig. 7: Average time (d) from hatching to pupation for individual black soldier flies ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). 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 diet (control).

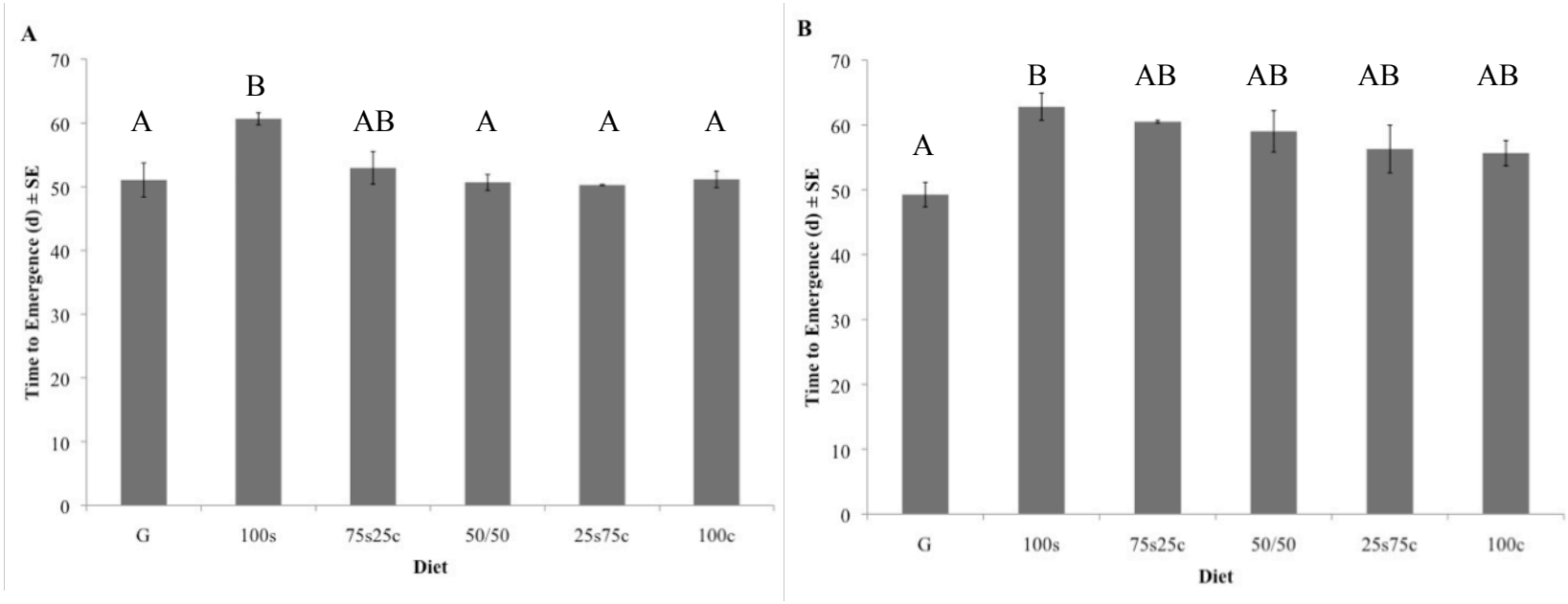

Fig. 8: Average time (d) from hatching to adult emergence for individual black soldier flies ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). 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 diet (control).

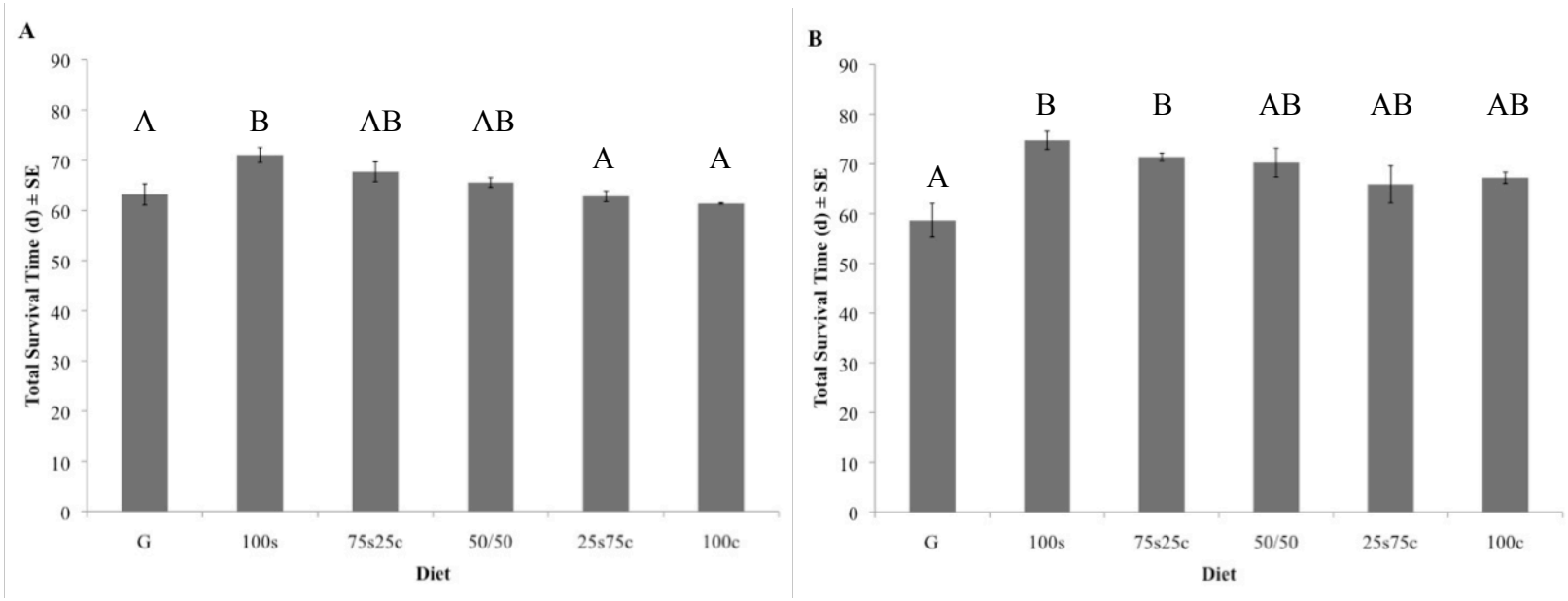

Fig. 9: Average time (d) from hatching to death for individual black soldier flies ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). 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 diet (control).

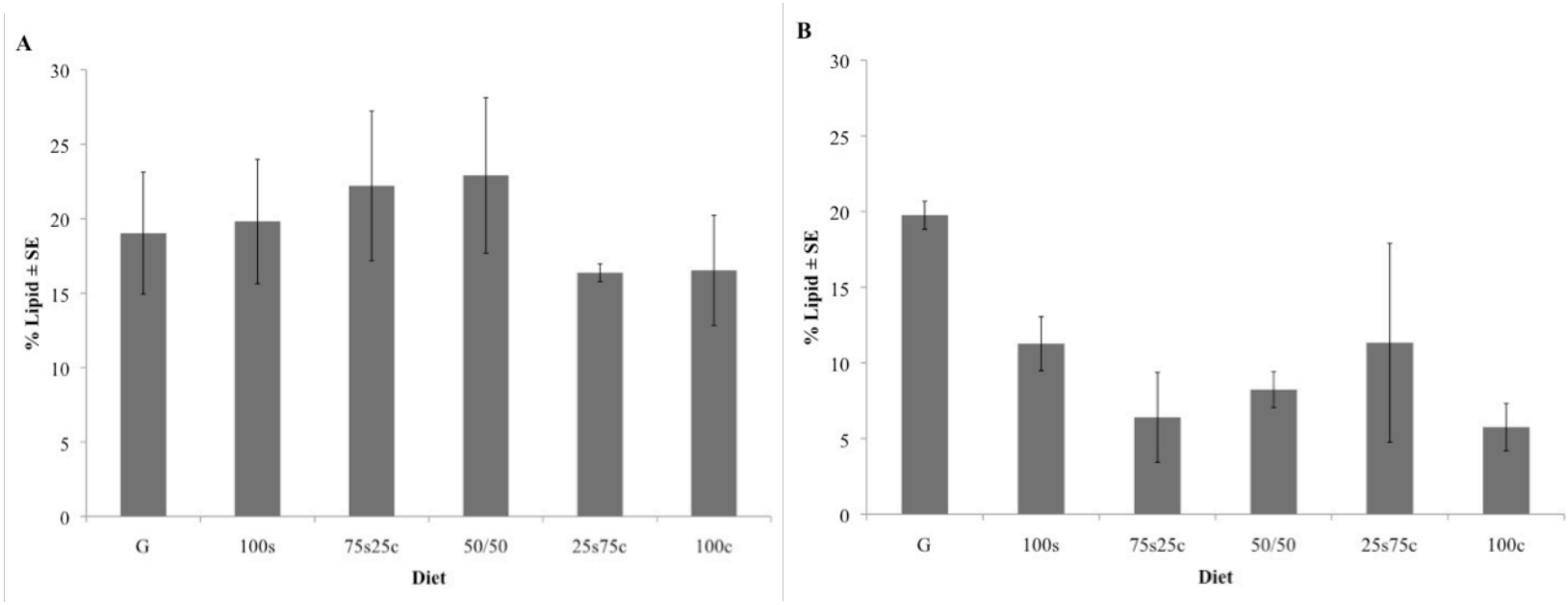

Fig. 10: Average percentage lipid in black soldier fly prepupae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Significant differences were not observed. Diets were 100c = 100% Cowpea, 100s = 100% Sorghum, 25s75c = 25% sorghum and 75% cowpea, 50/50 = 50% cowpea and 50% sorghum, 75s25c = 75% sorghum and 25% cowpea, and G = Gainesville diet (control).

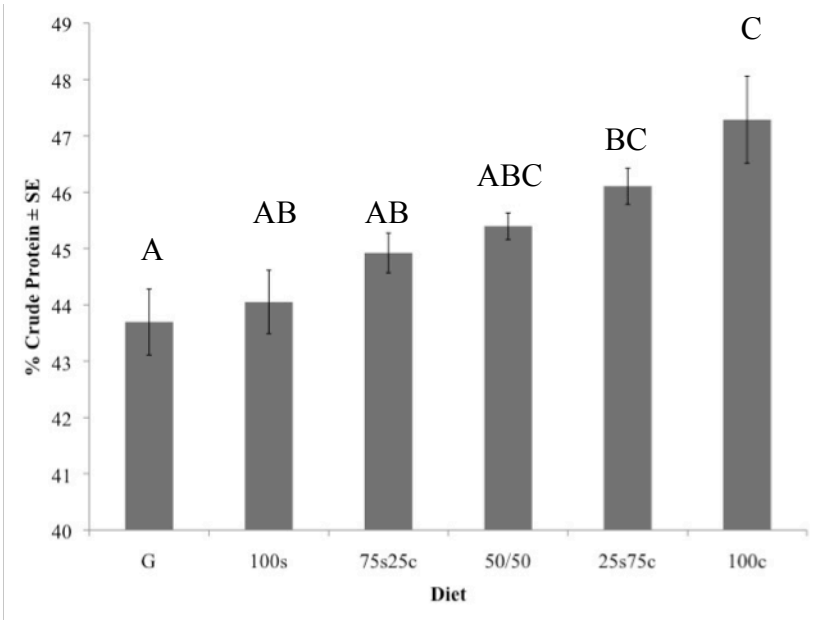

Fig. 11: Average percentage crude protein in black soldier fly prepupae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). 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 diet (control).

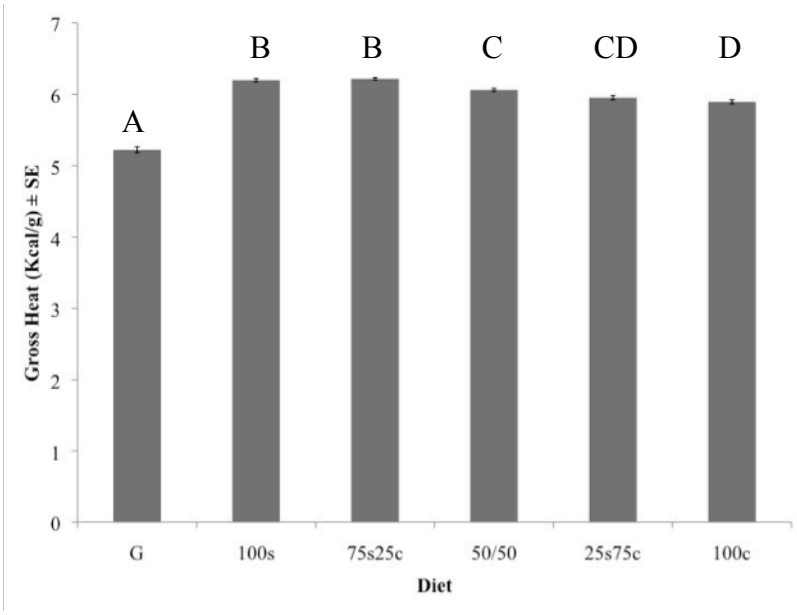

Fig. 12: Average gross energy (Kcal/g) in black soldier fly prepupae ± SE when fed different diets during trials (A and B) in a walk-in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Different letters above columns within a trial indicate significant differences ($P < 0.05$). Diets 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 diet (control).

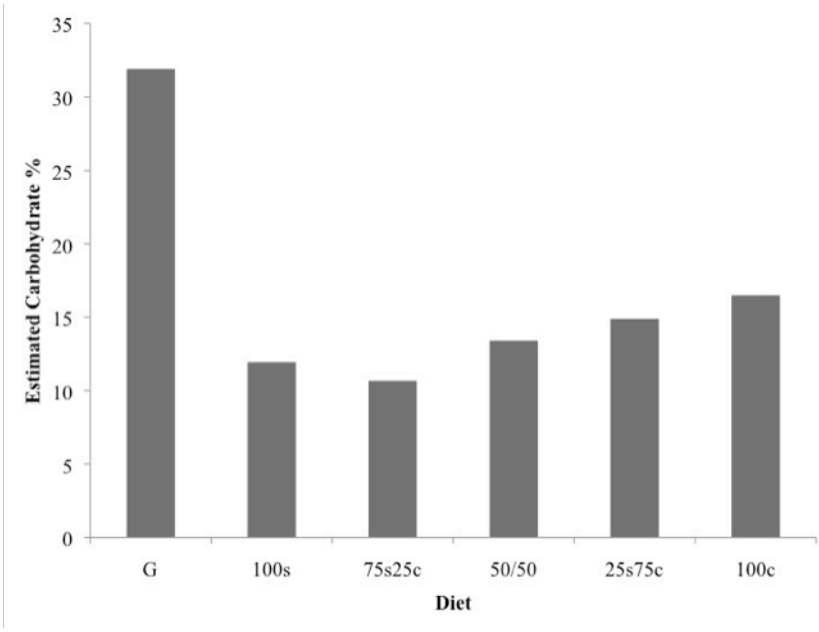

Fig 13: Estimated percentage of carbohydrate in black soldier fly prepupae when fed different diets in a walk in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Estimates for lipid and carbohydrate in Figs 13 and 14 were based on measured values of crude protein and gross energy. 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 diet (control).

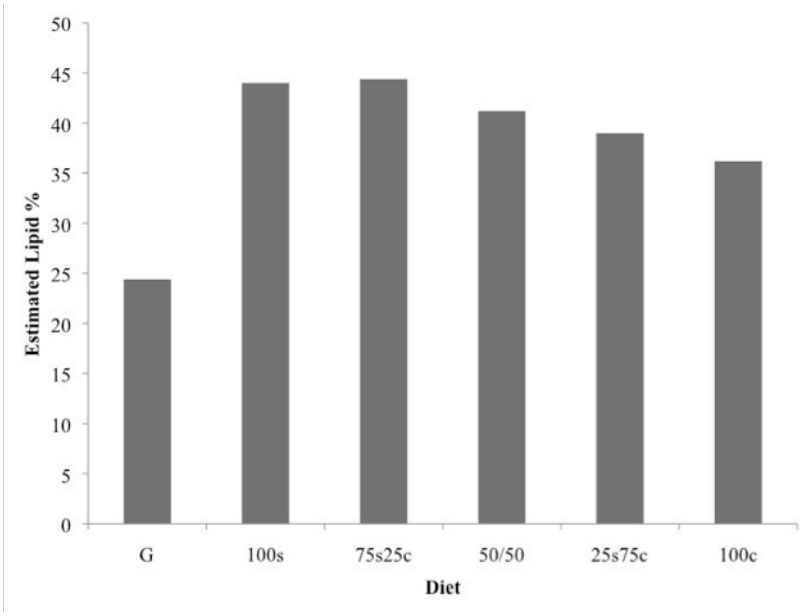

Fig 14: Estimated percentage of lipids in black soldier fly prepupae when fed different diets in a walk in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Estimates for lipid and carbohydrate in Figs 13 and 14 were based on measured values of crude protein and gross energy. Diets 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 diet (control).

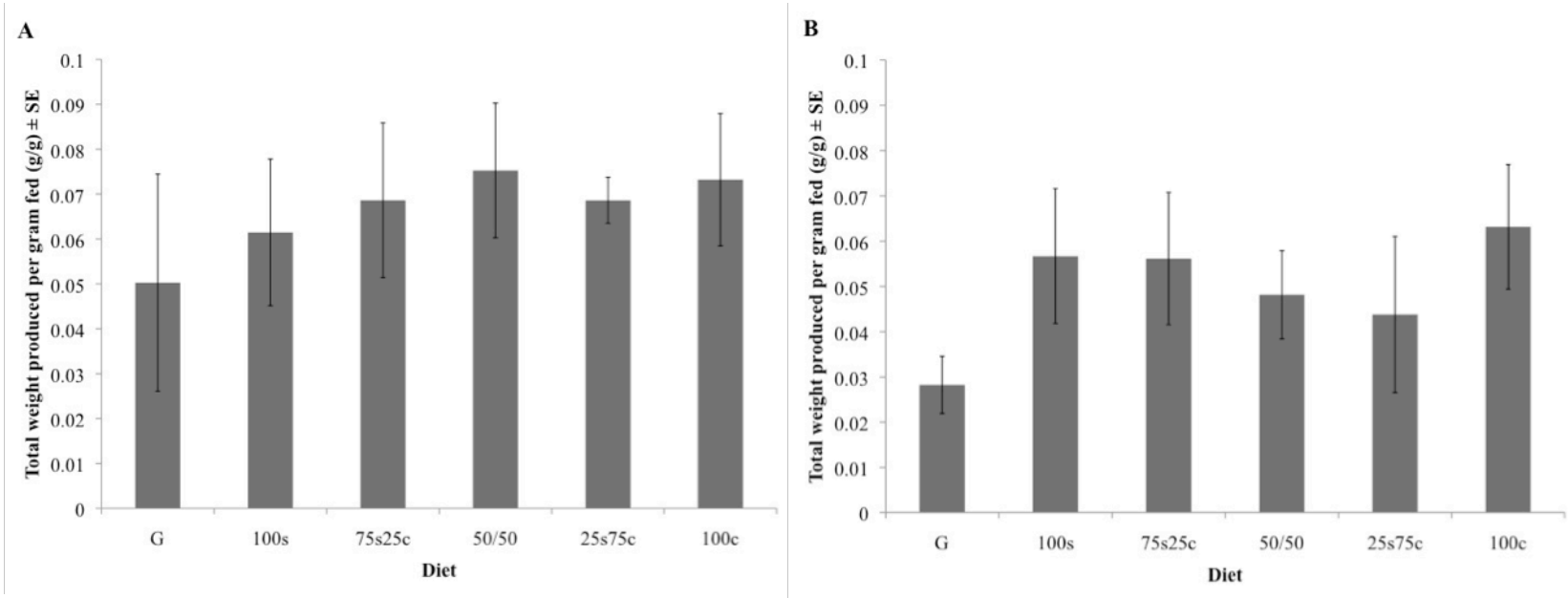

Fig 15: Total weight of black soldier fly prepupae produced per total weight of feed (g/g) in each container ± SE when fed different diets during trials (A and B) in a walk in incubator set at 28.0 ± 2.0°C, approximately 70% RH, and 14:10 L:D. Diets were 100c = 100% Cowpea, 100s = 100% Sorghum, 25s75c = 25% sorghum and 75% cowpea, 50/50 = 50% cowpea and 50% sorghum, 75s25c = 75% sorghum and 25% cowpea, and G = Gainesville diet (control).

Discussion

BSF were able to successfully complete larval development on all tested diets. There were subtle but discernable differences in development rates based on diet, particularly the diets containing a higher percentage of sorghum. In general, larvae on the sorghum diets developed more slowly (3-9 days longer from hatching to pupation) than those on the cowpea diets, which were higher in protein (Table 5). These differences are similar to those demonstrated by Nguyen et al. (2013), who observed faster (19-27 days difference during the wandering phase) development in higher-protein (4-18g more protein) diets (kitchen waste, liver) and slower development in lower-protein diets (fruits and vegetables, manure). Additionally, Tomberlin et al. (2002) observed that wild BSF, with different access to nutritional resources, were larger than lab-reared BSF. Diet did not have a significant impact on survivorship, indicating that any of the tested diets could potentially sustain a population of BSF (Tomberlin et al. 2002, Nguyen et al. 2013).

This variation in life history traits could be due to differences in dietary protein or energy. Nguyen et al. (2013), for example, 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. They also speculated that too much fat might be

detrimental to BSF development as the higher fat diets in their study had higher mortality rates than lower fat diets.

Table 5: Nutritional compositions of dietary components sorghum and cowpea fed to BSF in this study as found in the USDA's National Nutrient Database (US Department of Agriculture 2015).

		Diets	
Nutrient	Unit	Sorghum	Cowpea
Proximates	per 100g		
Water	g	71.41	70.04
Energy	kcal	119	116
Protein	g	3.51	7.73
Total lipid	g	1	0.53
Carbohydrate	g	23.67	20.76
Fiber	g	1.3	6.5
Sugars	g	0.13	3.3
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	0.4
Thiamin	mg	0.106	0.202
Riboflavin	mg	0.082	0.055
Niacin	mg	1.33	0.495
Vitamin B-6	mg	0.108	0.1
Folate, DFE	µg	19	208
Vitamin B-12	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

Higher protein diets translated to higher protein content 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 by diet as well (Table 5) (Anyango et al. 2011, US Department of Agruculture 2015). Lower protein diets led to higher gross energy prepupae in both trials. This 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). Lipid content of BSF pre-pupae was so diverse within treatments that diet is not a significant predictor of lipid content. This consistent inconsistency could mean this value could be standard for BSF raised on any table scraps, not necessarily just these diets. St-Hilaire et al. (2007b) include nutritional profiles for all feed ingredients they feed to fish, including BSF prepupae, for which they gave a standard lipid measurement of 13.9%.

Trial had a significant effect on all of the life history measurements, which suggests other sources of variation may have been involved, including the following. Different incubators were used for each trial because of an incubator malfunction during the first trial. Because of this, there may have been some variation in minute temperature and humidity variations, both of which could influence life history traits of BSF (Tomberlin et al. 2009, Holmes et al. 2012). Containers were removed from incubators when measurements were taken, and since trial one took place in the fall and trial two

took place in the summer, there may have been variation in ambient temperature and humidity, both of which could influence life history traits of BSF (Tomberlin et al. 2009, Holmes et al. 2012). Likewise, batches of diet were prepared in different seasons. The scales used for measuring larval and pupal weights, and preparing diets were moved and recalibrated multiple times during both trials, which could have caused inconsistency in measurements.

In this study, lipid content of prepupae varied greatly, but other studies found more consistent lipid measurements when using different lipid quantification methods (St-Hilaire et al. 2007a). Overall mean measured lipid content across all six treatments was 19.48% in trial A and 10.46% in trial B (Table 4, Fig. 10), neither of which aligns with the 13.9% listed by St-Hilaire et al. (2007b). Overall estimated lipid content, based on measured protein and gross energy values, was 38.2% (Table 4, Fig. 14) across all six treatments, which still does not agree with St-Hilaire et al. (2007b). 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). Carbohydrates, vitamins, and minerals of BSF were not measured at all in this study, though there may be differences by diet. This is particularly suggested by the inverse correlation between protein content and gross energy, as different macronutrients do have different energy densities (Sales 2009). There may be more to energy storage in insects. One study on grasshoppers demonstrated consistent storage of proteins and carbohydrates and varied storage of lipids based on diet (Hahn 2005). This suggests that these BSF may have been

storing carbohydrates more consistently than lipids. Estimated carbohydrate values, based on measured protein and gross energy values (Table 4, Fig. 13), seem to support this possibility.

This work is a piece in the puzzle of refining insect diets to improve insects 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 optimized to allow for cheaper mass production (van Huis 2013). Data collected here suggests that the quality of BSF prepupae as food or feed can be influenced drastically by diet, rather like traditional livestock (Maynard et al. 1979). These data represent further evidence of the viability of BSF for protein production, as they rank high in crude protein percentage as compared with common food insects listed by Bukkens (1997).

CHAPTER III

SUMMARY, LIMITATIONS, AND FUTURE RESEARCH

A few factors limit this study, as mentioned in the previous chapter. The use of different walk-in incubators for each trial could have contributed to the differences between trials, as could the different seasons during which the trials were performed. The experimental diets were cooked, while the control diet was uncooked. Cooked diets were prepared in batches, which were distributed across time, not treatment, to avoid causing differences based on this, but batches were not controlled between separate trials. Each of these raises questions about how drastically BSF might be affected by each of these variables.

Another limitation is the obvious issue with measurement of lipids. The lipid measurements described here yielded unreliable measurements that are not possible given gross energy and crude protein measurements in the same tissue. I suspect grinding of the larvae would help considerably with this inconsistency. However, different methods entirely may serve even better.

If BSF are to be reared in high enough densities to be a viable food source, it is imperative that we understand their nutritional needs in the same way we do traditional livestock. In this study diets with varied macronutrient and micronutrient compositions were compared. Since differences between diets were observed, a logical next step might be, if possible, to feed the larvae diets which only vary in one macronutrient or

micronutrient, to further control for possible sources of variation. Perhaps this could be done with enriched vs. non-enriched versions of the same diet.

With regard to nutrition, only gross energy, crude protein, and lipids were measured in this study. These unreliable lipid measurements decrease nutritional data to only gross energy and crude protein. While this information is still valuable, I look forward to the possibility of seeing more complete nutritional profiles of these insects, including all three macronutrients, and more specific spreads of the types of proteins (specific amino acids) present, as well as micronutrients. I am also curious about the possibility of gut loading for added nutritional benefit of using BSF as feed/food.

I think novelty candies in museum gift shops are just the beginning of acceptance. Insects are going to make it to the table of normal American families. Moving forward, insects will only become mainstream food if massive outreach efforts sway public opinion regarding this matter. More studies like this one must begin to calm the fears of consumers while contributing to efficiency and practicality of mass-rearing efforts. I believe that entomophagy broadens our ability to feed our growing global population.

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