LIFE-HISTORY TRAITS OF BLACK SOLDIER FLIES, *Hermetia illucens* (L.) (DIPTERA: STRATIOMYIDAE) AND HOUSE FLIES, *Musca domestica* L. (DIPTERA: MUSCIDAE) FED THREE MANURE TYPES

A Dissertation

by

CHELSEA DAWN MIRANDA

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Jeffery Tomberlin
Committee Members,	Pete Teel
	Mark Holtzapple
	Tawni Crippen
	Sonja Swiger
Head of Department,	Pete Teel

August 2019

Major Subject: Entomology

ABSTRACT

The United States is a major producer of livestock and poultry. As such, large volumes of manure are produced annually. This fact coupled with the lurking concern of the growing global human population and environmental impact of current manure storage practices calls for alternative methods to manage manure. Both black soldier flies (BSF), *Hermetia* illucens (L.) (Diptera: Stratiomyidae) and house flies (HF), *Musca* domestica L. (Diptera: Muscidae) are capable of reducing manure volume, as well as odorous compounds and pathogens and converting it into valuable biomass, but a better understanding of the bioconversion of different manure types (swine, dairy, or poultry) and nutrient reduction capabilities at different scales (small vs. large-scale production) is necessary to develop these systems. Results from the small-scale BSF study show that larvae fed poultry manure weighed more as prepupae (15–37%), developed faster (4–9 days), had higher survivorship to the prepupal stage (>94%), lived longer as adults (1–2 d), and converted more resource to biomass (3–4%) than those fed dairy or swine manure. A similar trend was found in the HF small-scale study for pupal weight, but more larvae survived to the pupal stage when fed swine manure (58–66%). When comparing the results from the small-vs-large-scale results, BSF larvae reared on a small-scale weighed less (30–45%), had a shorter development time (2–6 d) and higher survivorship (6–17%), and higher nutrient reductions (> 50%) compared to those reared on a larger scale. A similar pattern was found when comparing the HF studies, except lower survivorship (4–15%) was found in the small-sale study. In addition to these findings, the competitive interaction of these species on manure was evaluated as the presence of one species can impact the production of the other. Results demonstrate that HF are true pests, capable of surviving to the pupal stage on aged poultry manure (up to 6-d-old) with and without BSF; however, the presence of HF can negatively impact BSF survivorship and development. Collectively, these findings may assist in optimizing production of BSF or HF on manure.

DEDICATION

This dissertation is dedicated to the memory of Debbie Sharp. Although she loved to razz me about being an Aggie, I know she would be very proud of me. This is for her.

ACKNOWLEDGEMENTS

First, I would like to express my deepest appreciation to my advisor, Dr. Tomberlin, for his guidance and encouragement throughout the course of my research. I am grateful that he accepted me in his lab and provided me with numerous opportunities to grow as a scientist. It has truly been an honor to work with him.

I would also like to thank my committee members, Dr. Teel, Dr. Cammack, Dr. Holtzapple, Dr. Crippen, and Dr. Swiger for their willingness to serve on my committee and their helpful advice and expertise. Through their contributions, they taught me how to think critically. I believe the development of this skill culminated during my preliminary exam, which without a doubt, will have a lasting (positive) effect on me for the rest of my career.

I would also like to extend my sincerest thanks to past and present members and visitors of the F.L.I.E.S. Facility for making my time here enjoyable. I love you all as if you were my family. I would especially like to thank Ashleigh Faris, Dongmin Kim, Casey Flint, and Samantha Hays for always lending a helping hand or a shoulder to cry on without hesitation.

I cannot begin to express my thanks to my family for their unconditional love and support. I was not always able to attend family functions, but they understood and called (face-timed) me so that I was still included in all events. To my parents, Tony and Becky Holcomb, thank you for your unwavering moral, spiritual, and emotional support. I realize I would not be who I am or where I am today without you.

Thank you to my husband, Matthew (aka "Matty Man Love"), for always believing in me, especially when I did not believe in myself. Thank you for all of the sacrifices you have made. On mac-n-cheese, I love you more than I tell you, and I am grateful that you were by my side on this journey.

And finally, thank you Maxee Love, Lilly Banilly, Poundcake, Butterbean, and Besito Suavecito for your companionship. I realize this has also impacted your precious lives, and although you will never understand my expression of gratitude, you helped get me through one of the toughest chapters in my life.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Jeffery Tomberlin [advisor], Dr. Pete Teel, and Dr. Jonathan Cammack of the Department of Entomology, Dr. Mark Holtzapple of the Department of Chemical Engineering, Dr. Tawni Crippen of the United States Department of Agriculture, and Dr. Sonja Swiger of Texas A&M AgriLife Research and Extension Center. The work conducted for the dissertation was completed by the student independently.

Funding Sources

The research and authorship of this document was completed without receiving external funding.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The United States (US) is a major producer of livestock (USDA 2010, 2015, 2017) and poultry (USDA 2017). The value of production for the poultry, swine, and dairy industries in the US in 2017 was $42.7, $19.2, and $38.1 billion, respectively (USDA 2017). Recent structural changes in the size of concentrated animal feeding operations (CAFOs) in these industries have led to fewer, but larger, farms (higher concentration of animals). Traditionally, a majority of swine produced in the United States (US) were from small operations (<5000 head/operation) (USDA 2015); in 1994, approximately 70% of the annual pig crop came from small operations; however, by 2014, large operations accounted for 90% of annual production (USDA 2015). The dairy industry experienced similar trends in that the overall number of dairy operations decreased, but the number of operations with 500+ head increased. The progression of these industries to larger facilities raises concerns because the volume of manure produced annually in some areas is more than the amount that can be utilized by nearby land. Though manure is known to be a valuable resource for fertilization purposes, large volumes raises concerns about current management practices.

Manure

One of the by-products of producing animals is manure. The amount of manure produced is dependent on many factors, such as level of production (low or high), amount of feed intake, age, and sex of the animal (USDA 2008). The volume of manure produced is typically reported pounds (lbs) relative to animal units (AU), which is 1000 lbs of live animal weight (USDA 2008). In regard to the amount of manure produced as related to level of production, dairy cows producing 50 lbs of milk/d (low production) will excrete approximately 97 lbs of manure/d/1000-lb AU, whereas those producing 125 lbs of milk/d (high production) will excrete roughly 130 lbs of manure/d/1000-lb AU (USDA 2008). Likewise, age and sex can influence the amount of manure produced by an animal. For instance, gestating and lactating sows produce approximately 25 lbs and 59 lbs of manure/d/1000-lb AU, respectively, compared to boars and nursery pigs that produce on average 19 lbs and 88 lbs of manure/d/1000-lb AU, respectively (USDA 2008). In total, the US produces over 300 million tons (dry weight) of manure annually (USDA 2006). Thus, proper management of animal wastes is essential to avoid negative environmental impacts.

Manure Management

Lagoons are widely utilized to manage liquid manure because they are simple to operate and cost effective due to low labor requirements (Miller et al. 2011). However, there are drawbacks of utilizing a lagoon to manage wastes. For example, excessive rainfall may also cause a lagoon to flood or rupture, which is a concern as manure can be a source of pollution (Mallin et al. 1997). Additionally, rainfall determines when effluent can be sprayed on to fields, which limits the functionality of the system (Westerman et al. 1987). Another disadvantage of utilizing lagoons for waste management is that amount of effluent that can be sprayed onto fields is governed by the nitrogen (N) content in the manure and the extent of N assimilation by plants (Gutser et al. 2005). Though, proper application of manure based on nitrogen content may lead to overapplication of phosphorous (P) (Shober and Sims 2003). Overapplication of nutrients to land is a major cause of run-off, which leads to eutrophication if waterways

(Wright 1998). This is a major concern as nutrient overloading from agriculture via the Mississippi River has caused the second largest dead zone (low oxygen hypoxic environment) in the Gulf of Mexico (Rabalais et al. 2002), which kills marine life (Boesch et al. 2009) and impacts the seafood industry (Smith et al. 2017).

Alternative Applications of Manure Management

Manure can be utilized to generate biogas. This occurs via microbes (methane bacteria) that convert organic carbon in manure to methane, which can be used to generate energy. The process of energy production is carried out in a covered lagoon (anaerobic digester), which traps odorous compounds released and eliminates an undesirable consequence of uncovered (aerobic) lagoons, or it may be transferred to a central processing facility after solids have been separated, which can then be converted into fertilizer or directly applied to land (Carter-Young et al. 2003). Unfortunately, this technology is associated with large capital costs (Beddoes et al. 2007). In 2018, there were approximately 250 anaerobic digesters in the US, indicating that biogas production has not been widely adopted (EPA 2019). Furthermore, certain factors, such as pH, temperature, and concentration of manure contaminants (e.g. heavy metals and antibiotics) must be regulated because they impact survivorship of methane bacteria (Altaş 2009, MacDonald 2009, Lu et al. 2014). Another technology is to employ insects to digest the manure. This method provides a means to manage manure and offers multiple revenue streams, which will be discussed in more detail below.

Manure and Insects

From an ecological prospective, manure is a complex ephemeral resource and as such, is often the site of intense competition (manure as an ecological until). Various insects utilize manure as a resource, many of which are from the Orders Coleoptera and Diptera. Some insects that are associated with manure do not directly feed on the resource but are predacious on its inhabitants (Mohr 1943, Wingo et al. 1974). Among the coprophagous Coleopterans, dung beetles (Coleoptera: Scarabaeidae) have received a lot of attention for their positive attributes, such as dispersing seeds (Nichols et al. 2008), recycling nutrients (Nichols et al. 2008), reducing manure volume (Lee and Peng 1981, Jones et al. 2019), green-house gas emissions (Slade et al. 2016), pathogens (Jones et al. 2019) and parasites (Fincher 1973, Nichols et al. 2008). Yet, among the coprophagous Dipterans, majority of research focuses on pest control (Hall and Foehse 1980, Miller et al. 1981). However, dipterans may be also considered beneficial for their capabilities to degrade and transform wastes (van Huis 2019). Two species that utilize manure are the black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) and house fly (HF), *Musca domestica* L., (Diptera: Muscidae). Both are capable of degrading manure and may be valuable agents to employ for waste-management purposes.

Black Soldier Fly

The BSF is common in tropical and subtropical regions throughout the world and has gained a considerable amount of attention for food and feed purposes (van Huis et al. 2013, Makkar et al. 2014, van Huis et al. 2015, Lähteenmäki-Uutela et al. 2017). Newton et al. (1977) and Sheppard et al. (1994) concluded that larvae fed manure had a body composition consisting of 42% protein and 35% fat. (Arango Gutiérrez et al. 2004) suggested that BSF larvae meal has a higher fat content compared to fish meal, but the protein digestibility of larvae meal is similar. Black soldier fly larvae can convert poultry waste into a protein-rich biomass considered as quality feedstuff for commercial fish production (Bondari and Sheppard 1981, Diener et al. 2009, Li et al. 2017). Additionally, dried BSF larvae fed bovine manure can be ground into a meal and supplemented into swine diets (Newton et al. 1977) .

Black soldier fly larvae can be used to reduce can phosphorus by 61-70 % and nitrogen by 30–50 % in dairy manure (Myers et al. 2008). However, BSF may be more difficult to rear and take longer time to develop than other flies (Čičková et al. 2015). Tomberlin and Sheppard (2002) suggested that sunlight is necessary for BSF mating to occur. However, artificial lights may be used to stimulate mating as well (Zhang et al. 2010, Nakamura et al. 2016, Heussler et al. 2018) Time of day also influences BSF mating behavior; mating occurs more frequently early in the day (before 1500 h) and decreases as the day passes (Tomberlin and Sheppard 2002). Adults usually mate two days after eclosion and oviposit two days after mating (Tomberlin and Sheppard 2002) and do not need to feed for reproductive maturation, but providing a protein source may increase egg production (Bertinetti et al. 2019). Other factors known to influence mating and oviposition are temperature and humidity (Tomberlin and Sheppard 2002, Tomberlin et al. 2009). Tomberlin and Sheppard (2002) observed 80% of egg clutches were deposited when the humidity was greater than 60% and egg eclosion was greatest (73%) and took the least amount of time (71 hours) at 60% RH compared to 40% and 50% RH (Holmes et al. 2012). Under laboratory conditions, development time from egg to adult for individuals reared at 27°C ranged from 40 to 43 days, with the larval stage lasting 22–24 days (Tomberlin et al. 2002).

Black soldier fly larvae can be incorporated into a self-harvesting system due to the dispersal behavior of the prepupal stage (Sheppard et al. 1994). The last larval instar, known as prepupae, have modified mouthparts different from all other larval instars (Schremmer 1984). Young larvae are saprophagous using their mouthparts to feed, while non-feeding prepupae use their mouthparts for migration (Schremmer 1984). During the last larval instar, black soldier flies move away from the area where they were feeding to pupate in dryer environments. Locomotion is achieved by using their labrum, which is bent down like a hook, to anchor the head while the body contracts and moves forward (Schremmer 1984). Sheppard et al. (1994) demonstrated that BSF prepupae could self-harvest by placing plastic pipes under hen cages to direct migrating prepupae into holding containers. This system reduced manure accumulation by 50% and did not require laborious means or large investments in facilities to remove prepupae from waste (Sheppard et al. 1994).

Myers et al. (2008) was first to examine the development of the BSF on dairy manure. Black soldier fly larvae were randomly assigned to one daily feeding regimen of 27 g, 40 g, 54 g, or 70 g of dairy cow manure. The experiment was replicated four times and survivorship, sex, and pupal weights were recorded. Adults lived five days longer than those raised on artificial diets, but the adults fed the lowest daily feed rate (27 g) lived 3–4 days less than those fed the highest rate (70 g) (Myers et al. 2008). Larvae provided the least amount of manure took longer to develop to the prepupal stage, but needed less time to reach the adult stage (Myers et al. 2008). Additionally, larvae fed 27 g dairy manure per day reduced manure dry matter mass by 58%, where as those fed 70 g per day reduced dry matter by 33% (Myers et al. 2008). Myers et al. (2008)

suggested using lower feed rates to reduce dry matter and increase P and N reduction to most effectively use the BSF for waste management.

Black soldier flies offer a means to reduce the negative properties of manure (pathogens, pharmaceuticals, pesticides, heavy metals, and odors). Black soldier flies reduce *Salmonella* and *Enterococcus* spp. in human feces (Lalander et al. 2013) as well as *Escherichia col*i O157: H7 and *Salmonella enterica* (Erickson et al. 2004) in chicken manure. Additionally, BSF can decrease the half-life of pharmaceuticals (carbamazepine, roxithromycin, and trimethoprim) and fungicides (azoxystrobin and propiconazole) (Lalander et al. 2016). In regard to heavy metals, BSF reduce lead (Pb), nickel (Ni), boron (B), copper (Cu), chromium (Cr), cadmium (Cd), zinc (Zn), and mercury (Hg) in artificial diets (Cai et al. 2018). However, the impact on the metals on development has produced conflicting results. Cai et al. (2018) found that Pb, Ni, B, and Hg negatively impacted larval development and Cu, Cr, Zn, Cd, and Hg reduced larval survival. Yet, Diener et al. (2015) determined that increasing concentrations of Pb, Cd, and Zn did not impact pupal weight or development, but Pb and Zn bioaccumulated in larvae and Cd in prepupae. Though heavy metals have not been detected in extracted oil (Cai et al. 2018). Black soldier flies also reduce odorous compounds (phenols, indoles, and volatile fatty acids) (Beskin et al. 2018).

House Fly

House flies are a major pest of humans and livestock because they are known carriers of numerous pathogens (Malik et al. 2007). Transmission of pathogens occurs via direct contact, but can also be dispersed through regurgitation and excretion (Sasaki et al. 2000, Malik et al. 2007). Sasaki et al. (2000) showed *Escherichia coli* O157: H7 persisted in the HF crop for four days after consuming contaminated food. Additionally, the same study suggested that *E. coli* was harbored in the HF intestine and was excreted for three days after feeding.

The HF life cycle is well described. Adult emergence can occur in as little as 11–15 days (Khan et al. 2012). Egg deposition occurs 10–14 days post-emergence (Bishopp et al. 1915). Teotia and Miller (1973) suggested the number of eggs per gram of manure influences pupae yield. As the quantity of fly eggs per gram of poultry manure increases, larval development and pupal survival decrease due to competition and overcrowding (Teotia and Miller 1973, Barnard and Geden 1993). Laboratory-based experiments indicate population numbers can increase rapidly under optimal conditions (Khan et al. 2012) around 26.7˚C and 60% relative humidity (Hogsette 1992).

The idea of feeding HF feces to harvest proteins and lipids was proposed a century ago (Linder 1919). However, until recently, HF have received little attention in the US for this purpose. In a way, they are similar to BSF in the lack of information available on commercialization and mass production (Gold et al. 2018). Most of the research published on HF focuses on the species as a pest, concentrating on control and insecticide resistance (El Basheir 1967, Sawicki and Lord 1970, Georghiou 1972, Hayaoka and Dauterman 1982, Geden 2012). Although HF can degrade animal waste, they are still viewed from a negative perspective. However, if we are able to harness their ability to break down waste and produce value-added feed and foodstuff, we may be able to solve the major issues described above. Interestingly, BSF were historically revered as a pest in poultry houses (Axtell and Edwards 1970); however, today they are deemed beneficial insects, which begs the question if HF can be viewed from the same perspective (van Zanten et al. 2015).

House fly larvae can be used to process animal waste (Čičková et al. 2012, Zhang et al. 2012). Čičková et al. (2012) demonstrated that 177.7 ± 32.0 ml of HF eggs with an inoculation rate of 0.4–1.0 ml of eggs/kg of manure could biodegrade 178–444 kg of swine manure in 10 days. Čičková et al. (2012) concluded that the optimum density for successful biodegradation is 1 ml (11,000 eggs)/kg of manure. Zhang et al. (2012) developed a swine manure vermicomposting system using HF larvae and determined the total manure weight reduced to be 106 ± 17 kg/m^3/d). Both studies suggest that HF are capable of processing large amounts of manure. However, type of manure fed to HF impacts development and survivorship.

Type of manure fed to HF larvae affects their life-history performance (Farkas et al. 1998, Larrain and Salas 2008, Khan et al. 2012). Flies resulting from larvae fed livestock manure (e.g., poultry, swine, bovine) have shorter life cycles but have greater fecundity, and heavier pupae compared to horse, buffalo, sheep, and goat manures (Larrain and Salas 2008, Khan et al. 2012). Manure moisture seems to be a major factor regulating larval development. Moisture between 50–80% appears to be most appropriate for the HF (Farkas et al. 1998). Larvae reared on manure types with moisture levels outside of this range will have lower survivorship (Larrain and Salas 2008). For example, the moisture content of dog manure is 49%, which is low compared to milking calf (69%), swine (66%), and poultry manure (65%) (Larrain and Salas 2008). For the latter three manure types, more than 83% of larvae reached the pupal stage in six days compared to only 35% when reared on dog manure (Larrain and Salas 2008). Moreover, mortality for larvae fed milking calf, swine, or poultry manure ranged from 2.5–10.0%, whereas mortality for those provided dog excrement was 23.3% (Larrain and Salas 2008). In a similar study, Farkas et al. (1998) indicated that HF developed well in swine and poultry manure, but immature development was significantly greater in pig manure when moisture levels were 70–80%. When larvae were fed poultry manure at 70 and 80% moisture, the mean number to reach the pupal stage was 27.5 ± 0.7 and 24.5 ± 1.7, respectively out of 30 (Farkas et al. 1998). The same study examined the development of larvae on pig manure and determined the mean number to reach the pupal stage to be 29.5 ± 0.5 and 30 ± 0.0, respectively, suggesting HF larvae develop well in manures of 70–80% moisture levels (Farkas et al. 1998).

House fly pupae resulting from larvae fed manure may be substituted for soybean oil or fish meal in animal diets, because they are high in protein (60%), fatty acids, phosphorus and calcium (Calvert et al. 1969, Teotia and Miller 1974). Adult feeding is required to maintain a HF colony. Feeding prior to mating is essential because it prompts reproductive maturation (Morrison and Davies 1964). Protein, such as yeast or milk, increases colony egg yield (Pastor et al. 2011). Adult females lay 50% more eggs when fed a diet of sugar and powdered milk (2:1) when compared to sugar and yeast of the same proportion (Pastor et al. 2011). Compared to other flies with similar body composition, rearing HF may involve higher costs due to the required feeding by adults; however, in areas where BSF cannot be reared successfully, HF may be more suitable for mass-rearing.

Similar to BSF, HF can reduce pathogens (Wang et al. 2013, Nordentoft et al. 2017), odors (Wang et al. 2013), and heavy metals (Wang et al. 2017) in manure. In as little as 4 d, 100 3-d-old HF can reduce *Escherichia coli*, *Salmonella* Enteritidis and *Campylobacter jejuni* by 8 $\log_{10}$ (compared to 2 $\log_{10}$ reduction in untreated manure) in 100 g of poultry manure (Nordentoft et al. 2017). On a larger scale, Wang et al. (2013) 580,000 1-d-old larvae fed 25–30 kg/m^{-2}/d^{-1} swine manure reduced 3-methylindole (an odorous compound) and *E. coli* by over 90%. Also, when compared to untreated manure, 1 g of HF eggs fed 4 kg of poultry manure reduced C, Cr, Cd, S, and Zn below recommended heavy metal limits for fertilizer in 5 d (Wang et al. 2017).

The ability of BSF or HF to convert animal waste should not be disregarded as they promote a circular economy by reducing and recycling nutrients in manure. For example, biofuel can be extracted from BSF larvae fed swine dairy, and poultry manure (Li et al. 2011b) and from HF fed swine manure (Yang et al. 2014). Insect lipids can also be extracted to cultivate other naturally occurring compounds in the fat (fatty acids and fat-soluble vitamins) (Li et al. 2011a) or for cosmetic purposes (Verheyen et al. 2018) . The remaining larval residue after lipid extraction can be further refined for protein meal or antimicrobial compounds (Müller et al. 2017) and the spent media may be used as an organic fertilizer (Kováčik et al. 2014).

Competition

As discussed previously, manure can be the site of intense competition. Competition is the negative interaction that occurs among individuals of the same species (intraspecific) or members of different species (interspecific) that share a common resource (Begon et al. 2006). Understanding competitive interactions within and between species is necessary to optimize systems that utilize insects for manure degradation.

Intraspecific Competition

Intraspecific competition occurs when individuals of the same species compete for resources in an ecosystem. There are two basic responses of intraspecific competition due to overcrowding (Sullivan and Sokal 1963). The first response results in higher immature mortality with emerging adults maintaining their body weight, while the second response has lower immature mortality and reduced adult body weight (Sullivan and Sokal 1963). Larvae may be forced to metamorphose before adequate food reserve is obtained when food supply is exhausted, resulting in smaller adults (Sullivan and Sokal 1963, AMANO 1988). Sullivan and Sokal (1963) demonstrated that as larval density increases, adult weight decreases, and the mean number of days to adult emergence increases. Conversely, (AMANO 1988) showed that as *Orthellia pacifica* Zimin (Diptera: Muscidae) larval density increased, adult head width decreased, mortality increased, and development time decreased.

Interspecific Competition

Interspecific competition occurs when individuals of different species compete for the same resources in an ecosystem. Since BSF and HF exploit similar resources, they may interact. Under industrial conditions, either may be deemed a pest. Though BSF development time is longer than for HF, pupae are larger (220 mg/pupa) and thus, it is likely a single larva can consume more manure than can a HF (5–21 mg/pupa) (Čičková et al. 2015). Furman et al. (1959) explored the interspecific competition between HF and BSF and determined that the percent of successful development of HF decreased from 70% emergence in the control (without BSF larvae) to 0% HF emergence for the treatments with 400 and 600 actively feeding BSF larvae. In a similar study conducted by Bradley and Sheppard (1984), female HF adults readily oviposited in manure with lower BSF larval densities, but avoided higher densities. One hundred BSF larvae placed in 113 g cups with 75 g manure strongly inhibited HF oviposition (Bradley and Sheppard 1984). Additionally, the study concluded that the amount of time BSF larvae occupy the manure prior to HF exposure influences HF ovipositional responses (Bradley and Sheppard 1984). Fewer HF eggs were deposited in cups containing manure with BSF larvae placed 24 hours prior to HF exposure compared to BSF larvae placed 2.5 hours prior to HF exposure (Bradley and Sheppard 1984). Though the mechanism governing HF oviposition inhibition are not well-understood but is thought to be controlled by changes in the microbial communities by BSF (Erickson et al. 2004, Liu et al. 2008), which are responsible for volatile emissions that produce known attractants for HF oviposition (Tomberlin et al. 2016).

Research Objectives

The research presented was conducted to gain a better understanding of the development of BSF and HF on swine, dairy and poultry manure at two different scales (small vs. large scale) as well the changes in the chemical content of the manure as a result of larval feeding. In order to exploit BSF or HF as waste management agents, it is important to evaluate their performance at different scales to determine if results from small benchtop studies (few hundred larvae; incremental feeding) translate on a larger scale (thousands of larvae; single feeding). Additionally, BSF and HF both exploit manure as a resource and little is known about their competitive interactions. Therefore, the research presented encompasses seven objectives as described below.

Objectives 1 and 2 evaluate the development of BSF (Objective 1) and HF (Objective 2) fed swine, dairy, or poultry manure on a small-scale. Similar to most research conducted, these experiments were performed to gain a better understanding of the effects of intraspecific competition on life-history traits, DM reduction, and percent bioconversion. Results from these studies assisted in selection of a manure type for Objective 7, which evaluated the competitive interaction between BSF and HF.

Objective 1- Determine the effect of manure type and daily feed rate on black soldier fly life-history performance on a small-scale.

Hypotheses:

H_O: Manure type and feed rate do not alter life-history performance and development of black soldier flies.

H_A: Manure type and feed rate alter life-history performance and development of black soldier flies.

Objective 2- Determine the effect of manure type and daily feed rate on house fly life-history performance on a small-scale.

Hypotheses:

H_O: Manure type and feed rate do not alter life-history performance and development of house flies.

H_A: Manure type and feed rate alter life-history performance and development of house flies.

Objectives 3 and 4 evaluated the development of BSF (Objective 3) and HF (Objective 4) on a larger scale. Most research performed on these species is conducted on a small-scale; therefore, these experiments were performed to determine if results from small-scale studies translate on a larger scale (thousands of larvae; one single feeding). These data are valuable as they provide insight on limitations of small- and large-scale data.

Objective 3- Determine the effect of manure type and daily feed rate on black soldier fly life-history performance on a large-scale.

Hypotheses:

H_O: Manure type and feed rate do not alter life-history performance and development of black soldier flies.

$H_{A:}$ Manure type and feed rate alter life-history performance and development of black soldier flies.

Objective 4- Determine the effect of manure type and daily feed rate on house fly life-history performance on a large-scale.

Hypotheses:

H_O: Manure type and feed rate do not alter life-history performance and development of house flies.

$H_{A:}$ Manure type and feed rate alter life-history performance and development of house flies.

Objectives 5 and 6 evaluate the change in chemical content of the manure after larval processing from the first four objectives. This information is not known in BSF systems and not well-understood in HF. Data obtained from these studies provides information on differences in nutrient utilization, which is related to the biological data collected from the first four objectives. These objectives are combined into one manuscript.

Objective 5- Determine the change in chemical composition of swine, dairy, and poultry processed by black soldier flies on a small-and-large scale.

Hypotheses:

H_O: Chemical composition of swine, dairy, or poultry manure processed by black soldier flies is not different across diets or scales.

H_A: Chemical composition of swine, dairy, or poultry manure processed by black soldier flies is different across diets or scales.

Objective 6- Determine the change in chemical composition of swine, dairy, and poultry processed by house flies on a small-and-large scale.

Hypotheses:

H_O: Chemical composition of swine, dairy, or poultry manure processed by house flies is not different across diets or scales.

H_A: Chemical composition of swine, dairy, or poultry manure processed by house flies is different across diets or scales.

Objective 7 evaluates the competitive interaction between immature BSF and HF with a focus on colonization sequence as a factor governing BSF or HF development and survivorship to the prepupal (BSF) or pupal (HF) stage. Data may be useful for understanding biological differences and associated impacts due to the presence of conspecific larvae, which is valuable for industrialization purposes.

Objective 7: Evaluate the interspecific competition between black soldier flies and house flies with varying colonization sequences.

Hypothesis:

H_O: Interspecific competition does not exist between black soldier flies and house flies.

HA: Interspecific competition exists between black soldier flies and house flies.

References

Altaş, L. 2009. Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. Journal of Hazardous Materials 162: 1551-1556.

AMANO, K. 1988. Studies on the intraspecific competition in dung-breeding flies. IV. Effects of larval density on the development of *Orthellia pacifica* Zimin (Diptera: Muscidae). Applied Entomology and Zoology 23: 367-372.

Arango Gutiérrez, G. P., R. A. Vergara Ruiz, and H. Mejía Vélez. 2004. Compositional, microbiological and protein digestibility analysis of the larva meal of *Hermetia illuscens* L.(Diptera: Stratiomyiidae) at Angelópolis-Antioquia, Colombia. Revista Facultad Nacional de Agronomía, Medellín 57: 2491-2500.

Axtell, R., and T. Edwards. 1970. *Hermetia illucens* control in poultry manure by larviciding. Journal of Economic Entomology 63: 1786-1787.

Barnard, D. R., and C. J. Geden. 1993. Influence of larval density and temperature in poultry manure on development of the house fly (Diptera, Muscidae). Environmental Entomology 22: 971-977.

Beddoes, J. C., K. S. Bracmort, R. T. Burns, and W. F. Lazarus. 2007. An analysis of energy production costs from anaerobic digestion systems on US livestock production facilities. USDA NRCS Technical Note.

Begon, M., C. R. Townsend, and J. L. Harper. 2006. Ecology: from individuals to ecosystems.

Bertinetti, C., A. C. Samayoa, and S.-Y. Hwang. 2019. Effects of feeding adults of *Hermetia illucens* (Diptera: Stratiomyidae) on longevity, oviposition, and egg hatchability: Insights into optimizing egg production. Journal of Insect Science 19: 19.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Bishopp, F. C., W. E. Dove, and D. C. Parman. 1915. Notes on certain points of economic importance in the biology of the house fly. Journal of Economic Entomology 8: 54-71.

Boesch, D. F., W. R. Boynton, L. B. Crowder, R. J. Diaz, R. W. Howarth, L. D. Mee, S. W. Nixon, N. N. Rabalais, R. Rosenberg, and J. G. Sanders. 2009. Nutrient enrichment drives Gulf of Mexico hypoxia. Eos, Transactions American Geophysical Union 90: 117-118.

Bondari, K., and D. Sheppard. 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Bradley, S. W., and D. C. Sheppard. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. Journal of Chemical Ecology 10: 853-859.

Cai, M., R. Hu, K. Zhang, S. Ma, L. Zheng, Z. Yu, and J. Zhang. 2018. Resistance of black soldier fly (Diptera: Stratiomyidae) larvae to combined heavy metals and potential application in municipal sewage sludge treatment. Environmental Science and Pollution Research 25: 1559-1567.

Calvert, C. C., R. D. Martin, and N. O. Morgan. 1969. House fly pupae as food for poultry. Journal of Economic Entomology 62: 938-939.

Carter-Young, H., S. Cates, J. Chvosta, Y. Ji, A. Wossink, and K. Zering. 2003. Evaluation of swine producers' perceptions of alternative waste management systems: The Smithfield Foods-Premium Standard Farms Agreement with the North Carolina Attorney General. Special report prepared for the Animal and Poultry Waste Management Center, North Carolina State University.

Čičková , H., G. L. Newton, R. C. Lacy, and M. Kozanek. 2015. The use of fly larvae for organic waste treatment. Waste Management 35: 68-80.

Čičková , H., B. Pastor, M. Kozanek, A. Martinez-Sanchez, S. Rojo, and P. Takac. 2012. Biodegradation of pig manure by the house fly, *Musca domestica*: A viable ecological strategy for pig manure management. PloS ONE 7.

Diener, S., C. Zurbruegg, and K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management & Research 27: 603-610.

Diener, S., C. Zurbrügg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270.

El Basheir, S. 1967. Causes of resistence to DDT in Diazinon-selected and DDT-selected strain of house flies. Entomologia Experimentalis et Applicata 10: 111-126.

EPA. 2019. AGSTAR data and trends: Anaerobic digester facts and trends. United States Environmental Protection Agency.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. Journal of Food Protection 67: 685-690.

Farkas, R., J. A. Hogsette, and L. Börzsönyi. 1998. Development of *Hydrotaea aenescens* and *Musca domestica* (Diptera: Muscidae) in poultry and pig manures of different moisture content. Environmental entomology 27: 695-699.

Fincher, G. T. 1973. Dung beetles as biological control agents for gastrointestinal parasites of livestock. The Journal of Parasitology: 396-399.

Furman, D. P., R. D. Young, and E. P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52: 917-921.

Geden, C. J. 2012. Status of biopesticides for control of house flies. Journal of Biopesticides 5: 1.

Georghiou, G. P. 1972. The evolution of resistance to pesticides. Annual Review of Ecology and Systematics 3: 133-168.

Gold, M., J. K. Tomberlin, S. Diener, C. Zurbrügg, and A. Mathys. 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. Waste Management 82: 302-318.

Gutser, R., T. Ebertseder, A. Weber, M. Schraml, and U. Schmidhalter. 2005. Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. Journal of Plant Nutrition and Soil Science 168: 439-446.

Hall, R. D., and M. C. Foehse. 1980. Laboratory and field tests of CGA-72662 for control of the house fly and face fly in poultry, bovine, or swine manure. Journal of Economic Entomology 73: 564-569.

Hayaoka, T., and W. Dauterman. 1982. Induction of glutathione S-transferase by phenobarbital and pesticides in various house fly strains and its effect on toxicity. Pesticide Biochemistry and Physiology 17: 113-119.

Heussler, C. D., A. Walter, H. Oberkofler, H. Insam, W. Arthofer, B. C. Schlick-Steiner, and F. M. Steiner. 2018. Influence of three artificial light sources on oviposition and half-life of the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae): Improving small-scale indoor rearing. PloS ONE 13: e0197896.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Holmes, L., S. Vanlaerhoven, and J. Tomberlin. 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). Environmental Entomology 41: 971-978.

Jones, M. S., Z. Fu, J. P. Reganold, D. S. Karp, T. E. Besser, J. M. Tylianakis, and W. E. Snyder. 2019. Organic farming promotes biotic resistance to foodborne human pathogens. Journal of Applied Ecology.

Khan, H. A. A., S. A. Shad, and W. Akram. 2012. Effect of livestock manures on the fitness of house fly, *Musca domestica* L. (Diptera: Muscidae). Parasitology Research 111: 1165-1171.

Kováčik, P., M. Kozánek, P. Takáč, M. Galliková, and L. Varga. 2014. The effect of pig manure fermented by larvae of house flies on the yield parameters of sunflowers (*Helianthus annus* L.). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 58: 147-154.

Lähteenmäki-Uutela, A., N. Grmelová, L. Hénault-Ethier, M.-H. Deschamps, G. W. Vandenberg, A. Zhao, Y. Zhang, B. Yang, and V. Nemane. 2017. Insects as food and feed: Laws of the European Union, United States, Canada, Mexico, Australia, and China. European Food & Feed Law Review 12.

Lalander, C., S. Diener, M. E. Magri, C. Zurbrügg, A. Lindström, and B. Vinnerås. 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*)—From a hygiene aspect. Science of the Total Environment 458: 312-318.

Lalander, C., J. Senecal, M. G. Calvo, L. Ahrens, S. Josefsson, K. Wiberg, and B. Vinnerås. 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of the Total Environment 565: 279-286.

Larrain, P. S., and C. F. Salas. 2008. House fly (*Musca domestica* L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Lee, J. M., and Y.-S. Peng. 1981. Influence of adult size of *Onthophagus gazella* on manure pat degradation, nest construction, and progeny size. Environmental Entomology 10: 626-630.

Li, Q., L. Zheng, Y. Hou, S. Yang, and Z. Yu. 2011a. Insect fat, a promising resource for biodiesel. Journal of Petroleum and Environmental Biotechnology S2: S2.

Li, Q., L. Zheng, H. Cai, E. Garza, Z. Yu, and S. Zhou. 2011b. From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. Fuel 90: 1545-1548.

Li, S., H. Ji, B. Zhang, J. Zhou, and H. Yu. 2017. Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture.

Liu, Q., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37: 1525-1530.

Lu, X., G. Zhen, Y. Liu, T. Hojo, A. L. Estrada, and Y.-Y. Li. 2014. Long-term effect of the antibiotic cefalexin on methane production during waste activated sludge anaerobic digestion. Bioresource Technology 169: 644-651.

MacDonald, J. M. 2009. Manure use for fertilizer and for energy: report to congress, DIANE Publishing.

Makkar, H. P. S., G. Tran, V. Henze, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197: 1-33.

Malik, A., N. Singh, and S. Satya. 2007. House fly (*Musca domestica*): A review of control strategies for a challenging pest. Journal of Environmental Science and Health Part B 42: 453-469.

Mallin, M. A., J. M. Burkholder, M. R. McIver, G. C. Shank, H. B. Glasgow, B. W. Touchette, and J. Springer. 1997. Comparative effects of poultry and swine waste lagoon spills on the quality of receiving streamwaters. Journal of Environmental Quality 26: 1622-1631.

Miller, J. A., S. E. Kunz, D. D. Oehler, and R. W. Miller. 1981. Larvicidal activity of Merck MK-933, an avermectin, against the horn fly, stable fly, face fly, and house fly. Journal of Economic Entomology 74: 608-611.

Miller, R., J. Major, and P. Trinca. 2011. How a lagoon works for livestock Wastewater Treatment.

Mohr, C. O. 1943. Cattle droppings as ecological units. Ecological Monographs 13: 275-298.

Morrison, P. E., and D. M. Davies. 1964. Feeding of dry chemically defined diets + egg production in adult house-fly. Nature 201: 104-105.

Müller, A., D. Wolf, and H. O. Gutzeit. 2017. The black soldier fly, *Hermetia illucens*– a promising source for sustainable production of proteins, lipids and bioactive substances. Zeitschrift für Naturforschung C 72: 351-363.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Nakamura, S., R. T. Ichiki, M. Shimoda, and S. Morioka. 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. Applied Entomology and Zoology 51: 161-166.

Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. Journal of Animal Science 44: 395-400.

Nichols, E., S. Spector, J. Louzada, T. Larsen, S. Amezquita, M. Favila, and T. S. R. Network. 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biological Conservation 141: 1461-1474.

Nordentoft, S., C. Fischer, L. Bjerrum, L. Heckmann, and B. Hald. 2017. Reduction of *Escherichia coli*, *Salmonella* Enteritidis and *Campylobacter jejuni* in poultry manure by rearing of *Musca domestica* fly larvae. Journal of Insects as Food and Feed 3: 145-153.

Pastor, B., H. Čičková, M. Kozanek, A. Martinez-Sanchez, P. Takac, and S. Rojo. 2011. Effect of the size of the pupae, adult diet, oviposition substrate and adult population density on egg production in *Musca domestica* (Diptera: Muscidae). European Journal of Entomology 108: 587-596.

Rabalais, N. N., R. E. Turner, and W. J. Wiseman Jr. 2002. Gulf of Mexico hypoxia, aka “The dead zone”. Annual Review of Ecology and Systematics 33: 235-263.

Sasaki, T., M. Kobayashi, and N. Agui. 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera : Muscidae) in the dissemination of *Escherichia coli* O157 : H7 to food. Journal of Medical Entomology 37: 945-949.

Sawicki, R., and K. Lord. 1970. Some properties of a mechanism delaying penetration of insecticides into houseflies. Journal of Pesticide Science 1: 213-217.

Schremmer, F. 1984. The polymetabol development of the soldier fly larva *Hermetia illucens*.-A contribution to the metamorphosis of the Stratiomyidae. Annalen des Naturhistorischen Museums in Wien. Serie B für Botanik und Zoologie: 405-429.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value-added manure management-system using the black soldier fly. Bioresource Technology 50: 275-279.

Shober, A. L., and J. T. Sims. 2003. Phosphorus restrictions for land application of biosolids. Journal of Environmental Quality 32: 1955-1964.

Slade, E. M., T. Riutta, T. Roslin, and H. L. Tuomisto. 2016. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Scientific Reports 6: 18140.

Smith, M. D., A. Oglend, A. J. Kirkpatrick, F. Asche, L. S. Bennear, J. K. Craig, and J. M. Nance. 2017. Seafood prices reveal impacts of a major ecological disturbance. Proceedings of the National Academy of Sciences 114: 1512-1517.

Sullivan, R. L., and R. R. Sokal. 1963. Effects of larval density on several strains of house-fly. Ecology 44: 120-&.

Teotia, J. S., and B. F. Miller. 1973. Environmental conditions affecting development of house fly larvae in poultry manure. Environmental Entomology 2: 329-333.

Teotia, J. S., and B. F. Miller. 1974. Nutritive content of house-fly pupae and manure residue. British Poultry Science 15: 177-182.

Tomberlin, J. K., and D. C. Sheppard. 2002. Factors influencing mating and oviposition of black soldier flies (Diptera : Stratiomyidae) in a colony. Journal of Entomological Science 37: 345-352.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera : Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the Black Soldier Fly (Diptera: Stratiomyidae) in Relation to Temperature. Environmental Entomology 38: 930-934.

Tomberlin, J. K., T. L. Crippen, G. Wu, A. S. Griffin, T. K. Wood, and R. M. Kilner. 2016. Indole: An evolutionarily conserved influencer of behavior across kingdoms. BioEssays.

USDA. 2006. Annual report: Manure and by-product utilization. United States Department of Agriculture—Agricultural Research Service. 2005.

USDA. 2008. Agriculture Waste Management Field Handbook.

USDA. 2010. Overview of the United States dairy industry: Large operations increase share of inventory and milk production.

USDA. 2015. Overview of the United States hog industry: Breeding herd efficiency continues to increase.

USDA. 2017. Overview of U.S. livestock, poultry, and aquaculture production in 2017.

van Huis, A. 2019. Manure and flies: biodegradation and/or bioconversion? Wageningen Academic Publishers.

van Huis, A., M. Dicke, and J. Van Loon. 2015. Insects to feed the world. Wageningen Academic Publishers.

van Huis, A., J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, and P. Vantomme. 2013. Edible insects: future prospects for food and feed security, Food and Agriculture Organization of the United nations (FAO).

van Zanten, H. H., H. Mollenhorst, D. G. Oonincx, P. Bikker, B. G. Meerburg, and I. J. de Boer. 2015. From environmental nuisance to environmental opportunity: house fly larvae convert waste to livestock feed. Journal of Cleaner Production 102: 362-369.

Verheyen, G. R., T. Ooms, L. Vogels, S. Vreysen, A. Bovy, S. M. Van, and F. Meersman. 2018. Insects as an alternative source for the production of fats for cosmetics. Journal of Cosmetic Science 69: 187-202.

Wang, H., Z. Zhang, G. F. Czapar, M. K. Winkler, and J. Zheng. 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. Waste Management & Research 31: 223-231.

Wang, W., W. Zhang, X. Wang, C. Lei, R. Tang, F. Zhang, Q. Yang, and F. Zhu. 2017. Tracing heavy metals in ‘swine manure-maggot-chicken’ production chain. Scientific Reports 7: 8417.

Westerman, P., L. D. King, J. Burns, G. Cummings, and M. Overcash. 1987. Swine manure and lagoon effluent applied to a temperate forage mixture: II. Rainfall runoff and soil chemical properties 1. Journal of Environmental Quality 16: 106-112.

Wingo, C., G. Thomas, G. Clark, and C. Morgan. 1974. Succession and abundance of insects in pasture manure: relationship to face fly survival. Annals of the Entomological Society of America 67: 386-390.

Wright, R. J. 1998. Agricultural uses of municipal, animal, and industrial byproducts.

Yang, S., Q. Li, Y. Gao, L. Zheng, and Z. Liu. 2014. Biodiesel production from swine manure via house fly larvae (*Musca domestica* L.). Renewable Energy 66: 222-227.

Zhang, J., L. Huang, J. He, J. K. Tomberlin, J. Li, C. Lei, M. Sun, Z. Liu, and Z. Yu. 2010. An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. Journal of Insect Science (Tucson) 10: 1-7.

Zhang, Z., H. Wang, J. Zhu, S. Suneethi, and J. Zheng. 2012. Swine manure vermicomposting via house fly larvae (*Musca domestica*): The dynamics of biochemical and microbial features. Bioresource Technology 118: 563-571.

2. LIFE-HISTORY TRAITS OF THE BLACK SOLDIER FLY, *Hermetia illucens* (L.) (DIPTERA: STRATIOMYIDAE), REARED ON THREE MANURE TYPES[*]

Overview

Structural changes and growth of animal production systems have resulted in greater manure volumes. Current manure storage methods pose a potential environmental threat. Lessening these issues is a key concern for the industry. The primary aim of this research was to evaluate black soldier fly (BSF) performance when fed poultry, swine, or dairy manure at different rates (18 or 27 g/ 2 d until 40% prepupation). Results indicated larvae fed the control diet (Gainesville diet) resulted in the heaviest larvae (+ 31–70%); however, for other life-history traits, those fed the higher feed rate of poultry manure produced comparable results to the control. Larvae fed more resource, regardless of manure type, weighed more as larvae (+ 3–9%), pupae (+ 22–48%), and adults (+ 18–42%), developed faster (up to 3–4 d), had a higher percentage reach the prepupal stage (+ 2–16%), lived longer as adults (+ 1 d), and converted more resource to biomass (up to 1% more) than those fed at the lower rate. Yet, no difference was detected in dry matter (DM) reduction across feed rate for a given manure type. Based on these results, all three manure types can be digested by black soldier fly larvae thus demonstrating its potential for waste management.

---

[*] Reprinted with permission from "Life-history traits of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), reared on three manure types" by Chelsea Miranda, Jonathan Cammack, and Jeffery Tomberlin, 2019. *Animals* 9, 281. Copyright [2019] by Chelsea Miranda, Jonathan Cammack, and Jeffery Tomberlin.

Introduction

Continued growth of the human population raises concern with regards to food security. Soymeal, fishmeal, and other protein sources may become limited, thus increasing the demand for alternative diet components for livestock and poultry (Boland et al. 2013, Makkar et al. 2014). Similarly, the recent increase in the number of confined animal facilities (USDA 2010, 2015) due to changes in production costs, results in large quantities of manure that must be properly managed. For example, in the United States (US) in 2012, factory-farmed livestock produced approximately 13 times the amount of sewage produced by the entire US population (FWW 2015).

Currently, alternative methods for waste management are being sought out to avoid issues with handling wastes. Manure from confined operations is typically stored in lagoons for long periods of time (6–12 months) (Pfost et al. 2000), posing an environmental risk. Rearing insects on animal manure could be an efficient solution for managing wastes, while also producing protein, but a better understanding of the bioconversion of different manure types by targeted insects is necessary to develop efficient systems.

One potential insect that could be utilized for waste management is the black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) (Sheppard et al. 1994, Myers et al. 2008, Oonincx et al. 2015, ur Rehman et al. 2017b). The black soldier fly is widely distributed in temperate and tropical regions throughout the world (James 1935, Callan 1974, Martínez-Sánchez et al. 2011, Tsagkarakis et al. 2015). Often found near confined animal feeding facilities, BSF recycle manure, thus reducing associated nitrogen (N), phosphorus (P) and dry matter (DM) by 50% or more (Sheppard 1983, Myers et al. 2008, Oonincx et al. 2015). Additionally, BSF larvae convert manure into a 35–44% protein (Sheppard et al. 1994, Zhou et al. 2013, Wang and Shelomi 2017) and 28 –35% fat biomass (dry weight basis) (Sheppard et al. 1994, Wang and Shelomi 2017). Some suggest they are a suitable substitute for fishmeal for aquaculture production, such as catfish (Ictalurus punctatus Rafinesque) (Bondari and Sheppard 1981), rainbow trout (Oncorhynchus mykiss Walbaum) (St-Hilaire et al. 2007, Sealey et al. 2011), blue tilapia (Tilapia aurea Steindachner) (Bondari and Sheppard 1981), and turbot (Psetta maxima Linnaeus) (Kroeckel et al. 2012). Additionally, BSF can be incorporated into poultry (Hale 1973), and swine (Newton et al. 1977) diets. The BSF is an ideal candidate for waste management because it is capable of reducing pathogens such as *Escherichia coli* (Liu et al. 2008) and *Salmonella spp*. (Lalander et al. 2015) and is known to reduce offensive odors such as phenols, indoles, and volatile fatty acids (Beskin et al. 2018). Lastly, lipids can be extracted from the larvae for biofuel production (Zheng et al. 2012), and the spent media remaining after larval feeding can be used as fertilizer (Čičková et al. 2015), providing other potential applications of this species. Though, one of the biggest obstacles BSF-fed-manure systems faces in the US is legislation. Currently, BSF are being sought out as alternative feed additives, but larvae fed manure are not permitted for food and feed purposes. Instead, BSF have been approved by the Association of American Feed Control Officials (AAFCO) for inclusion in aquaculture and poultry diets, but they must be fed 'feed grade materials' exclusively. This constraint for manure-fed BSF systems is due to the fact that manure is not regarded as safe as it is possible that larvae can bioaccumulate harmful compounds from manure. Charlton et al. (2015) investigated the safety potential of BSF exposed to different contaminants via manure (e.g., veterinary medications, pesticides, heavy metals, mycotoxins, dioxins, polychlorinated biphenyls, and polybrominated diphenyl ethers) and found that all levels for all compounds in the larvae were below the European Union or Codex regulatory limits. However, this is the only known study to explore these concerns and it was for one population in Ghana; therefore, more research should be conducted before BSF fed manure are deemed safe and incorporated into animal diets. Despite the legal limitations for protein production from BSF-fed-manure in the US, they are utilized to manage manure and produce protein in other areas of the world, such as China (personal communication, Tomberlin).

The purpose of this study was to evaluate select life-history trait (from egg to adult) performance of BSF larvae fed three types of manure (poultry, swine, and bovine) as well as their ability to reduce dry matter (DM) and convert it into protein. As discussed previously, this species is capable of reducing manure, which may be an efficient solution to current management concerns. Furthermore, BSF can convert animal waste into a protein-rich biomass, which may also provide alternative means for protein production. Results will supplement existing data on how BSF may be utilized in waste management systems.

Materials and Methods

Acquisition of BSF

This experiment was conducted using BSF larvae from a colony (established in 2014) maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University. This colony originated in 1998 from a colony at the Coastal Plain Experiment Station (University of Georgia) in Tifton, Georgia USA. The colony maintained at the F.L.I.E.S. Facility is reared according to methods detailed by Sheppard et al. (Sheppard et al. 2002).

Acquisition of Manure

Swine manure less than 12-h-old was collected from a local farm, in Anderson, TX, USA. Similarly, dairy manure was collected from a commercial dairy located in Stephenville, TX, USA, and poultry manure was collected from layer hens housed at the Poultry Science Research, Teaching and Extension Center (Texas A&M University, College Station, TX, USA). Manure was collected before each trial. Once collected, manure was placed into 18.9 L buckets with lids (Home Depot®, LeaktiteTM, Leominster, MA, USA) and transported to the F.L.I.E.S Facility, where it was homogenized (vigorously mixed by hand for approximately 5 minutes) and transferred to 3.76 L Ziploc® Freezer Bags and stored at -20°C until used. Manure was allowed to thaw at room temperature for 24 hours before initiation of the experiment. Manure not used on day one was placed in 1.9 L Reditainer™ EXTREME FREEZE™ deli containers (Clear Lake Enterprises, Port Richey, FL, USA) and stored at 4°C until used. Three 10 g samples of thawed manure of each type were used to determine initial moisture content gravimetrically (Franson 1989).

Chemical Content of Manure

Chemical content (total percent N, and minerals (ppm)) of manure was analyzed at the Texas A&M AgriLife Extension Service Soil, Water, and Forage Testing Laboratory in College Station, TX, USA. Total N, is determined by a combustion process and mineral (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) are determined by an inductive coupled plasma spectrometry of a nitric acid digest. The chemical composition of the manures used in this study are detailed in Table 2.1

Table 2.1 Chemical composition (mean ± SE, [1]n = 6) of swine, dairy, and poultry manure for the black soldier fly experiment.

%	**Swine**	**Dairy**	**Poultry**
N	2.10 ± 0.02^{A2}	2.10 ± 0.03^{A}	2.40 ± 0.04^{B}
P	2.42 ± 0.08^{A}	0.66 ± 0.11^{B}	2.43 ± 0.05^{A}
K	0.01 ± 0.02^{A}	0.57 ± 0.00^{B}	2.44 ± 0.01^{C}
Ca	4.36 ± 0.05^{A}	1.96 ± 0.19^{B}	14.0 ± 0.47^{C}
Mg	0.87 ± 0.03^{A}	0.67 ± 0.00^{B}	0.56 ± 0.00^{B}
Na	0.55 ± 0.01^{A}	0.32 ± 0.02^{B}	0.58 ± 0.00^{A}
Zn	0.08 ± 0.00^{A}	0.02 ± 0.00^{B}	0.04 ± 0.00^{C}
Fe	0.01 ± 0.00^{A}	0.27 ± 0.04^{B}	0.19 ± 0.02^{B}
Cu	0.01 ± 0.00^{A}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
Mn	0.05 ± 0.00^{A}	0.02 ± 0.00^{B}	0.05 ± 0.00^{A}
S	0.78 ± 0.01^{A}	0.41 ± 0.02^{B}	0.86 ± 0.00^{C}
B	0.08 ± 0.00^{A}	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}

[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments ([2]α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Chelsea Miranda, Jonathan Cammack, and Jeffery Tomberlin [2019].

Experiment Design

Adults were maintained in a 1.2 x 1.2 x 2.4 m wooden cage with each side of the cage lined with wire mesh (256 x 112 cm) in a greenhouse (25 –45°C, >50% RH) to provide natural sunlight for mating and oviposition. Corrugated cardboard was cut into 4.0 x 4.0 x 0.5 cm pieces and three pieces of cardboard were taped together to form a bundle. Three bundles of corrugated cardboard were placed on the lid of 5.7 L Sterilite® container with a 15 x 7 cm hole covered with wire cloth. The Sterilite® container was filled with 1000 g Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% corn meal) (Hogsette 1992) saturated with RO water and contained approximately 500 actively feeding larvae to enhance attraction and oviposition. Every four hours the cardboard was checked for egg clutches. The cardboard containing eggs was dissected and egg clutches removed and placed in a 0.5 L plastic container, covered with a paper towel held in place with a rubber band, and stored in a Rheem Environmental Chamber (Asheville, NC, USA) at 29˚C, 60% RH, and 16L:8D until larvae eclosed. Newly-hatched larvae were fed 200 g of Gainesville diet as described by Sheppard et al. (2002) to decrease larval mortality prior to use in the experiment.

Replicates consisted of 100 4-d-old larvae placed in 88 ml Great Value® Brand bathroom cups (Wal-Mart® Stores, Inc., Bentonville, AR, USA) and provided manure as described below. Cups were covered with a paper towel held in place with a rubber band and stored in the growth chamber described above. As a control, 100 4-d-old larvae were placed on Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% cornmeal) (Hogsette 1992) and the methods described by Tomberlin et al. (2002) were followed. Three replicates of each treatment and control were used and two trials were conducted.

Every other day, larvae in a treatment were fed their assigned manure type at a specified feed rate (18 g or 27 g) identified in preliminary experiments, or 27 g of Gainesville diet (10 g diet with 17 mL of water) (Tomberlin et al. 2002). Individual cups were each placed inside a 1.9 L Reditainer™ EXTREME FREEZE™ deli containers (Clear Lake Enterprises, Port Richey, FL, USA) covered with tulle fabric (Wal-Mart®

Stores, Inc., Bentonville, AR, USA) held in place with a rubber band. The containers were placed in a complete randomized block design in the incubator under the same conditions as previously described. The contents of the bathroom cups were dumped into the 1.9 L Reditainer™ EXTREME FREEZE™ deli containers (Clear Lake Enterprises, Port Richey, FL, USA) on day four due to concerns with moisture. Post-feeding larvae (identified in previously publications as prepupae) (Sheppard et al. 1994) were removed daily and feeding discontinued when 40% reached the prepupal stage as described by Tomberlin et al. (2002).

In order to minimize the effect of handling, three of the largest larvae observed were selected from each replicate every three days, individually weighed on an OHaus® Adventurer™ Pro AV64 balance (OHaus® Corporation, Pine Brook, NJ, USA), and returned to their respective container. The mean larval weight (of three larvae) recorded on the day that 40% of the larvae within a treatment reach the prepupal stage was recorded as the final larval weight.

Prepupae were removed daily from all treatments and weighed on the scale described above. Half of the prepupae produced were frozen at -20°C (in the freezer described above). Those remaining were placed individually in 35 ml cups, covered with a plastic lid with a cotton ball inserted through a hole in the center of the lid, and returned to the growth chamber as a means to evaluate adult emergence time and associated longevity (monitored daily). Tomberlin et al. (2002) suggested that individual adult flies provided 0.125 ml of water lived longer than flies not provided water. Therefore, the cotton ball was dampened daily with RO water and served as a source of water for the adults upon eclosion.

Percent DM Reduction

Percent DM reduction was determined as described by Zhou et al. (2013). The percent DM reduction was calculated with:

$$((W1-W2) / W1) \times 100\%$$

with W1 being the DM provided during the experiment and W2 being the DM remaining at the end of the experiment.

Bioconversion of Manure

To determine bioconversion, the total mass of all prepupae produced (wet weight) and amount of manure fed (wet weight) to each replicate over the course of the experiment were recorded. Percent bioconversion was calculated with:

$$(\text{total g of all prepupae} / \text{total g of manure fed}) \times 100\%$$

Protein Content of Larvae

To determine protein content of prepupae, samples were sent to SDK Laboratories in Hutchinson, KS, USA. Approximately 5 g of prepupae per replicate were needed for the protein analysis, which could not be obtained for the swine or dairy treatments. Therefore, statistical analyses were conducted only on prepupae fed poultry manure and Gainesville diet.

Statistical Analysis

Moisture content of manure, life-history traits (final larval weight, development time from newly-hatched larvae to prepupal stage, percent prepupation prepupal weight, adult male and female weight, adult male and female longevity), DM reduction, percent bioconversion, and protein content of prepupae, were analyzed across treatments. An ANOVA was performed for each parameter listed above using JMP 9.0.0 (SAS Institute

Inc., Cary, NC, USA). Levine's test for equal variances was used to check unequal variances and Tukey's HSD (honest significant difference) was used for mean separation ($p \leq 0.05$).

Results

Moisture Content of Manure

Initial moisture content differed significantly ($F_{3,20} = 296.1$; $p < 0.0001$) across manure types. No significant treatment by trial interaction ($F_{1, 20} = 0.1101$; $p = 0.9535$) was detected or trial effect ($F_{1,20} = 0.1278$; $p = 0.7241$) was detected. Initial moisture content ranged from approximately 74–84 %, with the highest found in dairy manure and lowest found in swine manure (Table 2.2).

Final Larval Weight

Final larval weight was significantly different ($F_{6, 28} = 10.95$; $p < 0.0001$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 1.510$; $p = 0.2113$) was found; however, a significant trial effect was ($F_{1, 28} = 19.75$; $p < 0.0001$). In general, individuals in trial one were 22% heavier than those in trial two. Furthermore, those provided Gainesville diet were the heaviest (0.20 g/larva) across trials (Table 2.3). With regards to treatment effect, those fed at the 18 g rate of poultry manure were the heaviest (0.14 g/larva). Those provide dairy or swine manure weighed 8% and 11% less than those provided the poultry manure, respectively. For those fed at the 27 g rate, the same pattern occurred. However, when comparing across feed rate for a manure type, larvae were in general 3-9% larger when provided more resource.

Table 2.2 Initial moisture contents (mean ± SE, [1]n = 3) of swine, dairy, and poultry manure, and Gainesville diet (Hogsette 1992) for the black soldier fly experiment.

Manure Type	Feed Amount (g/ 2 days)	Initial (%)
Swine	18	73.9 ± 0.41^{A}
	27	
Dairy	18	83.9 ± 0.63^{B}
	27	
Poultry	18	77.2 ± 0.29^{C}
	27	
Gainesville[2]	27	70.0 ± 0.56^{D}

[1] n = number of replicates per treatment

Different letters within a column indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

[2] Larvae were fed the standard diet (10 g of dry Gainesville diet + 17 mL of water) following the methods described by Tomberlin et al. (2002).

Reprinted with permission from Miranda et al. (2019).

Time to First Prepupation

Time to first observed prepupation was significantly different ($F_{6, 28} = 62.27$; $p < 0.0001$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 1.5775$; $p = 0.1906$) was found. However, a significant trial effect was found ($F_{1, 28} = 11.0423$; $p = 0.0025$). In general, individuals in trial two reached the prepupal stage 1 d faster than those in trial one. Furthermore, those provided Gainesville diet were the first to reach the prepupal stage, at 9 d after the experiment began (Table 2.3). With regards to treatment effect for those fed manure at the 18 g rate, larvae fed poultry manure reached the prepupal stage in 12 d. Those provide dairy or swine manure required 7 and 9 d longer to reach the prepupal stage than those provided the poultry manure, respectively. For those fed at the 27 g rate, the same pattern occurred. However, when comparing across feed rate for a manure type, larvae reached the prepupal stage up to 3–4 d faster when provided more resource.

Prepupal Weight

Prepupal weight was significantly different ($F_{6, 28} = 47.94$; $p < 0.0001$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 1.430$; $p = 0.2366$) was found, but a significant trial effect ($F_{1, 28} = 19.32$; $p < 0.0001$) was. In general, individuals in trial one were approximately 13% larger than those in trial two. Furthermore, those provided Gainesville diet were the heaviest (0.13 g/larva) across trials (Table 2.3). With regards to treatment effect, those fed at the 18 g rate, larvae reared on poultry manure were the heaviest (0.09 g/larva). Those provided dairy or swine manure weighed 23% and 22% less than those provided the poultry manure, respectively. For those fed at the 27 g rate, the same pattern occurred. Still, when comparing across feed rate for a manure type, larvae fed higher feed amounts were 22 –48% larger.

Percent Prepupation

Percent pupation was significantly different ($F_{6, 28} = 6.170$; $p = 0.0004$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 0.250$; $p = 0.9545$) or trial effect ($F_{1, 28} = 1.940$; $p = 0.1751$) were found. With regards to treatment effect, larvae fed the Gainesville diet resulted in 86% pupation (Table 2.3), whereas for those fed manure at the 18 g rate, larvae reared on poultry manure resulted in the highest pupation percentage (94%), while those fed dairy (77%) or swine (70%) manure had fewer individuals reach the prepupal stage. A similar trend was observed for the 27 g rate. When comparing across feed rate for manure type, pupation in general was approximately 2–16% higher when larvae were provided more resource.

Adult Male Weight

Adult male weight was significantly different across larval diets and feed rates ($F_{6, 28} = 55.08$; $p < 0.0001$). No significant treatment by trial interaction ($F_{6, 28} = 2.330$; $p = 0.0596$) was found, but a significant ($F_{1, 28} = 21.41$; $p < 0.0001$) trial effect was. In general, individuals in trial one were approximately 14% heavier than those in trial two. Furthermore, those provided Gainesville diet were the heaviest (0.05 g/adult) (Table 2.3). With regards to treatment effect, for those fed at the 18 g rate, males fed poultry manure were the heaviest (0.04 g/adult), while those provide dairy or swine manure weighed 27% and 26% less, respectively. For those fed at the 27 g rate, the same pattern occurred. However, when comparing across feed rate for a manure type, males were in general 18 –30% larger when provided more resource.

Adult Male Longevity

Diet and feed rate had a significant impact on adult male longevity ($F_{6, 28} = 21.92$; $p < 0.0001$). No significant treatment by trial interaction ($F_{6, 28} = 2.170$; $p = 0.0758$) or trial effect ($F_{1, 28} = 2.960$; $p = 0.0962$) were found. Individuals provided Gainesville diet lived approximately 8 days (Table 2.3). In regard to treatment effect, for those fed at the 18 g rate, larvae fed poultry manure lived approximately 7 d, while those provided dairy or swine manure lived 29% and 32% less, respectfully. Similar results were observed for those fed the 27 g rate. However, when comparing across feed rate for a manure type, males lived in general 12 –14% longer when provided more resource.

Table 2.3 Comparison of life-history traits (mean ± SE, [1]n = 6) of black soldier fly larvae fed swine, dairy, or poultry manure at two feed rates or Gainesville diet (Hogsette 1992) every other day at 29˚C, 60% RH, and 16L:8D.

Treatment		Final Larval Weight (g)	Time to First Prepupation (d)	Prepupal Weight (g)	Percent Prepupation	Adult Male Weight (g)	Adult Male Longevity (d)	Adult Female Weight (g)	Adult Female Longevity (d)
Swine	18	0.1264 ± 0.0063^{A}	20 ± 1.1^{A}	0.0678 ± 0.0029^{A}	70 ± 5. 3^{A}	0.0273 ± 0.0016^{A}	4.9 ± 0.3^{A}	0.0320 ± 0.0018^{A}	4.7 ± 0.1^{A}
	27	0.1189 ± 0.0132^{A}	17 ± 0.6^{BC}	0.0827 ± 0.0009^{AB}	84 ± 6.0^{ABC}	0.0321 ± 0.0019^{B}	5.5 ± 0.2^{A}	0.0405 ± 0.0028^{BC}	5.1 ± 0.2^{AB}
Dairy	18	0.1306 ± 0.0093^{A}	19 ± 0.7^{AB}	0.0668 ± 0.0016^{A}	77 ± 3.4^{AB}	0.0271 ± 0.0004^{A}	5.1 ± 0.2^{A}	0.0330 ± 0.0009^{BC}	5.9 ± 0.2^{A}
	27	0.1339 ± 0.0118^{A}	16 ± 0.6^{C}	0.0990 ± 0.0054^{BC}	93 ± 3.1^{BC}	0.0334 ± 0.0017^{BC}	5.7 ± 0.4^{A}	0.0427 ± 0.0023^{B}	6.0 ± 0.2^{BC}
Poultry	18	0.1419 ± 0.0089^{A}	12 ± 0.2^{D}	0.0866 ± 0.0016^{AB}	94 ± 2.7^{BC}	0.0371 ± 0.0012^{C}	7.2 ± 0.2^{B}	0.0420 ± 0.0019^{BC}	6.5 ± 0.2^{CD}
	27	0.1541 ± 0.0168^{A}	11 ± 0.3^{DE}	0.1137 ± 0.0085^{CD}	95 ± 1.7^{C}	0.0483 ± 0.0022^{D}	8.2 ± 0.3^{C}	0.0598 ± 0.0044^{C}	7.2 ± 0.3^{D}
Gainesville[2]	27	0.2019 ± 0.0020^{B}	9.0 ± 0.0^{E}	0.1324 ± 0.0008^{D}	86 ± 2.9^{ABC}	0.0516 ± 0.0014^{D}	8.1 ± 0.1^{BC}	0.0653 ± 0.0014^{C}	6.7 ± 0.3^{CD}

[1] n = treatments per replicate.

Different letters within a column indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

[2] Larvae were fed the standard diet (10 g of dry Gainesville diet + 17 mL of water) following the methods described by Tomberlin et al. (2002).

Reprinted with permission from Miranda et al. (2019).

Adult Female Weight

Adult female weight was significantly different ($F_{6, 28} = 27.47$; $p < 0.0001$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 2.110$; $p = 0.0837$) was found. However, a significant ($F_{1, 28} = 14.78$; $p = 0.0006$) trial effect was found. In general, females in trial one were 15% heavier than those in trial two. Individuals provided Gainesville diet were the heaviest (0.07 g/adult) (Table 2.3.). In regard to treatment effect, for those fed at the 18 g rate, larvae fed poultry manure were the heaviest (0.04 g/adult), while those provide dairy or swine manure weighed 21% and 24% less, respectively. The same pattern occurred for those fed the 27 g rate. Similarly, when comparing across feed rate for a manure type, females were in general 27 –42% larger when provided more resource.

Adult Female Longevity

Adult female longevity was significantly different ($F_{6, 28} = 21.66$; $p < 0.0001$) across larval diets and feed rates. No significant treatment by trial interaction ($F_{6, 28} = 2.040$; $p = 0.0942$) was found. Additionally, no significant ($F_{1, 28} = 0.020$; $p = 0.8883$) trial effect was found. Individuals provided Gainesville diet lived approximately 6.7 d. In regard to treatment effect (Table 2.3.), for those fed at the 18 g rate, larvae fed poultry manure lived 6.5 d, while those provided dairy or swine manure lived 9% and 28% less, respectfully. For those fed at the 27 g rate, the same trend occurred. Though, when comparing across feed rate for a manure type, females lived in general 2–11% longer when provided more resource.

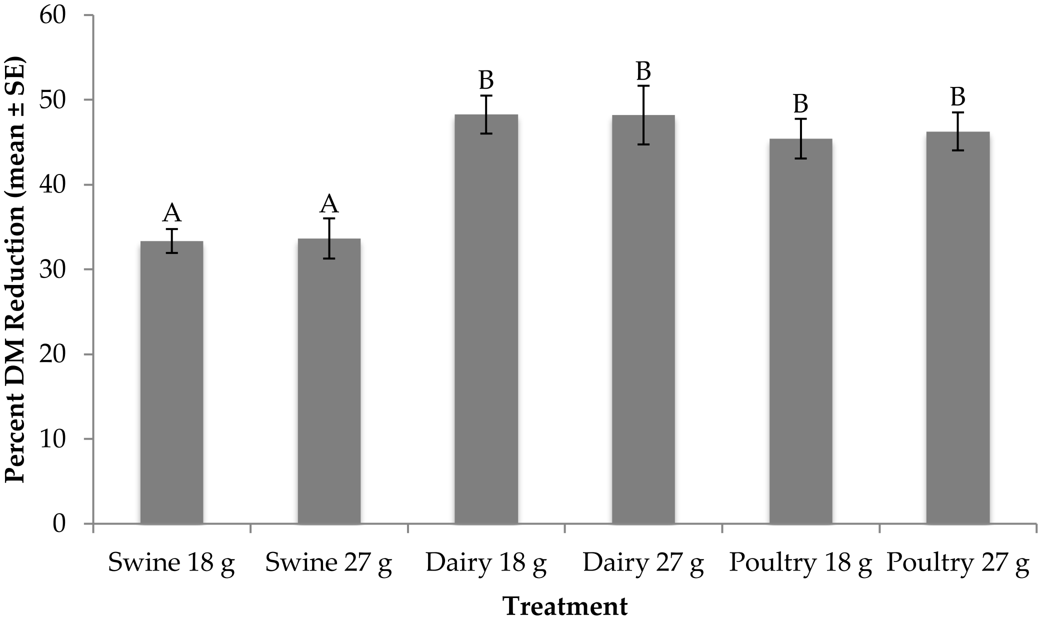
Percent DM Reduction

Percent DM reduction was significantly different across larval diets and feed rates ($F_{5, 24} = 7.780$; $p = 0.0002$). No significant treatment by trial interaction ($F_{5, 24} = 0.060$; $p = 0.9971$) or trial effect ($F_{1, 24} = 2.960$; $p = 0.0984$) was found. In regard to treatment effect, for those fed the 18 g rate, larvae provided dairy manure resulted in the highest DM reduction (48%), while those provided poultry and swine reduced the DM by 2% and 15% less, respectively (Figure 2.1). A similar trend occurred for those fed the 27 g rate. Yet, when comparing across feed rate, individuals fed less dairy or swine manure reduced DM by 1 –2% more; however, more DM was reduced for those fed the higher feed rate of poultry manure.

Percent Bioconversion

Significant differences ($F_{5, 24} = 44.23$; $p < 0.0001$) in percent bioconversion were found across larval diets and feed rates. No significant treatment by trial interaction ($F_{5, 24} = 2.340$; $p = 0.0727$) or trial effect ($F_{1, 24} = 1.250$; $p = 0.2742$) were found. In regard to treatment effect, for those fed the 18 g rate, larvae provided poultry manure resulted in the highest bioconversion (5.6%), while those fed swine and dairy converted 1.8% and 2.1% of the manure into biomass, respectively (Figure 2.2). A similar trend occurred for those fed the 27 g rate. Yet, when comparing across feed rate for a manure type, larvae fed more manure converted up to 1% more resource to biomass than those fed less.

Figure 2.1 Percent dry matter (DM) reduction (mean ± SE, [1]n = 6) for black soldier fly larvae fed swine, dairy or poultry manure at two feed rates every other day at 29 °C, 60% RH, and 16L:8D.

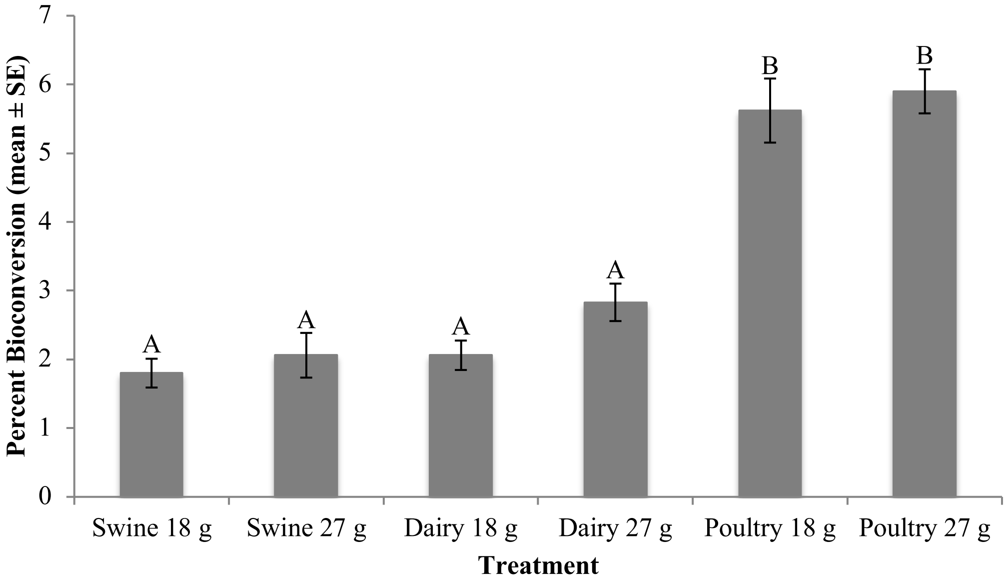


[1] n = number of replicates per treatment

Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Reprinted with permission from Miranda et al. (2019).

Figure 2.2 Percent bioconversion (mean ± SE, [1]n = 6) for black soldier fly larvae fed swine, dairy or poultry manure at two feed rates every other day at 29 °C, 60% RH, and 16L:8D.

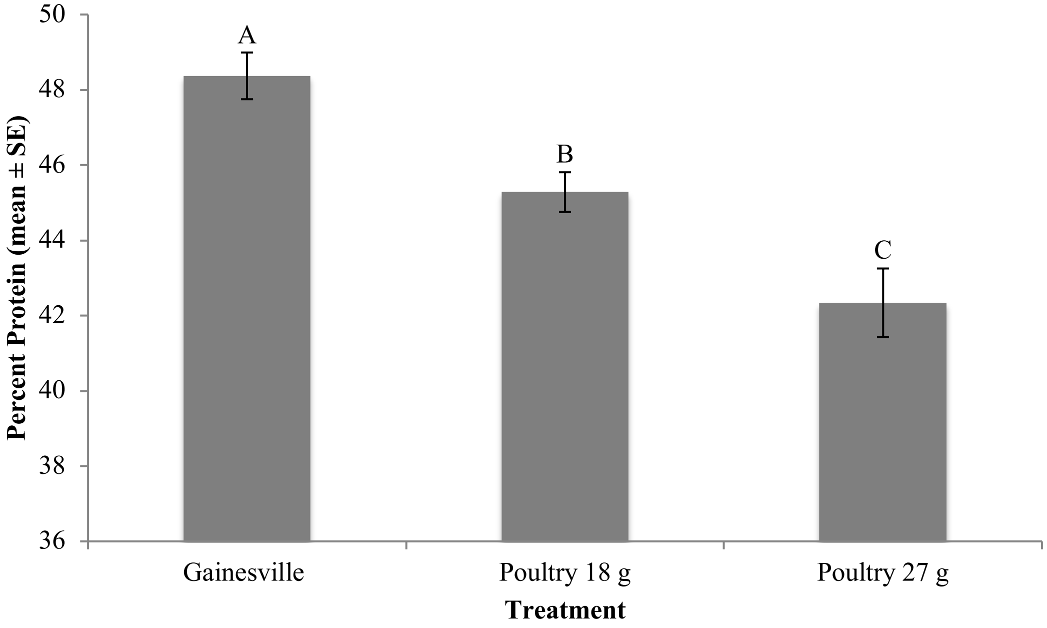


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Miranda et al. (2019).

Protein Content of Prepupae

Protein content of prepupae was significantly affected by diet ($F_{2, 12} = 18.70$; $p = 0.0002$). No significant treatment by trial interaction ($F_{2, 12} = 1.410$; $p = 0.2827$) or trial effect ($F_{1, 12} = 0.570$; $p = 0.4657$) were found. Prepupae produced from the Gainesville diet contained approximately 48% protein, while those produced from the 18 or 27 g of poultry treatments contained approximately 3 points and 6 points less, respectively (Figure 2.3). When comparing across feed rate for poultry manure, prepupae produced on the lower feed rate had higher protein content than those fed more manure.

Figure 2.3 Percent protein (mean ± SE, [1]n = 6) for black soldier fly larvae fed Gainesville diet (Hogsette 1992) or poultry manure at two feed rates every other day at 29 °C, 60% RH, and 16L:8D.



[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Miranda et al. (2019).

Discussion

Results from this study indicate manure type and feed rate significantly influence the development of BSF. Regardless of feed rate, larvae fed poultry manure weighed more as prepupae (15 –37%), developed faster (4–9 days), had a higher percentage reach the prepupal stage (2–24%), lived longer as adults (1–2 d) and converted more resource to biomass (3–4%) than those fed dairy or swine manure (Table 2.3). Furthermore, when comparing individuals within a manure type, larvae fed the higher feed rate weighed more as larvae (3–9%), pupae (22–48%), and adults (18–42%), developed faster (up to 3–4 d), had a higher percentage reach the prepupal stage (2 –16%), lived longer as adults

(1 d), and converted more resource to biomass (up to 1% more) than those fed at the lower rate. However, no differences were detected across feed rate for a given manure type for DM reduction. These data are necessary in optimizing biomass production and waste management practices.

There are only two known studies that concurrently compared the development of BSF on different manure types (Zhou et al. 2013, Oonincx et al. 2015). Results from the current study agree with those reported by Zhou et al. (2013), who found that larvae of three different BSF strains (one from Texas and two from China) fed poultry manure, weighed on average 17 –28% and 134 –208% more than those fed swine and dairy manure, respectively. Specifically, larvae that originated from the Texas strain fed poultry manure weighed 28% and 208% more than those fed swine and dairy manure, respectively. Larvae from the current study fed poultry manure weighed 9 –12% and 15 –30% more than those fed swine or dairy manure, respectively. However, larvae from the previous study were dried, whereas our results are reported on a wet-weight basis and our measurements are based on the average weight of three larvae, which impacts the mean value. Additionally, in the previous study, 300 6-d-old larvae were fed ad libitum, which differs from the age of larvae initially inoculated into the manure, larval density, and feeding regimen used in the current study. Still, a similar trend occurred when comparing the studies demonstrating that those fed poultry manure weighed more than those fed swine or dairy manure. In a similar study, Oonincx et al. (2015) placed 100 newly-hatched larvae on poultry, swine, and dairy manure, that had been previously dehydrated and then rehydrated to 66% moisture. This study focused on different life-history traits than the study by Zhou et al. (2013): survivorship and development time to the first prepupae. They found that larvae fed swine manure resulted in higher larval survivorship (97%) compared to those fed poultry (82%) and dairy (88%) manures. Although Oonincx et al. (2015) measured larval survivorship to the first prepupa was detected and our study measured total number to successfully reach the prepupal stage, results from the current study do not agree with those reported by Oonincx et al. (2015) as larvae fed poultry manure resulted in higher percent prepupation (94 –95%) compared to those fed swine (70 –84%) and dairy (77 –93%) (Table 2.3). Additionally, Oonincx et al. (2015) found that the development time for those fed swine manure was not significantly different from than to those fed poultry manure, which was not the case in our study (Table 2.3); larvae reared on swine manure took 5 –8 days more than when reared on poultry manure. Differences in the results between the current study and Oonincx et al. (2015) could be due to differences in the chemical composition of the manures tested or methods employed. The N content in the chicken manure used by Oonincx et al. (2015) was higher (4.8 vs 2.1%) compared to the current study but was similar for swine and dairy. However, for P, manure used in the current study was higher for poultry manure (2.43 vs 1.24%). Also, they dehydrated and then rehydrated the manures, which likely reduced the microorganisms and significantly extended the BSF development to 144 –215 d. It is known that microorganisms impact BSF development (Yu et al. 2011) and moisture impacts microbial growth (Ishii et al. 2009); therefore, drying the manure may have influenced the results reported by Oonincx et al. (2015). However, if BSF fed manure are intended to be used as a feed additive, a pre-treatment of the manure, such as drying seems necessary to circumvent safety concerns. Future research should explore pre-treatment options for BSF fed manure.

Past research has indicated nutritional composition of the diet can impact immature (Cammack and Tomberlin 2017) and adult life-history traits (Gobbi et al. 2013). Black soldier flies are able to develop in various types of wastes, which makes them ideal candidates to manage multiple waste streams. The larvae are considered generalist feeders capable of digesting a wide range of organic wastes (Nguyen et al. 2013). However, when compared to other substrates, such as poultry feed, kitchen wastes, liver, and fish renderings, BSF fed swine manure consumed the resource at a slower rate (Nguyen et al. 2015) and weighed 8 –40% less (Nguyen et al. 2013). These results are expected as manure contains less energy and nutrients than the other waste types (Nguyen et al. 2013, Nguyen et al. 2015).

Different types of manure have different chemical contents (Table 2.1). As such, variations in observed life-history traits of flies fed different manure types are expected. For example, poultry manure is typically higher in protein, amino acids, and minerals and lower in fiber than dairy manure (Martin et al. 1983, Chen et al. 2003). Furthermore, these manures vary in the ratio of nutrients and moisture (Chen et al. 2003), which is known to impact BSF development. Black soldier flies fed a balanced diet of protein and carbohydrates (21:21, each at 21% of the diet) developed faster and consumed less compared to those fed protein- (35:7) or carbohydrate- (7:35) rich diets (Cammack and Tomberlin 2017). Additionally, moisture content of the substrate influences BSF development (Cammack and Tomberlin 2017, Cheng et al. 2017), and this may have also impacted our results, as initial moisture content for the manures used in this study varied by 7 –10 % (Table 2.2). Larval nutrition is important, especially for a species like H. illucens that rely on their fat bodies acquired during larval development to sustain their adult livelihood. For example, Gobbi et al. (2013) showed that larval diet influenced adult wing size and ovarian development in BSF, providing evidence that larval nutrition can impact population dynamics. Still, inconclusive results have been reported for the effects of different larval diets on other adult traits such as longevity and egg production (Cammack and Tomberlin 2017). This is important because past research on other flies has suggested that longevity and egg production may be inversely related (Fletcher et al. 1990) and it has been postulated that they may be influenced by the duration of emergence time (Cammack and Tomberlin 2017) or delays in mating (Tomberlin et al. 2002) as resources are being reallocated to sustain livelihood, rather than directed towards reproductive purposes. In the current study, larvae fed poultry manure resulted in heavier adults with increased longevity (Table 2.3). As such, future studies should investigate differences in ovipositional preference for those fed manure to optimize production systems.

Amount of resource provided to larvae can influence life-history traits. Myers et al. (2008) fed 300 4-d-old larvae 27, 40, 54, or 70 g of dairy manure daily. For the purposes of comparing the aforementioned results with ours, we will focus on those fed 27 g and 40 g daily, as these feed rates are most similar to ours. Results from the study by Myers et al. (2008) indicate that feeding more resource (40 vs. 27 g/d) decreased development time by 6%, increased prepupal weight by 33%, increased adult weight by 35% and longevity by 15 %; however, 22% more DM was reduced by feeding less resource. Our results show larvae fed more dairy manure also have shorter development times (by 3d), are larger as prepupae (by 48%) and adults (by 23 –29%) and live longer as adults (by 2 –12%). Albeit, those fed more dairy manure reduced less DM in our study, the difference across feed rates was not as great as reported by Myers et al. (2008) and may be considered negligible (0.12% difference). Other differences between Myers et al. (2008) and our study may be due to differences in the density (100 vs. 300 larvae) and feeding frequency (feeding daily vs. every other day). Additionally, different results may be expected in systems that utilize bulk-feeding regimes as Banks et al. (2014) showed that 100 larvae fed one bulk feeding (of human feces) develop slower but weigh 19% more than those fed incrementally, but there was no significant difference in waste reduction capabilities between the two feeding regimes. However, significant differences ($p \leq 0.05$) were detected for waste reduction for other densities tested (1 and 10 larvae/treatment) with higher reductions occurring in incremental systems. These findings show that feeding regimen is relevant in terms optimizing biomass production or waste management as incremental systems may extend development by one day compared to batch feeding systems (Meneguz et al. 2018). Nevertheless, higher biomass is desired, feeding one bulk feeding (Banks et al. 2014) or more resource (in incremental systems) (Myers et al. 2008) is recommended. However, for waste management purposes, increased DM reduction is achieved in incremental systems (Banks et al. 2014) with less resource (Myers et al. 2008).

From an industrial perspective, co-digestion of different wastes may be an alternative method to increase larval production efficiency on lower quality substrates. In the current study, larvae fed dairy manure reduced 2 –15% more DM than those fed poultry or swine manure (Figure 2.1). However, more resource was converted to larval biomass for those fed poultry manure (Figure 2.2). Dry matter and bioconversion were not measured for the control diet in this study, which was an oversight. Such data should be included in future studies with similar questions being addressed. Still, co-digestion of dairy manure with poultry manure offers a way to manage multiple waste streams while increasing DM reduction and bioconversion, as well as increasing survivorship and decreasing development time (ur Rehman et al. 2017b). A similar trend for increased DM reduction, bioconversion, and survivorship, along with decreased development time was observed when dairy manure was co-digested with soybean curd (ur Rehman et al. 2017a). Likewise, mixing wastes offers the opportunity to manipulate the nutrient content of the larvae. For example, fish offal can be mixed with dairy manure to increase larval biomass as well as enrich larvae with omega-3 fatty acids (St-Hilaire et al. 2007). Such systems that involve co-digestion should be further investigated to improve BSF production and waste management systems.

Sheppard et al. (1994) suggested utilizing BSF for on-farm poultry manure management. This species is an attractive means to manage animal waste because they can break down cellulose, lignocellulose, and hemicellulose (ur Rehman et al. 2017b). Additionally, they deter house fly, *Musca domestica* L. (Diptera: Muscidae) oviposition (Furman et al. 1959), reduce manure accumulation and nutrients by 50% or more (Sheppard 1983, Myers et al. 2008, Oonincx et al. 2015), and recycle it into a protein-rich biomass (Sheppard et al. 1994). In this study, larvae fed Gainesville had a higher percent protein (48%) than those fed the lower fed rate (45%) or higher feed rate (42%) of poultry manure (Figure 2.3). Although not tested in the current study, differences in those fed different manure types is expected, as Zhou et al. (2013) showed that protein content of larvae from three different BSF strains fed poultry, swine or dairy manure varied; specifically, those fed poultry manure had higher protein contents than those fed the other two manure types. Past research has also suggested that larval diet influences protein (Lalander et al. 2019) and lipid accumulation (Pimentel et al. 2017) and as previously discussed, ultimately influences adult life-history traits (Gobbi et al. 2013), which in turn, impacts population dynamics. Therefore, in order to utilize these insects for waste management or food and feed purposes, a better understanding of their life history parameters is necessary to develop efficient systems.

In this study, results show significant differences in weight, development and survivorship across the manure types tested. In all cases, BSF could be used to recycle these wastes and produce protein. However, these results are based on a bench-top experiment and may not easily translate on a larger production scale. Future research should examine the development of BSF fed different manures under mass rearing conditions

References

Banks, I. J., W. T. Gibson, and M. M. Cameron. 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Tropical Medicine International Health 19: 14-22.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Boland, M. J., A. N. Rae, J. M. Vereijken, M. P. Meuwissen, A. R. Fischer, M. A. van Boekel, S. M. Rutherfurd, H. Gruppen, P. J. Moughan, and W. H. Hendriks. 2013. The future supply of animal-derived protein for human consumption. Trends in Food Science & Technology 29: 62-73.

Bondari, K., and D. Sheppard. 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Callan, E. 1974. *Hermetia illucens* (L.)(Diptera, Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. Entomol Mon Mag.

Cammack, J. A., and J. K. Tomberlin. 2017. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). Insects 8: 56.

Charlton, A., M. Dickinson, M. Wakefield, E. Fitches, M. Kenis, R. Han, F. Zhu, N. Kone, M. Grant, and E. Devic. 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1: 7-16.

Chen, S., W. Liao, C. Liu, D. C. Elliott, M. D. Brown, and A. E. Solana. 2003. Value-added chemicals from animal manure, Pacific Northwest National Laboratory.

Cheng, J. Y., S. L. Chiu, and I. M. Lo. 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste Management 67: 315-323.

Čičková , H., G. L. Newton, R. C. Lacy, and M. Kozanek. 2015. The use of fly larvae for organic waste treatment. Waste Management 35: 68-80.

Fletcher, M., R. Axtell, and R. Stinner. 1990. Longevity and fecundity of *Musca domestica* (Diptera: Muscidae) as a function of temperature. Journal of Medical Entomology 27: 922-926.

Franson, M. A. H. 1989. Total solids dried at 103-105C: Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Furman, D. P., R. D. Young, and E. P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52: 917-921.

FWW. 2015. Factory Farmed Nation: 2015 Edition.

Gobbi, P., A. Martínez-Sánchez, and S. Rojo. 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). European Journal of Entomology 110: 461.

Hale, O. 1973. Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. Journal of the Georgia Entomological Society.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Ishii, S., T. Yan, H. Vu, D. L. Hansen, R. E. Hicks, M. J. J. M. Sadowsky, and Environments. 2009. Factors controlling long-term survival and growth of naturalized *Escherichia coli* populations in temperate field soils. Microbes and Environments.

James, M. 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bulletin of the Brooklyn Entomological Society 30: 165-170.

Kroeckel, S., A.-G. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, and C. Schulz. 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute—growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364: 345-352.

Lalander, C., S. Diener, C. Zurbrügg, and B. Vinnerås. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). Journal of Cleaner Production 208: 211-219.

Lalander, C. H., J. Fidjeland, S. Diener, S. Eriksson, and B. Vinnerås. 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agronomy for Sustainable Development 35: 261-271.

Liu, Q., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37: 1525-1530.

Makkar, H. P. S., G. Tran, V. Henze, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197: 1-33.

Martin, J. H., R. C. Loehr, and T. E. Pilbeam. 1983. Animal manures as feedstuffs: nutrient characteristics. Agricultural Wastes 6: 131-166.

Martínez-Sánchez, A., C. Magana, M. Salona, and S. Rojo. 2011. First record of *Hermetia illucens* (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. Forensic Science International 206: e76-e78.

Meneguz, M., L. Gasco, and J. K. Tomberlin. 2018. Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. PloS ONE 13: e0202591.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019. Life-History Traits of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), Reared on Three Manure Types. Animals 9: 281.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Newton, G. L., C. V. Booram, R. W. Barker, and O. M. Hale. 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. Journal of Animal Science 44: 395-400.

Nguyen, T. T., J. K. Tomberlin, and S. Vanlaerhoven. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology 44: 406-410.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2013. Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. Journal of Medical Entomology 50: 898-906.

Oonincx, D., A. van Huis, and J. van Loon. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. Journal of Insects as Food and Feed 1: 131-139.

Pfost, D. L., C. D. Fulhage, and D. Rastorfer. 2000. Anaerobic lagoons for storage/treatment of livestock manure.

Pimentel, A. C., A. Montali, D. Bruno, and G. Tettamanti. 2017. Metabolic adjustment of the larval fat body in *Hermetia illucens* to dietary conditions. Journal of Asia-Pacific Entomology 20: 1307-1313.

Sealey, W. M., T. G. Gaylord, F. T. Barrows, J. K. Tomberlin, M. A. McGuire, C. Ross, and S. St-Hilaire. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. Journal of the World Aquaculture Society 42: 34-45.

Sheppard, C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environmental Entomology 12: 1439-1442.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value-added manure management-system using the black soldier fly. Bioresource Technology 50: 275-279.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera : Stratiomyidae). Journal of Medical Entomology 39: 695-698.

St-Hilaire, S., C. Sheppard, J. K. Tomberlin, S. Irving, L. Newton, M. A. McGuire, E. E. Mosley, R. W. Hardy, and W. Sealey. 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 38: 59-67.

St-Hilaire, S., K. Cranfill, M. A. McGuire, E. E. Mosley, J. K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, and S. Irving. 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38: 309-313.

Tomberlin, J. K., D. C. Sheppard, and J. A. Joyce. 2002. Selected life-history traits of black soldier flies (Diptera : Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386.

Tsagkarakis, A. E., E. Arapostathi, and G. Strouvalis. 2015. First record of the black soldier fly, *Hermetia illucens*, in Greece. Entomologia Hellenica 24: 27-30.

ur Rehman, K., A. Rehman, M. Cai, L. Zheng, X. Xiao, A. A. Somroo, H. Wang, W. Li, Z. Yu, and J. Zhang. 2017a. Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (*Hermetia illucens* L.). Journal of Cleaner Production 154: 366-373.

ur Rehman, K., M. Cai, X. Xiao, L. Zheng, H. Wang, A. A. Soomro, Y. Zhou, W. Li, Z. Yu, and J. Zhang. 2017b. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). Journal of Environmental Management 196: 458-465.

USDA. 2010. Overview of the United States dairy industry: Large operations increase share of inventory and milk production.

USDA. 2015. Overview of the United States hog industry: Breeding herd efficiency continues to increase.

Wang, Y.-S., and M. Shelomi. 2017. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 6: 91.

Yu, G., P. Cheng, Y. Chen, Y. Li, Z. Yang, Y. Chen, and J. K. Tomberlin. 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environmental Entomology 40: 30-35.

Zheng, L., Q. Li, J. Zhang, and Z. Yu. 2012. Double the biodiesel yield: rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. Renewable Energy 41: 75-79.

Zhou, F., J. K. Tomberlin, L. Zheng, Z. Yu, and J. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of Medical Entomology 50: 1224-1230.

3. LIFE-HISTORY TRAITS OF HOUSE FLY, *Musca domestica* L. (DIPTERA: MUSCIDAE), REARED ON THREE MANURE TYPES[†]

Overview

Houseflies are typically revered as pests because they are a nuisance and carriers of pathogens; however, they are capable of converting animal wastes into valuable biomass that may be suitable for inclusion in animal diets, which has been explored for industrialization outside of the United States. The purpose of this study was to evaluate the life-history performance, dry matter reduction, and bioconversion of three different manure types fed to housefly larvae. Treatments consisted of 100 1-day old larvae fed either 9 or 18 g of swine, dairy, or poultry manure every other day until 40% pupation. Gainesville diet was used as a control and produced superior results across all parameters examined. When comparing across feed rate for a manure type, larvae weighed more (4–16%) when provided the higher feed rate. A similar trend occurred for pupal (16–25%) and adult (8–25%) weight, as well as adult longevity (7–28%). In regard to those fed manure, significant differences ($p < 0.05$) were detected for time to pupation, percent pupation, adult weight and adult longevity, dry matter reduction, and bioconversion, across feed rates and manure types. Specifically, among those provided manure, larvae fed poultry manure delivered comparable results to those provided Gainesville diet except for time to pupation and percent pupation. Data from this study are necessary to advance industrial systems for managing animal wastes with houseflies. Conversely,

---

[†] Part of this chapter is reprinted with permission from "Life-history traits of the house fly, *Musca domestica* L. (Diptera: Muscidae), reared on three manure types" by Chelsea Miranda, Jonathan Cammack, and Jeffery Tomberlin, 2019. *Journal of Insects as Food and Feed.* Copyright [2019] by Wageningen Academic Publishers.

these data provide insight on potential population dynamics for houseflies in confined animal facilities, which could be beneficial for integrated pest management practices.

Introduction

Structural changes in the size of confined animal facilities (CAFOs) in the swine, dairy, and poultry industries have led to fewer, but larger, farms (higher concentration of animals). Traditionally, a majority of swine produced in the United States (US) were from small operations (<5000 head/operation) (USDA, 2015). For example, in 1994, approximately 70% of the annual pig crop came from small operations, whereas large operations (5000+ head) produced roughly 30% of the annual pig crop. However, in 2014, large operations accounted for 90% of annual production and small operations produced the remaining 10% (USDA, 2015). The dairy industry experienced similar trends (overall number of dairy operations declined 33% from 2001 to 2009), while at the same time, the number of head increased 1 % (from 9.1 million head to 9.2 million head) (USDA, 2010a). Despite the overall number of dairy operations decreasing, the number of operations with 500+ head increased by 20%. For poultry, majority of chickens are produced by a small number of large companies, yet the USDA does not provide approximations on the number of companies or their exact contribution to the industry (USDA, 2010b). However, from 1950-2007, the number of broiler farms decreased by an estimated 98%, but the number of chickens increased by approximately eight billion birds (Group, 2011). The evolution of these industries to larger facilities has amplified concerns regarding waste management as the volume of manure produced annually in some areas outweighs the amount that may be utilized by nearby land for fertilization. While manure is typically considered a valuable resource, too much can be a major issue and current management practices are an environmental hazard.

Manure from CAFOs is typically stored outdoors in earthen basins or lagoons (Hribar and Schultz, 2010). This method of management is environmentally risky as lagoons are vulnerable to natural disasters and may rupture (Hribar and Schultz, 2010). Lagoons may also increase antimicrobial resistance in bacteria as they often harbor antimicrobials administered to animals (Zhang *et al.*, 2013). Currently, alternative methods for waste management are being developed with the concept of recycling wastes, with insects becoming a potential solution. However, a better understanding of the bioconversion of different manure types is necessary to develop efficient systems.

In most regions of the world, the housefly, *Musca domestica* L. (Diptera: Muscidae) is viewed as a pest (Malik *et al.*, 2007). The housefly is distributed globally and has a well-described life cycle. Houseflies are ecologically associated with humans and domestic animals as they feed on foodstuffs and wastes. Therefore, they are often thought of as a nuisance and are capable of transmitting hundreds of pathogens either by direct contact (Malik *et al.*, 2007) or through regurgitation and excretion (Sasaki *et al.*, 2000). However, researchers are now suggesting the housefly could be used to recycle animal waste if properly managed. Khan *et al.* (2012) demonstrated adults could emerge in as little as 11-15 d in laboratory-based experiments. Additionally, Čičková *et al.* (2012) showed housefly larvae can process 178-444 kg of swine manure (at an inoculation rate of 0.4-1.0 mL of eggs (4400-11,000 eggs)/kg of manure) in as little as 10 d. Moreover, Hussein *et al.* (2017) showed houseflies can reduce nitrogen and phosphorus in dairy manure by 25% and 6%, respectively, and can also reduce odor and pathogens (Wang *et al.*, 2013). Furthermore, housefly pupae resulting from larvae fed poultry manure may be substituted for soybean oil or fish meal in animal diets, as they are high in protein (60%), fatty acids, phosphorus, and calcium(Calvert *et al.*, 1969; Teotia and Miller, 1974). Likewise, larvae fed dairy manure could be used to produce a meal high in monounsaturated fatty acids that has a balanced amino acid composition, lending to their potential use as a high-quality feed for livestock and aquaculture (Hussein *et al.*, 2017). Due to the fact that houseflies can reduce nutrients and odor, as well as recycle large amounts of waste into a high protein and lipid biomass in a relatively short time, this species may be utilized for waste management. To date, the use of houseflies for waste conversion has received limited attention in the US. The purpose of this study was to evaluate the life-history performance of housefly larvae fed poultry, swine, and dairy manure provided at different rates.

Materials and Methods

Acquisition of HF

This experiment was conducted using housefly larvae from a colony maintained at the F.L.I.E.S. Facility at Texas A&M University, College Station, TX, USA. This colony originated from adults collected in 2014 from dairy and swine facilities in Stephenville, TX, USA. The housefly colony is maintained by providing water as well as sugar mixed with milk powder (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA) (1:1 ratio by mass) *ad libitum*.

Acquisition of Manure

Acquisition of manure followed methods described in the previous section. Swine, dairy and poultry manure less than 12-h-old was collected for use in this experiment. Swine manure was collected from a local farmer, in Anderson, TX, USA, dairy manure was collected from a commercial dairy located in Stephenville, TX, USA, and poultry manure was collected from layer hens housed at the Poultry Science Research, Teaching and Extension Center at Texas A&M University, in College Station, TX, USA. Once collected, manure was placed into 18.9 L buckets, covered with lids (Home Depot®, Leaktite™, Leominster, MA, USA), and transported to the F.L.I.E.S. Facility, where it was homogenized in the buckets (mixed vigorously by hand for approximately 5 minutes) and transferred to 3.76 L Ziploc® Freezer Bags and stored at -20°C until used. Manure was allowed to thaw at room temperature for 24 h before initiation of the experiment. Manure not used on day one was placed in 1.9 L Reditainer™ EXTREME FREEZE™ deli containers (Clear Lake Enterprises, Port Richey, FL, USA) and stored in a refrigerator (4°C) until used. Three 10 g samples of thawed manure of each type were used to gravimetrically determine moisture content at the beginning of the experiment (Franson 1989). Final moisture content of manure was taken when all individuals within a treatment reached the pupal stage or when 5 d passed without remaining larvae reaching the pupal stage.

Experiment Design

Housefly eggs were collected by balling up a Kimwipe® (Kimberly-Clark Corp., Irving, TX) saturated with Evaporated Milk (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA), and placing the Kimwipe® in an 88 ml bathroom cup (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA). The cup was placed inside a 30 x 30 x 30 cm cage (Bioquip Products, Rancho Dominquez, CA, USA) that housed gravid adult flies. The cup was checked for egg deposition every four hours and was removed from the cage when there appeared to be a sufficient number of eggs. To collect the eggs from the Kimwipe®, the wipe was unfolded and rinsed with distilled water over a 100 ml beaker. Viable housefly eggs sank to the bottom of the beaker and were collected with a Pasteur pipette. The eggs were transferred to a 118 ml container (Glad® Brand, The Clorox Company, Oakland, CA, USA) filled with 10 g of Gainesville diet and moistened with water (1:1 ratio by volume) (Hogsette, 1992) and stored in a Rheem Environmental Chamber (Asheville, NC, USA; at 33˚C, 60% RH, and 16L:8D) until larval eclosion.

Replicates consisted of 100 1-d-old larvae placed into 88 ml bathroom cups and provided manure as described below. Cups were covered with a paper towel held in place with a rubber band and stored in the growth chamber described above. The control diet followed the methods of Hogsette (1992) and consisted of 7.4 g of Gainesville diet mixed with 16.6 mL of distilled water. Three replicates of each treatment and control were used and the experiment was replicated twice.

Every other day, larvae in a treatment were fed their assigned manure type at a specified feed rate (9 g or 18 g) identified in preliminary experiments. Individual cups were each placed inside a 1.89 L Reditainer™ EXTREME FREEZE™ deli containers covered with tulle fabric (Wal-Mart® Stores, Inc., Bentonville, AR, USA) held in place with a rubber band. The containers were placed in a randomized complete block design in the environmental chamber previously described. The bathroom cups were dumped into the 1.9 L Reditainer™ EXTREME FREEZE™ deli containers on day four due to concerns with moisture. Pupae were removed daily, and the addition of feed stopped when 40% reached the pupal stage.

In order to minimize the effect of handling, three of the largest larvae observed were selected from each replicate every three days, weighed individually on an OHaus Adventure Pro AV64 balance (OHaus Corporation, Pine Brook, NJ, USA), and returned to their respective container. The mean larval weight recorded on the day that 40% of the larvae within a treatment reach the pupal stage was recorded as the final larval weight.

Pupae were removed daily from all treatments, weighed individually using methods described above, and placed in individual 35 ml cups, covered with a plastic lid with a cotton ball inserted through a hole in the center of the lid, and returned to the growth chamber. The cotton ball was dampened daily with deionized water and served as a source of water for the adults upon eclosion. Adult emergence and longevity were monitored daily and recorded.

To determine bioconversion, the total mass of larvae produced, and amount of manure fed to each replicate over the course of the experiment were recorded. Bioconversion was (total g of larvae/ total g of manure fed) X 100%. Dry matter reduction was determined in the same manner as described by (Zhou *et al.*, 2013). The dry matter reduction was (W1-W2) / W1 X 100%, with W1 being the dry matter provided during the experiment and W2 being the dry matter remaining at the end of the experiment.

Statistical Analysis

Moisture content of manure, percent dry matter (DM) reduction, larval development over time, final larval weight, time to pupariation, pupal weight, percent pupariation, adult male and female weight, survivorship, adult longevity, and bioconversion percent (wet weight basis) across treatments were analyzed. Statistics were performed using JMP PRO 12 (SAS Institute Inc., Cary, NC, USA). Normality was checked using Shapiro Wilk Test and equal variances were checked using Bartlett's test. Tukey's HSD (honest significant difference) was used for mean separation ($p \leq 0.05$). Replicates with only one adult individual for either sex were removed from all analyses involving life-history performance.

Results

Moisture Content of Manure

Initial moisture content differed significantly ($F_{2, 12} = 14.95$; $p = 0.0006$) across manure types, but no significant treatment by trial interaction ($F_{2, 12} = 0.623$; $p = 0.9399$) or trial effect ($F_{1, 12} = 0.1197$; $p = 0.7353$) was detected. Similarly, final moisture content differed significantly ($F_{5, 15} = 27.18$; $p < 0.0001$) across manure types and feed rates, but no significant treatment by trial interaction ($F_{5, 15} = 1.0241$; $p = 0.4383$) or trial effect ($F_{1,15} = 1.91$; $p = 0.1867$) was detected. Initial and final moisture contents for swine, dairy and poultry manure are displayed in Table 3.1. Initial manures had moisture contents ranged from 74–84 %, while final moisture contents for ranged from 9–55% and 10–42% for the 9 g and 18 g feed rate, respectively.

Table 3.1 Mean initial and final moisture contents (mean ± SE, $^1n = 3$) of swine, dairy, or poultry manure at two different feed rates before and after house fly larval digestion.

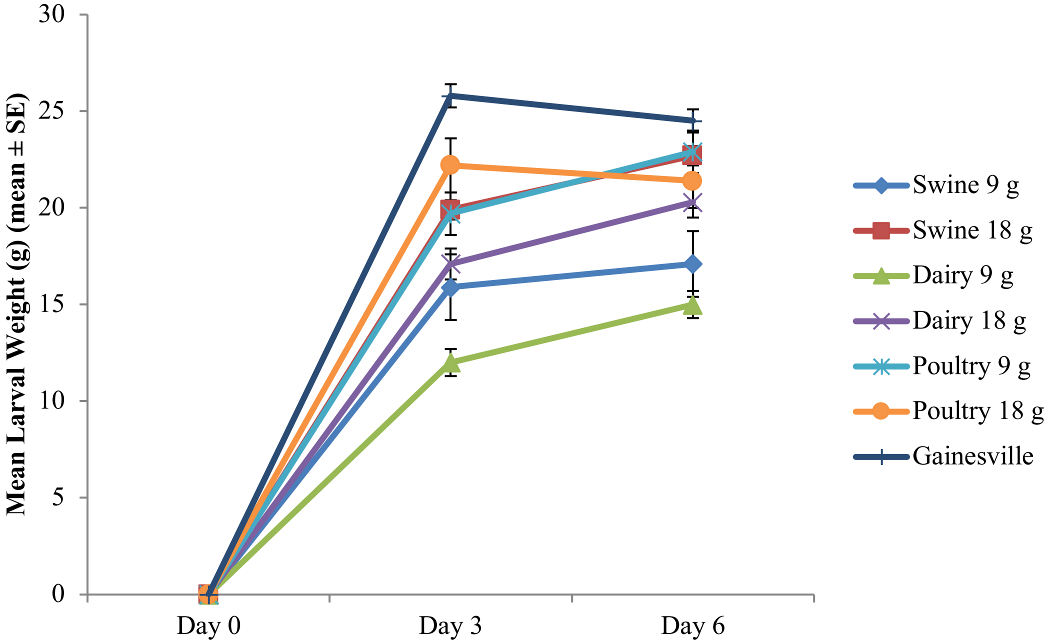
Manure Type	Feed Amount (g)	Initial (%)	Final (%)
Swine	9	73.7 ± 1.52^{A}	9.00 ± 3.96^{A}
	18		10.0 ± 2.95^{A}
Dairy	9	83.8 ± 1.27^{B}	55.3 ± 3.96^{C}
	18		41.5 ± 3.74^{CB}
Poultry	9	76.6 ± 1.36^{A}	19.2 ± 2.64^{C}
	18		34.3 ± 2.64^{B}

^{1}n = number of replicates per treatment.
Different letters within a column indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Miranda et al. (2019).

Larval Development

Larval development differed significantly ($F_{6,\,84} = 18.04$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6,\,84} = 0.76$; $p = 0.6007$) or trial effect ($F_{1,\,84} = 0.087$; $p = 0.7691$) was detected. Larvae fed the control diet weighed 16–115% and 7–63% more than those fed manure on day three and day six, respectively (Figure 3.1). Of those fed 9 g of manure, larvae fed poultry manure weighed 24% and 64% more than those fed swine or dairy manure, respectively on day three and 34% and 53% more, respectively, on day six. A similar trend occurred for those fed 18 g of manure.

Figure 3.1 Mean larval weight (g) (mean ± SE, [1]n = 6) of houseflies fed a standard diet (Hogsette 1992) or swine, dairy, or poultry manure at two feed rates at 33 °C, 60% RH, and 16L:8D**.**



[1]n = number of replicates per treatment.
Reprinted with permission from Miranda et al. (2019).

Final Larval Weight

Final larval weight differed significantly ($F_{6, 25} = 5.69$; p = 0.0008) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 0.87$; p = 0.5315) or trial effect ($F_{1, 25} = 0.25$; p = 0.6168) was detected. Furthermore, larvae provided Gainesville diet weighed 21–45% more than those provided manure (Table 3.2). With regards to treatment effect, those fed at the 9 g rate, larvae reared on dairy manure were the heaviest (17.1 mg/larva). Those provided swine or poultry manures weighed approximately 1% and 7% less than those provided the dairy manure, respectively. However, for those fed at the 18 g rate, larvae provided dairy weighed 8% and 4% more than those provided swine and poultry manure, respectively (Table 2). When comparing across feed rate for a manure type, larvae were 4–16% larger when provided more resource.

Time to First Pupariation

Days to first pupariation differed significantly ($F_{6, 25} = 11.07$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 1.80$; $p = 0.1391$). However, a significant trial effect ($F_{1, 25} = 25.81$; $p = 0.0023$) was detected. In general, individuals in trial one reached pupariation approximately 1 d faster than those in trial two. Furthermore, those provided Gainesville diet reached the pupariation in approximately 5.5 d. With regards to treatment effect for those fed manure at the 9 g rate, larvae fed swine manure reached pupariation in 5 d. Those provided poultry or dairy manure required approximately 1 and 2 d longer than those provided the swine manure, respectively (Table 3.2). For those fed at the 18 g rate, a similar pattern occurred except for those provided dairy, which reached pupariation approximately 1 d earlier than those provided poultry manure. When comparing across feed rate for a manure type, larvae reached pupariation 1.3 and 1.8 d earlier when provided less swine or poultry manure, respectively; though, no difference was detected for those fed dairy manure.

Pupal Weight

Puparium weight differed significantly ($F_{6, 25} = 48.52$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 1.53$; $p = 0.2085$) was detected. However, a significant ($F_{1, 25} = 5.70$; $p = 0.0249$) trial effect was detected. In general, individuals in trial one were 7% larger than those in trial two. Furthermore, those provided Gainesville diet weighed 28–104% more than those fed manure (Table 3.2). With regards to treatment effect, those fed at the 9 g rate, larvae reared on poultry manure were the heaviest (13.6 mg/larva). Those provide dairy or swine manure weighed approximately 27% and 12% less than those provided the poultry manure, respectively. For those fed at the 18 g rate, a similar pattern occurred. However, when comparing across feed rate for a manure type, puparia were 16 –25% larger when provided more resource.

Percent Pupariation

Percent pupariation differed significantly ($F_{6, 25} = 8.63$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 0.79$; $p = 0.5837$) or trial effect ($F_{1, 25} = 0.76$; $p = 0.3914$) was detected. In regard to treatment effect, larvae fed the Gainesville diet resulted in 58% pupariation (Table 3.2). Whereas for those fed manure at the 9 g rate, larvae reared on swine manure resulted in the highest percent pupariation (58%), while those fed dairy (46%) or poultry (53%) manure had fewer individuals reach pupariation, although these differences were not significantly different. A similar trend was observed for the 18 g feed rate, where the highest percent pupation occurred for swine manure (66%); however, more individuals pupated when reared on dairy manure (42%) than those fed poultry manure (23%). Nevertheless, when comparing across feed rates for dairy and poultry manures, pupariation was 4% and 29% higher, respectively, when larvae were provided less resource. However, in regard to swine manure, pupariation was 8% higher in the higher feed rate.

Figure 3.2 Comparison of life-history traits (mean ± SE) of house fly larvae fed one of three types of manure at two feed rates every other day or Gainesville diet (Hogsette 1992) at 29°C, 60% RH, and 16:8 L:D.

Treatment		n	Final Larval Weight (mg)	Time to Pupariation (d)	Pupal Weight (mg)	Percent Pupariation	Adult Male Weight (mg)	Adult Male Longevity (d)	Adult Female Weight (mg)	Adult Female Longevity (d)
Swine	9 g	6	16.9 ± 1.0^{A}	5.0 ± 0.4^{A}	11.9 ± 0.5^{AB}	58.0 ± 4.1^{AB}	8.0 ± 0.7^{AB}	1.4 ± 0.1^{A}	8.2 ± 0.7^{A}	1.4 ± 0.1^{AB}
	18 g	6	17.6 ± 1.0^{A}	6.3 ± 0.2^{AB}	13.9 ± 0.5^{BC}	65.7 ± 4.1^{A}	10.2 ± 0.7^{BCD}	1.6 ± 0.1^{AB}	10.2 ± 0.7^{AB}	1.7 ± 0.1^{AB}
Dairy	9 g	5	17.1 ± 1.1^{A}	7.0 ± 0.4^{BC}	9.9 ± 0.5^{A}	46.2 ± 4.6^{ABC}	6.1 ± 0.8^{A}	1.2 ± 0.1^{A}	8.0 ± 0.7^{A}	1.4 ± 0.1^{A}
	18 g	6	19.1 ± 1.0^{AB}	6.6 ± 0.2^{BC}	12.4 ± 0.5^{B}	42.2 ± 4.1^{BC}	7.8 ± 0.7^{AB}	1.4 ± 0.1^{A}	8.6 ± 0.7^{A}	1.5 ± 0.1^{AB}
Poultry	9 g	6	15.9 ± 1.0^{A}	6.0 ± 0.3^{AB}	13.6 ± 0.5^{BC}	53.3 ± 4.1^{AB}	9.7 ± 0.7^{BC}	1.7 ± 0.1A^{AB}	10.2 ± 0.7^{AB}	1.8 ± 0.1^{AB}
	18 g	4	18.4 ± 1.2^{AB}	7.8 ± 0.3^{C}	15.8 ± 0.6^{C}	23.8 ± 5.0^{C}	11.9 ± 0.8^{CD}	2.0 ± 0.1^{B}	12.8 ± 0.9^{BC}	2.3 ± 0.1^{C}
Gainesville[3]	--	6	23.1 ± 1.0^{B}	5.5 ± 0.3^{AB}	20.2 ± 0.5^{D}	57.7 ± 4.1^{AB}	12.8 ± 0.7^{D}	2.0 ± 0.1^{B}	13.7 ± 0.7^{C}	2.0 ± 0.1^{BC}

[1] n = number of replicates per treatment

Different letters within a column indicate significant differences between treatments (α=0.05), ANOVA followed by Tukey's HSD.

[3] Followed methods described by Hogsette (1992) for flies fed Gainesville diet.

Reprinted with permission from Miranda et al. (2019).

Adult Male Weight

Adult male weight significantly differed ($F_{6, 25} = 10.13$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 2.63$; $p = 0.1174$) or trial effect ($F_{1, 25} = 0.65$; $p = 0.6882$) was detected. Males produced from Gainesville diet weighed 12.8 mg, which was 8–110% more than those provided manure (Table 3.2). With regards to treatment effect, for those fed at the 9 g rate, males produced from poultry manure were the heaviest (9.7 mg), while those provided dairy or swine manure weighed approximately 37% and 18% less, respectively. Likewise, similar trends were observed for those fed at the 18 g rate. However, when comparing across feed rate for a manure type, males, in general, were 23–28% larger when provided more resource.

Adult Male Longevity

Adult male longevity differed significantly ($F_{6, 25} = 6.71$; $p = 0.0003$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 2.05$; $p = 0.0959$) was detected. However, a significant ($F_{1, 25} = 9.30$; $p = 0.0054$) trial effect was detected. In general, males in trial two lived approximately 17% longer than those in trial one. Individuals produced from Gainesville diet lived approximately 2 d, which was 0–67% longer than those provided manure (Table 3.2). Likewise, of those fed the 9 g rate, larvae fed poultry manure lived approximately 1.7 d, while those provided dairy or swine manure lived approximately 29% and 18% less, respectfully. For those fed at the 18 g rate, the same pattern occurred. Though, when comparing across feed rate for a manure type, males lived in general 14–18% longer when provided more resource.

Adult Female Weight

Adult female weight differed significantly ($F_{6, 25} = 8.53$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 0.59$; $p = 0.7350$) or trial effect ($F_{1, 25} = 1.10$; $p = 0.3032$) was detected. Individuals provided Gainesville diet weighed 13.7 mg/larva, which was 7–71% more than those provided manure (Table 3.2). In regard to treatment effect, for those fed at the 9 g rate, larvae fed poultry manure were the heaviest (10 mg/adult), while those provide dairy or swine manure weighed approximately 22% and 20% less, respectively. A similar pattern occurred for the 18 g feed rate. Yet, when comparing across feed rate for a manure type, females were in general 8–25% larger when provided more resource.

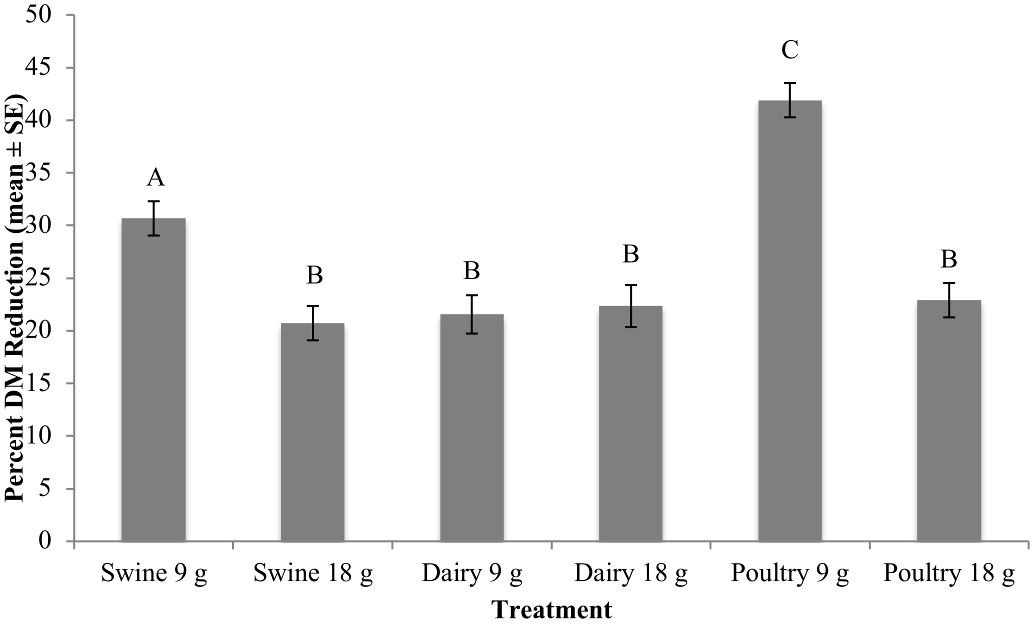
Adult Female Longevity

Adult female longevity differed significantly ($F_{6, 25} = 7.56$; $p = 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{6, 25} = 2.24$; $p = 0.0729$) was detected. However, a significant ($F_{1, 25} = 19.41$; $p = 0.0002$) trial effect was detected. In general, individuals in trial two lived approximately 23% longer than those in trial one. Individuals provided Gainesville diet lived approximately 2 d, which was 11–43% longer than those provided manure (Table 3.2). In regard to treatment effect, for those fed at the 9 g rate, larvae fed poultry manure lived approximately 2 d, while those provided dairy or swine manure lived approximately 22% less. For those fed at the 18 g rate, the same pattern occurred. Though, when comparing across feed rate for a manure type, females lived in general 7–28% longer when provided more resource.

Percent DM Reduction

Percent dry matter reduction differed significantly ($F_{5, 21} = 24.83$; $p < 0.0001$) across larval diets, but no significant treatment by trial interaction ($F_{5, 21} = 0.37$; $p = 0.8538$) or trial effect ($F_{1, 21} = 1.52$; $p = 0.2312$) was detected. Those provided 9 g of poultry manure reduced the greatest amount of dry matter (42%), whereas larvae provided 9 g of dairy manure reduced the least amount of dry matter (21%) (Figure 3.2). A similar trend occurred for those fed 18 g of manure, although no significant differences ($p < 0.05$) were detected when comparing across manure types. When comparing across feed rates for a given manure type, no significant difference was detected for those fed dairy manure; however, there were significant differences detected for those fed poultry (42% vs 23%; $p < 0.0001$) and swine (31% vs 21%; $p = 0.0023$) manure with greater reductions occurring at the lower feed rate. Specifically, for these manure types, those provided less resource reduced dry matter by an additional 10 –18%.

Figure 3.3 Mean percent dry matter (DM) reduction (mean ± SE, [1]n = 6) for house flies fed swine, dairy or poultry manure two feed rates at 33 °C, 60% RH, and 16L:8D.

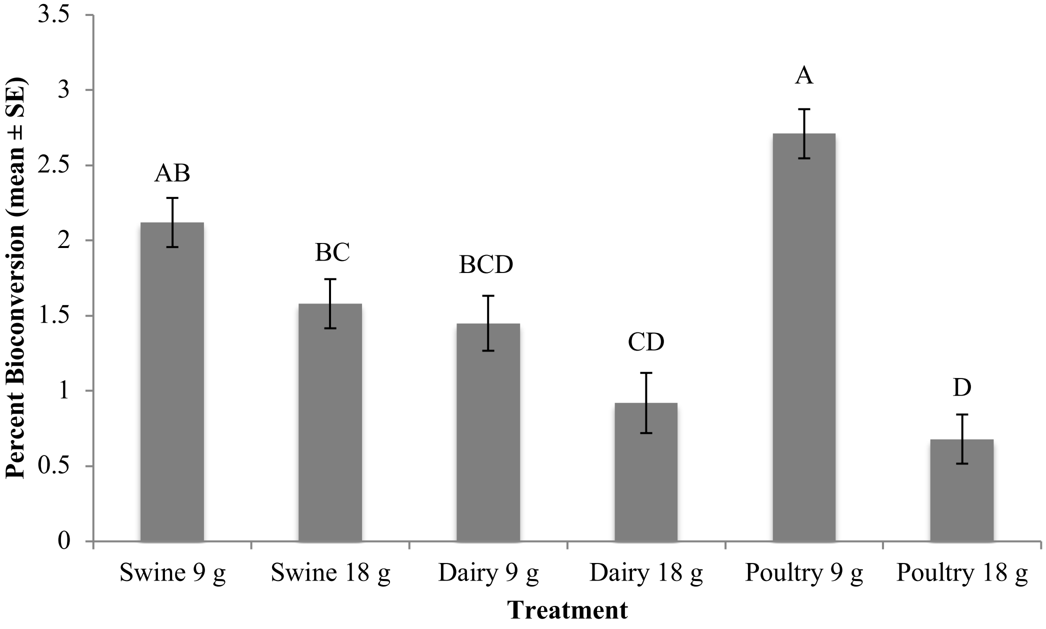


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Miranda et al. (2019).

Bioconversion of manure

Bioconversion of manure differed significantly ($F_{5, 20} = 16.11$; $p < 0.0001$) across larval diets (Figure 4). No significant treatment by trial interaction ($F_{5, 20} = 0.99$; $p = 0.4462$) or trial effect ($F_{1, 20} = 0.10$; $p = 0.7535$) was detected. In regard to those fed 9 g of manure, the highest bioconversion rate was observed for larvae provided poultry manure (2.71%) and the lowest from larvae fed dairy manure (1.45%) (Figure 3.3). Conversely, for larvae fed 18 g of manure, those provided swine manure produced the highest bioconversion rate (1.58%), whereas those provided poultry manure resulted in the lowest (0.68%). When comparing across feed rate for a manure type, larvae fed less resource, in general, resulted in greater (0.5–2%) bioconversion rates.

Figure 3.4 Mean percent bioconversion (mean ± SE, ^{1}n = 6) for house flies fed swine, dairy, or poultry manure two different feed rates at 33 °C, 60% RH, and 16L:8D



^{1}n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.
Reprinted with permission from Miranda et al. (2019).

Discussion

The primary aim of this research was to evaluate the life-history performance of houseflies fed poultry, swine, and dairy manure as well as Gainesville diet (control) at two feed rates. As expected, Gainesville diet was the superior diet for growing houseflies as this diet was specifically formulated for optimal housefly production (Hogsette, 1992). Such data provide a suitable comparison for other resources, such as manure in this case, and could allow for optimal production methods to be developed or implementation of life-history data in integrated pest management plans.

With regards to larvae fed manure, results demonstrate larval diet influenced life-history traits. Specifically, results show that larvae reared on poultry manure were similar to those fed Gainesville diet (Table 3.2). Additionally, individuals fed more resource (18 g every other day until 40% pupation), regardless of manure type, had greater final larval (4–16%), pupal (16–25%) and adult (8–25%) weights, as well as lived longer as adults (7–28%) than those fed the lower feed rate (9 g every other day) (Table 3.2). However, providing more resource resulted in lower percent dry matter reduction (Figure 1) and subsequent bioconversion (Figure 3.3). Previous research has detected similar findings for percent dry matter reduction by the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), when fed 27 g/d compared to those fed 70 g/d (Myers *et al.*, 2008). It is possible that the greater percent moisture in the higher feed amount impacted survivorship, which may have influenced bioconversion. From an industrial viewpoint, these results may be used to optimize systems for mass production of houseflies to be utilized as food and feed.

Larrain and Salas (2008) found that housefly larvae fed poultry and swine manure (compared to dairy, dog, calf, horse and goat manure) resulted in the heaviest pupae, while those fed dairy manure resulted in lower pupal weights (approximately 50% less than those fed poultry and swine manure) and had longer development time (only 3% of the individuals emerged on Day 11 compared to 89% and 76% for poultry and swine, respectively). Similar results were found by (Khan *et al.*, 2012). Larvae fed poultry manure weighed approximately 11% more and developed approximately 19% faster than those fed dairy manure; whereas larvae fed 9 g of poultry manure in this study weighed 37% more and developed 14% faster than those fed dairy manure. Percent differences between the current study and previous studies may be due to differences in methods. Khan *et al.* (2012) fed 30 neonate larvae a lump sum feeding of 100 g of manure; whereas 100 neonates and incremental feeding was utilized in this study. However, results with regards to pupal weight and development time generated in the current study agree with previous studies, as those fed poultry and swine manure weighed more as larvae and took less time to reach the pupal stage than those fed dairy manure (Table 3.2).

While not measured in the current study, microbial community variation across manure types could explain differences observed in the current study as related to housefly life-history traits. Manure is a complex matrix of microbes; however, some bacterial genera are commonly found in swine, dairy and poultry manures. Key bacterial genera commonly found in these manures are *Clostridium* (Lu *et al.*, 2003; Pandey *et al.*, 2018; Zhu, 2000), *Escherichia* (Barreiro *et al.*, 2013; Chen and Jiang, 2014; EPA, 2015), *Staphylococcus* (Chen and Jiang, 2014; Smith, 2015; Zhu, 2000) and *Streptococcus* (Chen and Jiang, 2014; Wang *et al.*, 2004; Zhu, 2000). Interestingly, these bacteria have also been isolated from housefly digestive tract (Gupta *et al.*, 2012; Zurek *et al.*, 2000) indicating a close ecological relationship between flies and bacteria. These bacteria are known to break down carbohydrates (Zhu, 2000), degrade fiber, and cycle nitrogen and sulfur (e.g., *Clostridium*) (Lu *et al.*, 2003). They also aid in development of housefly larva, especially *Escherichia, Staphylococcus, Streptococcus* (Schmidtmann and Martin, 1992) or can produce volatiles that serve as fly attractants to oviposition sites (e.g., *Escherichia*) (Tomberlin *et al.*, 2016). However, it should be noted that bacterial prevalence in manure varies based on several factors, such as animal diet (Spiehs and Goyal, 2007; Van Weelden *et al.*, 2016), animal age (EPA, 2015), temperature (Wang *et al.*, 2004; Zhu, 2000), moisture (Wang *et al.*, 2004), pH (Zhu, 2000), location (Bahrndorff *et al.*, 2017; Terzich *et al.*, 2000), as well as storage and treatment methods implemented at CAFOs (Pandey *et al.*, 2018; Spiehs and

Goyal, 2007). Likewise, the impacts of refrigerating or freezing the manure as applied in this study on fly development are not known; however, Pan *et al.* (2009) suggested that refrigeration (4°C) had the strongest impact on manure N composition compared to freezing (-20°C), freeze-drying (-45°C), or acidification. Conversely, (Pratt *et al.*, 2014) suggested that refrigerating (4°C) or freezing (-20°C) beef or poultry manures for up to 8 weeks did not impact several physiochemical properties (such as pH, moisture, nutrients, and minerals) or overall presence of viable microbes. Still, there may be an effect of freezing other types and this should be further examined.

Bacteria impact fly development and survivorship. Schmidtmann and Martin (1992) evaluated growth and development of housefly larvae reared on blood agar inoculated with one of several different species of bacteria. Of those identified, *Staphylococcus epidermidis, Staphylococcus xylosus, Streptococcus bovis, Bacillus cereus, Klebsiella pneumonia,* and *Escherichia coli* resulted in greater pupal weights and percent eclosion than those reared on sterile blood agar. Likewise, Rochon *et al.* (2004) compared the development of housefly and stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) larvae fed an artificial diet inoculated with different concentrations of *E. coli* and suggested that houseflies rely on the bacterium as a source of food because the bacteria in the gut declined when larvae were fed high concentrations. However, the opposite occurred with stable flies suggesting they do not rely on *E. coli* as a dietary source.

As previously mentioned, another aspect of manure that could impact fly development is nutritional quality of the manure. For example, dairy manure typically has lower nitrogen (N), phosphorous (P), and potassium (K) concentrations than swine and poultry manures. For example, Brown (2008) suggested that poultry manure contains

2.71% N, 1.32% P, and 1.45% K (as-is basis); whereas swine is comprised of 0.93%, 0.49%, and 0.57%, respectively, and dairy manure contains 0.72%, 0.20%, and 0.61%, respectively. Furthermore, the percent N of the total solids (TS) differs among poultry, swine, and dairy manures. For example, N accounts for 8.95% of the TS in poultry manure compared to 3.92% and 2.46% for swine and dairy, respectively (Li *et al.*, 2015). Nitrogen is important for the development, survivorship, and reproduction of houseflies. Barnard *et al.* (1995) demonstrated that increasing fecal N in poultry manure (by increasing dietary nitrogen for laying hens at 0.96, 2.08, 3.04, and 4.00%) resulted in increased housefly survival, emergence, and fecundity. Interestingly, they also found P concentrations (in the same manner as above at 0.33, 1.67, ad 3.00%) negatively impacted pupal mass, fecundity, and natality. Additionally, as related to previous discussion, these elements also impact microbial communities in manure. Previous research has shown that limiting N (Chen and Strevett, 2003), P (Mallette *et al.*, 1964), and K (Weiden *et al.*, 1967) restricts growth of *E. coli,* which potentially hinders housefly utilization of the bacteria as a resource. Levinson (1960) suggested houseflies rely on the cellular constituents of *E. coli* rather than the metabolic products of the bacteria and postulated that substrates within housefly diets are actually food for bacteria, which then themselves become food for the larvae. However, Chang and Wang (1958) demonstrated that development was prolonged for a subspecies of the housefly, the oriental housefly, *Musca domestica vicinia* Macquart (Diptera: Muscidae), by feeding larvae sterile diets lacking ribonucleic acid or vitamins A, C, and D. Furthermore, they showed oriental houseflies do not rely on symbiotic bacteria if all nutritional requirements are met. It appears likely that the common housefly relies on both nutrient acquisition from bacteria as a direct food source as well as their metabolic products (Zurek *et al.*, 2000).

Fiber content of manure could also explain the differences in life-history traits of houseflies reared on different manure types. Dairy manure typically contains approximately 50% fiber on a dry matter basis; whereas fiber in poultry (aged 17-40 weeks) and swine (finisher pigs) manure accounts for approximately 35% and 40% of the dry matter, respectively (Chen *et al.*, 2003). Unlike other insects, such as eastern subterranean termites, *Reticulitermes flavipes* (Kollar) (Blattodea: Rhinotermitidae), silverfish, *Ctenolepisma lineata* (Fabricius) (Thysanura: Lepismatidae) or leafcutter ants, *Atta colombica* Linnaeus (Hymenoptera: Formicidae), (Sun and Zhou, 2013), houseflies are not known digest fiber; therefore, dairy manure contains more indigestible substrates and may be less nutritious to the fly. Additionally, there are differences in the proportion of fiber types (cellulose or hemicellulose) across different manure types. Approximately half of the fiber content for dairy manure is cellulose, while hemicellulose constitutes over half of the fiber content in poultry and swine manures (Chen *et al.*, 2003); although type of fiber (cellulose or hemicellulose) in manure can vary based on the fiber source provided in the animal diet (Kerr and Shurson, 2013), which can ultimately influence the microorganisms in the manure (Van Weelden *et al.*, 2016). As previously discussed, microbes are important for fly development; therefore, these differences in the composition of manure may explain the variation in life-history traits for houseflies.

Conversely, increased larval density could potentially improve development and productivity by the fly. Fly larvae typically feed en masse, which provides individual benefits. For example, oral excretions produced by larvae are used to breakdown food particles before the larvae themselves consume them. Thus, more larvae could accelerate the process. Additional benefits include reduced risk for predation or parasitism, Zi-zhe *et al.* (2017) demonstrated that total larval biomass increased approximately 325% as larval inoculation rates increased from 1000 – 8000 larvae/kg poultry manure, but decreased by 33% with inoculation rates greater than 8000 larvae/kg manure. Additionally, this study suggested 8000 larvae/kg poultry manure yielded the greatest bioconversion rate of approximately 11% (Zi-zhe *et al.*, 2017). In a similar study on a larger scale, (Wang *et al.*, 2013) produced an average larval yield of 95-120 kg by inoculating 580,000 larvae/m^2 into a bioreactor loaded with 25 – 30 kg/m^2/d, which resulted in a 21% bioconversion rate of swine manure by houseflies. Though, Hussein *et al.* (2017) suggested 4 eggs/g of manure was the optimal density for houseflies fed dairy manure and reported 2% bioconversion, which is very similar to our findings for 9 g of dairy manure. However, this was expected as the larval density and amount fed was similar to our study (approximately 3 eggs/ g manure). Nevertheless, previous research indicates that increasing larval inoculation rates and amount of resource available can increase bioconversion in houseflies. For this reason, on a separate occasion, we inoculated three replicates of 1 kg of poultry manure with 8000 larvae following the recommendation described by Zi-zhe *et al.* (2017) and found the bioconversion rate increased to approximately 10%. As such, future research should consider performing experiments on larger scales, as results from bench-top studies may not be scalable.

The idea of feeding waste to houseflies originated almost 100 years ago by Lindner (1919), who suggested feeding human excrement to houseflies for protein and lipid production. More recently, the interest in utilizing insects in human and animal diets has grown exponentially (van Huis *et al.*, 2013). Although utilizing insects for food and feed has not been a major focus in developed countries, the nutritional value is similar to conventional meat and they may serve as feed additives for fish, swine, and chickens (van Huis *et al.*, 2013). Due to the expected increase in the global population, coupled with the high demand for protein from consumers, sustainable production of protein will be a major challenge. Likewise, it may be expected that animal production may increase to meet these demands, in which more manure may be produced. Therefore, more research that focuses on utilizing insects as waste management agents and sources of protein and lipids is necessary. Recent research has explored the use of houseflies as a replacement for fishmeal (Đorđević *et al.*, 2008; Hussein *et al.*, 2017; van Huis *et al.*, 2013). Houseflies may be a suitable fishmeal replacement for Hybro-G broilers (Đorđević *et al.*, 2008), juvenile whiteleg shrimp (*Litopenaeus vannamei*) (Cao *et al.*, 2012), and Nile tilapia (*Oreochromis nilticus*) (Wang *et al.*, 2017). Furthermore, housefly larvae fed cattle manure are high in calcium and phosphorus and are composed of approximately 60% protein and 20% lipids (Hussein *et al.*, 2017). Exploiting the ability of this species to convert organic wastes, such as manure, into valuable biomass may be a potential solution to concerns about future food production; however, understanding housefly development and resource quality is imperative to improve industrialization of this species to manage waste or produce protein.

References

Bahrndorff, S., N. de Jonge, H. Skovgård, and J. L. Nielsen. 2017. Bacterial communities associated with houseflies (*Musca domestica* L.) sampled within and between farms. PloS ONE 12: e0169753.

Barnard, D., R. Harms, and D. Sloan. 1995. Influence of nitrogen, phosphorus, and calcium in poultry manure on survival, growth, and reproduction in house fly (Diptera: Muscidae). Environmental Entomology 24: 1297-1301.

Barreiro, C., H. Albano, J. Silva, and P. Teixeira. 2013. Role of flies as vectors of foodborne pathogens in rural areas. ISRN Microbiology 2013.

Brown, C. 2008. Available nutrients and value for manure from various livestock types, Ontario Ministry of Agriculture, Food and Rural Affairs.

Calvert, C. C., R. D. Martin, and N. O. Morgan. 1969. House fly pupae as food for poultry. Journal of Economic Entomology 62: 938-939.

Cao, J., J. Yan, Y. Huang, G. Wang, R. Zhang, X. Chen, Y. Wen, and T. Zhou. 2012. Effects of replacement of fish meal with house fly maggot meal on growth performance, antioxidant and non-specific immune indexes of juvenile *Litopenaeus vannamei*. Journal of Fisheries of China 36: 529-537.

Chang, J. and Wang, M., 1958. Nutritional requirements of the common housefly, *Musca domestica* vicina Macq. Nature 181: 566.

Chen, G., and K. Strevett. 2003. Impact of carbon and nitrogen conditions on *E. coli* surface thermodynamics. Colloids and Surfaces B: Biointerfaces 28: 135-146.

Chen, S., W. Liao, C. Liu, D. C. Elliott, M. D. Brown, and A. E. Solana. 2003. Value-added chemicals from animal manure, Pacific Northwest National Laboratory.

Chen, Z., and X. Jiang. 2014. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. Agriculture 4: 1-29.

Čičková , H., B. Pastor, M. Kozanek, A. Martinez-Sanchez, S. Rojo, and P. Takac. 2012. Biodegradation of pig manure by the house fly, *Musca domestica*: A viable ecological strategy for pig manure management. PloS ONE 7.

Đorđević, M., B. Radenković-Damnjanović, M. Vučinić, M. Baltić, R. Teodorović, L. Janković, M. Vukašinović, and M. Rajković. 2008. Effects of substitution of fish meal with fresh and dehydrated larvae of the house fly (*Musca domestica* L) on productive performance and health of broilers. Acta Veterinaria 58: 357-368.

EPA. 2015. Literature review of contaminants in livestock and poultry manure and implications for water quality, Createspace Independent Pub. United Sates Environmental Protection Agency.

Franson, M. A. H. 1989. Total solids dried at 103-105C: Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Group, P.E., 2011. Big chicken: pollution and industrial poultry production in America. Pew Environment Group.

Gupta, A. K., D. Nayduch, P. Verma, B. Shah, H. V. Ghate, M. S. Patole, and Y. S. Shouche. 2012. Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). FEMS Microbiology Ecology 79: 581-593.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Hribar, C., and M. Schultz. 2010. Understanding concentrated animal feeding operations and their impact on communities. Bowling Green, OH: National Association of Local Boards of Health. Retrieved February 18: 2013.

Hussein, M., V. V. Pillai, J. M. Goddard, H. G. Park, K. S. Kothapalli, D. A. Ross, Q. M. Ketterings, J. T. Brenna, M. B. Milstein, and H. Marquis. 2017. Sustainable production of house fly (*Musca domestica*) larvae as a protein-rich feed ingredient by utilizing cattle manure. PloS ONE 12: e0171708.

Kerr, B. J., and G. C. Shurson. 2013. Strategies to improve fiber utilization in swine. Journal of Animal Science and Biotechnology 4: 11.

Khan, H. A. A., S. A. Shad, and W. Akram. 2012. Effect of livestock manures on the fitness of house fly, *Musca domestica* L. (Diptera: Muscidae). Parasitology Research 111: 1165-1171.

Larrain, P. S., and C. F. Salas. 2008. House fly (*Musca domestica* L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Levinson, Z. 1960. Food of house fly larvae. Nature 188: 427.

Li, K., R. Liu, and C. Sun. 2015. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. Bioresource Technology 198: 133-140.

Lindner, P. 1919. Extraction of fat from small animals. Zootechnica Biologica 7: 213-220.

Lu, J., S. Sanchez, C. Hofacre, J. J. Maurer, B. G. Harmon, and M. D. Lee. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. Applied and Environmental Microbiology 69: 901-908.

Malik, A., N. Singh, and S. Satya. 2007. House fly (Musca domestica): a review of control strategies for a challenging pest. Journal of Environmental Science and Health Part B 42: 453-469.

Mallette, M., C. I. Cowan, and J. Campbell. 1964. Growth and survival of *Escherichia coli* in medium limited in phosphate. Journal of Bacteriology 87: 779-785.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019. Life-History Traits of Housefly, *Musca domestica* L. (Diptera: Muscidae), Reared on Three Manure Types. Journal of Insects as Food and Feed.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Pan, J., J. Fadel, R. Zhang, H. El-Mashad, Y. Ying, and T. Rumsey. 2009. Evaluation of sample preservation methods for poultry manure. Poultry science 88: 1528-1535.

Pandey, P., C. Chiu, M. Miao, Y. Wang, M. Settles, N. S. Del Rio, A. Castillo, A. Souza, R. Pereira, and R. Jeannotte. 2018. 16S rRNA analysis of diversity of manure microbial community in dairy farm environment. PloS ONE 13: e0190126.

Pratt, C., M. Redding, J. Hill, S. R. Mudge, M. Westermann, C. Paungfoo-Lonhienne, and S. Schmidt. 2014. Assessing refrigerating and freezing effects on the biological/chemical composition of two livestock manures. Agriculture, Ecosystems & Environment 197: 288-292.

Rochon, K., Lysyk, T. and Selinger, L., 2004. Persistence of *Escherichia coli* in immature house fly and stable fly (Diptera: Muscidae) in relation to larval growth and survival. Journal of Medical Entomology 41: 1082-1089.

Sasaki, T., M. Kobayashi, and N. Agui. 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157: H7 to food. Journal of Medical Entomology 37: 945-949.

Schmidtmann, E. and Martin, P., 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. Journal of Medical Entomology 29: 232-235.

Smith, T. C. 2015. Livestock-associated *Staphylococcus aureus*: the United States experience. PLoS Pathogens 11: e1004564.

Spiehs, M. J., and S. M. Goyal. 2007. Best management practices for pathogen control in manure management systems. Minnesota Extension.

Sun, J. and Zhou, X.J., 2013. Lignocellulolytic Systems of Insects and their Potential for Viable Biofuels. Biological Conversion of Biomass for Fuels and Chemicals: Exploration from Natural Utilization Systems. Cambridge, UK: Royal Society of Chemistry: 195-222.

Teotia, J. S., and B. F. Miller. 1974. Nutritive content of house-fly pupae and manure residue. British Poultry Science 15: 177-182.

Terzich, M., M. J. Pope, T. E. Cherry, and J. Hollinger. 2000. Survey of pathogens in poultry litter in the United States. Journal of Applied Poultry Research 9: 287-291.

Tomberlin, J.K., Crippen, T.L., Wu, G., Griffin, A.S., Wood, T.K. and Kilner, R.M., 2016. Indole: An evolutionarily conserved influencer of behavior across kingdoms. BioEssays.

USDA, 2010a. Overview of the United States dairy industry: large operations increase share of inventory and milk production, United States Department of Agriculture, National Agriculture Statistics Service.

USDA, 2010b. Overview of U.S. livestock, poultry, and aquaculture production in 2010 and statistics on major commodities, United States Department of Agriculture.

USDA. 2015. Overview of the United States hog industry: Breeding herd efficiency continues to increase. United States Department of Agriculture, National Agricultural Statistics Service.

van Huis, A., J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, and P. Vantomme. 2013. Edible insects: future prospects for food and feed security, Food and Agriculture Organization of the United nations (FAO).

Van Weelden, M., D. Andersen, B. Kerr, S. Trabue, and L. Pepple. 2016. Impact of fiber source and feed particle size on swine manure properties related to spontaneous foam formation during anaerobic decomposition. Bioresource Technology 202: 84-92.

Wang, H., Z. Zhang, G. F. Czapar, M. K. Winkler, and J. Zheng. 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. Waste Management & Research 31: 223-231.

Wang, L., K. Mankin, and G. Marchin. 2004. Survival of fecal bacteria in dairy cow manure. Transactions of the ASAE 47: 1239.

Wang, L., J. Li, J. Jin, F. Zhu, M. Roffeis, and X. Zhang. 2017. A comprehensive evaluation of replacing fishmeal with house fly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): growth performance, flesh quality, innate immunity and water environment. Aquaculture Nutrition 23: 983-993.

Weiden, P. L., W. Epstein, and S. G. Schultz. 1967. Cation transport in *Escherichia coli*: VII. Potassium requirement for phosphate uptake. The Journal of General Physiology 50: 1641-1661.

Zhang, Y., C. Zhang, D. B. Parker, D. D. Snow, Z. Zhou, and X. Li. 2013. Occurrence of antimicrobials and antimicrobial resistance genes in beef cattle storage ponds and swine treatment lagoons. Science of the Total Environment 463: 631-638.

Zhou, F., J. K. Tomberlin, L. Zheng, Z. Yu, and J. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of Medical Entomology 50: 1224-1230.

Zhu, J. 2000. A review of microbiology in swine manure odor control. Agriculture, Ecosystems & Environment 78: 93-106.

Zi-zhe, C., Y. De-po, W. Sheng-qing, W. Yong, M. J. Reaney, Z. Zhi-min, Z. Long-ping, S. Guo, N. Yi, and Z. Dong. 2017. Conversion of poultry manure to biodiesel, a practical method of producing fatty acid methyl esters via house fly (*Musca domestica* L.) larval lipid. Fuel 210: 463-471.

Zurek, L., C. Schal, and D. Watson. 2000. Diversity and contribution of the intestinal bacterial community to the development of *Musca domestica* (Diptera: Muscidae) larvae. Journal of medical entomology 37: 924-928.

4. MASS PRODUCTION OF THE BLACK SOLDIER FLY, *Hermetia illucens* (L.), (DIPTERA: STRATIOMYIDAE) REARED ON THREE MANURE TYPES

Overview

Recent interest in the mass production of black soldier fly (BSF) larvae has resulted in many studies being conducted on its life cycle when fed various organic by-products. However, a majority of the studies are bench-top, or small scale, experiments, and results generated from such studies may not translate to large-scale production. The current study was conducted at a conventional large scale, using 10,000 larvae/treatment fed 7 kg to determine impact on selected life-history traits, when fed a control diet (50% wheat bran, 30% alfalfa meal, 20% corn), swine, dairy, or poultry manure. Larval and prepupal weight did not differ significantly ($p > 0.05$) across diets; however, development time and survivorship did ($p < 0.0001$). Larvae fed dairy manure required 1 –2 d longer to develop to prepupation when compared to those fed poultry or swine manure, with 45% survivorship to prepupation compared to >70% for those fed swine or poultry manure. Data from this study may be valuable for industrialization of BSF, as companies using a scale varying from previously published work, including this study, should conduct pilot studies to optimize their system prior to implementation.

Introduction

Insects provide a variety of goods and services for human exploitation (Kellert 1993). They may be reared for medicinal purposes, such as wound therapy (Sherman 2003), or to engineer antibodies (Verma et al. 1998) and vaccines (Krammer and Grabherr 2010). They may be cultivated for other well-known purposes such honey (Zumla and Lulat 1989), silk (Peigler 1993), and dye production (Barayovits 1978), or can be used to control insect pest populations (Orr and Sriyanka 2014). Insects may also be used for food and feed purposes (van Huis et al. 2013); however, this industry is still in its infancy in terms of operating on an industrial scale. Recent interest in exploiting insects in this manner has prompted numerous studies (Hwangbo et al. 2009, Oonincx et al. 2015, Van Broekhoven et al. 2015, Morales-Ramos et al. 2018, Ssepuuya et al. 2018), which helps progress the idea and refine the industry; however, most of the published studies are based on small-scale (bench-top experiments), which may not truly represent what occurs on a larger scale.

Recently, the black soldier fly (BSF), *Hermetia illucens* (L.), (Diptera: Stratiomyidae) has gained a considerable amount of attention. As discussed in previous publications, this species is distributed globally (i.e., tropics and temperate regions) and is an ideal candidate for industrialization purposes because it offer a means to manage a variety of waste (Nguyen et al. 2013, Nguyen et al. 2015, Lalander et al. 2019) as well as provide multiple revenue streams, such as production of animal feed (van Huis et al. 2013) and biofuel (Li et al. 2011a, Li et al. 2011b). In systems that utilize manure as a resource, BSF reduce dry matter (Myers et al. 2008), pathogens (Erickson et al. 2004, Liu et al. 2008), and odors (Beskin et al. 2018). However, most of the previously-published work on BSF is performed on a small-scale (e.g., serval hundred larvae per replicate tray), which may not translate to an industrial-scale (thousands to tens of thousands of larvae per tray).

Methods used in laboratory studies are typically different than those employed by mass-production facilities (larval numbers in the thousands consuming kilograms rather than grams over time). Both population size and feeding rate are known to impact development. For example, Banks et al. (2014) showed that bulk feeding increased development time and larval weight across three densities (1, 10, and 100 larvae) compared to those fed incrementally. Similarly, Barragan-Fonseca et al. (2018) added nutrient content of the diet as a factor and reported decreased development time by 2 –4 d for those fed a high nutrient (protein., fat, and non-cellulose carbohydrate) diet across all densities tested (50, 100, 200, and 400 larvae) and by 7–11 d for those fed a low nutrient diet for the lower densities compared to those fed incrementally; but, larvae fed the bulk low nutrient diet at higher densities (200 and 400) took longer (45 d) to develop. Even the authors of this study have conducted such studies (Cammack and Tomberlin 2017, Miranda et al. accepted May 2019). Yet, these studies can still be considered small-scale when compared to practices in industry (personal communication, Cammack).

Larval density can hinder, or in some instances enhance, insect performance. Bryant and Sokal (1967) showed that low (80 eggs/18 g of diet) and high (640 eggs/18 g of diet) densities of house flies (HF), *Musca domestica* L., (Diptera: Muscidae) faced different challenges during development. Low densities may result in poor conditioning of the diet (via metabolites produced by larvae), which impacts yeast growth and ultimately availability of food for HF (Bryant and Sokal 1967). However, increased larval density may intensify effects of competition leading to reduced survivorship (Sullivan and Sokal 1963, Bryant and Sokal 1967). Still, larvae feed in aggregates, which generates heat (Slone and Gruner 2007), and temperature is known to impact BSF development and survivorship. Black soldier fly larvae reared at 30°C developed the fastest (13 d), had the shortest prepupal development (8 –10 d), and highest larval survivorship (90%) compared to those reared on temperatures that ranged from 10 –42°C (Chia et al. 2018). Additionally, it is possible that higher densities excrete more oral secretions (gut microbiota) that aid in cooperative digestion a resource (Zhao et al. 2017). As such, larval density is a major factor that influences BSF performance.

The purpose of this study was to evaluate selected life-history traits (larval development, development time to first prepupation, percent survivorship to prepupation, and prepupal weight) of BSF fed swine, dairy, or poultry manure by using methods based on industrial standards (Zurbrügg et al. 2018). Most of the data available on this species originates from small scale-studies, which may not translate on a larger production scale, as previously discussed. Results from this study may be valuable by providing a basis to compare findings from previous small-scale studies as well as a paradigm to optimize mass-rearing conditions of BSF fed manure.

Methods

Acquisition of BSF

Methods for this study were based on those conducted by Miranda et al. (accepted May 2019). Black soldier flies were obtained from a colony that is maintained at the F.L.I.E.S (Forensic Laboratory for Investigative Entomological Sciences) Facility at Texas A&M University in College Station, TX, USA. The colony originated from one maintained in Tifton, GA, USA, and follows modified methods proposed by Sheppard et al. (2002).

Acquisition of Manure

Manure less than 12-h-old was used in this study. Swine manure was collected from a local farmer, in Anderson, TX, USA, dairy manure was collected from a commercial dairy located in Stephenville, TX, USA, and poultry manure was collected from layer hens housed at the Poultry Science Research, Teaching and Extension Center (Texas A&M University, College Station, TX, USA) Manure was placed into 18.9 L buckets, covered with lids (Home Depot®, Leaktite™, Leominster, MA, USA), and transported to the F.L.I.E.S. Facility, where it was homogenized by hand, transferred to 3.7 L Ziploc® Freezer Bags (S.C. Johnson & Son, Racine, WI, USA) and stored at -20°C until used. Manure was allowed to thaw at room temperature for 24 h before initiation of the experiment. Moisture content of manure was measured gravimetrically from three 10 g samples, following methods described by Franson (1989). Initial moisture contents for swine, dairy, and poultry were 72%, 83%, and 77%.

Experiment Design

Black soldier fly adults were maintained at the F.L.I.E.S facility in a 260 x 116 x 129 cm wooden cage lined with fiberglass window screen (18x16 mesh size) in a greenhouse (25 -45°C, >50% RH). To collect eggs, a 5.7 L Sterilite® container (Townsend, MA, USA) was filled with 500 g Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% corn meal) (Hogsette 1992) saturated with RO water and contained approximately 500 actively feeding larvae. The lid of the container had a 15 x 7 cm hole that was covered with wire mesh. Corrugated cardboard was cut into 5 x 2 x 0.5 cm strips, and 5 pieces of cardboard were stacked and taped together to form a bundle, which was placed on the lid of the container described above. The container remained in the cage for 8 h, after which the cardboard was removed and placed in 0.9 L Ball® mason jar (Ball Corporation, Broomfield, CO, USA) covered with a paper towel secured with the metal ring of the mason jar lid. The jar with the cardboard containing eggs was stored in a Rheem Environmental Chamber (Asheville, NC, USA; 29˚C, 60% RH, and 16L:8D light cycle) until larvae eclosed. Approximately 10,000 newly hatched larvae per replicate (n=6) were weighed on an OHaus® Adventurer™ Pro AV64 (OHaus® Corporation, Pine Brook, NJ, USA), and placed in 0.5 L deli food storage container (Amazon.com Inc., Seattle, WA, USA) without a lid. To decrease larval mortality prior to use in the experiment, larvae were fed 150 g of Gainesville diet (70% moisture) for four days.

Treatments consisted of 10,000 4-d-old larvae fed 7 kg of swine, dairy or poultry manure. Larvae in control groups were fed 7 kg of Gainesville diet (70% moisture). Larval diets were placed in the center of a 30 L Sterilite® ClearView latch storage container (Townsend, MA, USA) with 700 g of dry Gainesville diet (Hogsette 1992) placed around the perimeter of the manure to serve as a pupation substrate and to prevent developing larvae from escaping. Ten larvae per replicate were weighed prior to placement on the manure or Gainesville diet to determine that their weights were not significantly different. The deli containers with developing larvae were poured on top of the manure, and the 30 L containers (with the larvae and waste) were placed inside the environmental chamber described above.

Larvae were allowed to feed on the manure or Gainesville diet for two days prior to measuring daily larval weight, as they were too small to find without significantly disturbing the media. Daily larval weight was measured by selecting 10 of the largest larvae for 9 days, and development time to first prepupation was recorded upon observation of the first prepupae in each replicate container. Survivorship was calculated by dividing the total weight of all living larvae from each treatment by the average final larval weight, which was recorded on the day the first prepupa was observed. Lastly, prepupal weight was measured by selecting 10 of the largest prepupae from each treatment when 40% reached the prepupal stage. All weights were measured using the scale described above.

Statistical Analyses

Larval development, development time from placement on manure to prepupation, percent survivorship to the prepupal stage, and prepupal weight were analyzed across treatments using an ANOVA. Statistics were performed using JMP PRO 14 (SAS Institute Inc., Cary, NC, USA). Normality was checked using the Shapiro-Wilk

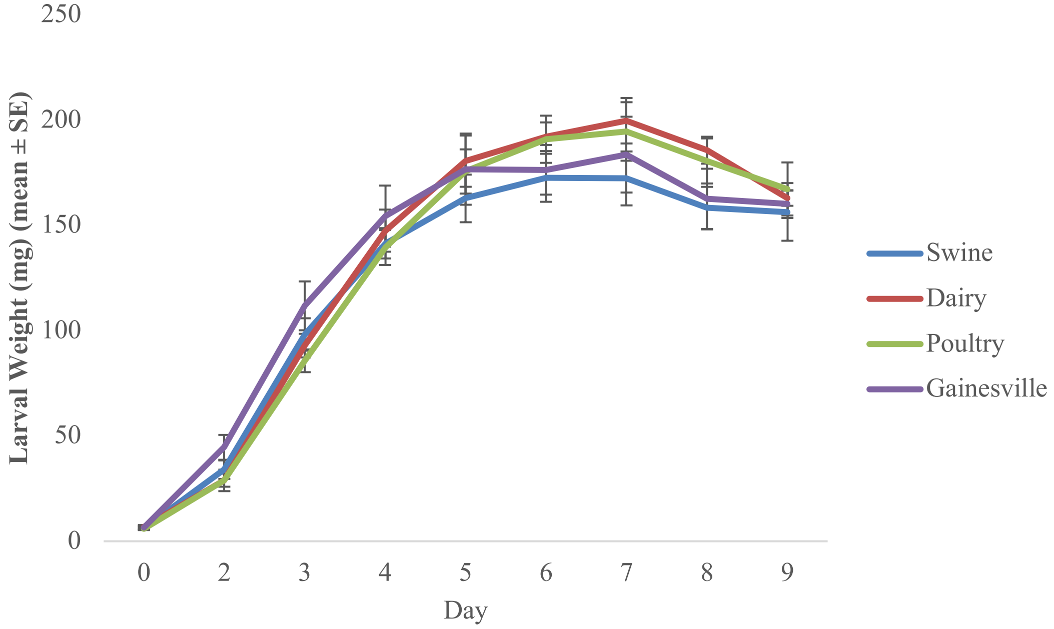
Test and homogeneity of variance was checked using Bartlett's test. Tukey's HSD was used for mean separation ($p \leq 0.05$).

Results

Larval Development

Larval development did not differ significantly ($F_{3,142} = 1.9318$; $p = 0.1263$) across larval diets (Figure 4.1). A significant trail effect ($F_{1,142} = 163.6540$; $p < 0.0001$) was found, but no significant treatment by trial interaction ($F_{27,142} = 0.7314$; $p = 0.8178$) was found. In general, individuals in trial two weighed 22% more than those in trial one.

Figure 4.1 Mean larval weight (mg) (mean ± SE[1]n = 6) of black soldier flies fed 7 kg of standard diet (Hogsette 1992) or swine, dairy, or poultry at 29 °C, 60% RH, and 16L:8D**.**

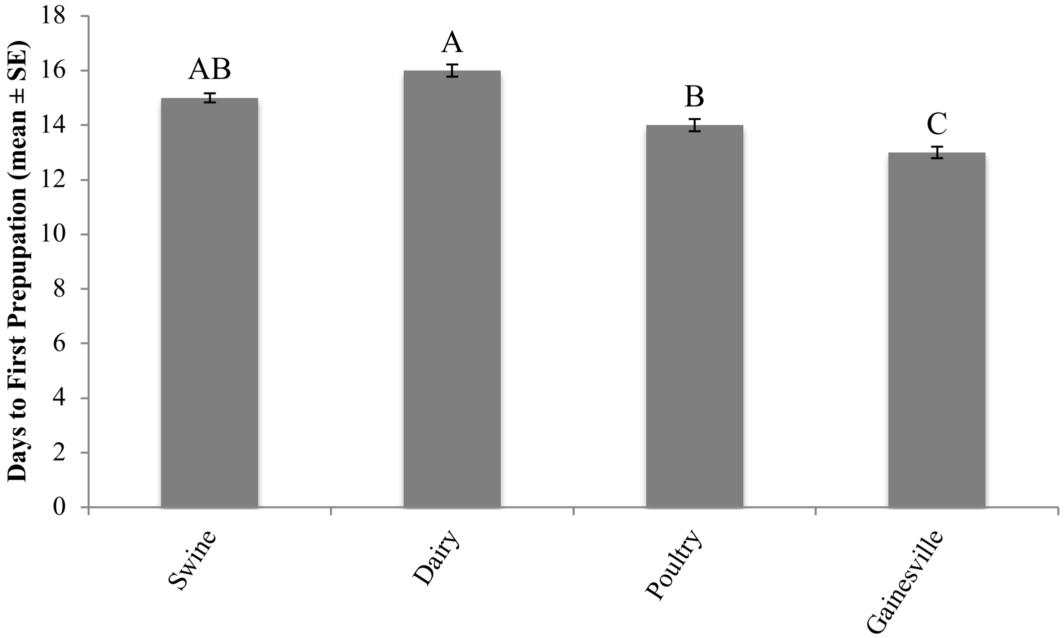


[1]n = number of replicates per treatment.

Development Time to First Prepupation

Development time to first prepupation differed significantly ($F_{3,16} = 16.9048$; $p < 0.001$) across larval diets. No trial effect ($F_{1,16} = 0.1429$; $p = 0.7104$) or treatment by trial interaction ($F_{3,16} = 0.5238$; $p = 0.6721$) was found. The shortest development time was found for those fed Gainesville diet (13 d). In regard to those fed manure, the shortest development time was found for those fed poultry (14 d) and swine manures (15 d); whereas those fed dairy manure reached the prepupal stage in 16 d (Figure 4.2).

Figure 4.2 Development time to first prepupation (mean ± SE, $^1n = 6$) of black soldier fly larvae fed 7 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 29˚C, 60% RH, and 16L:8D.

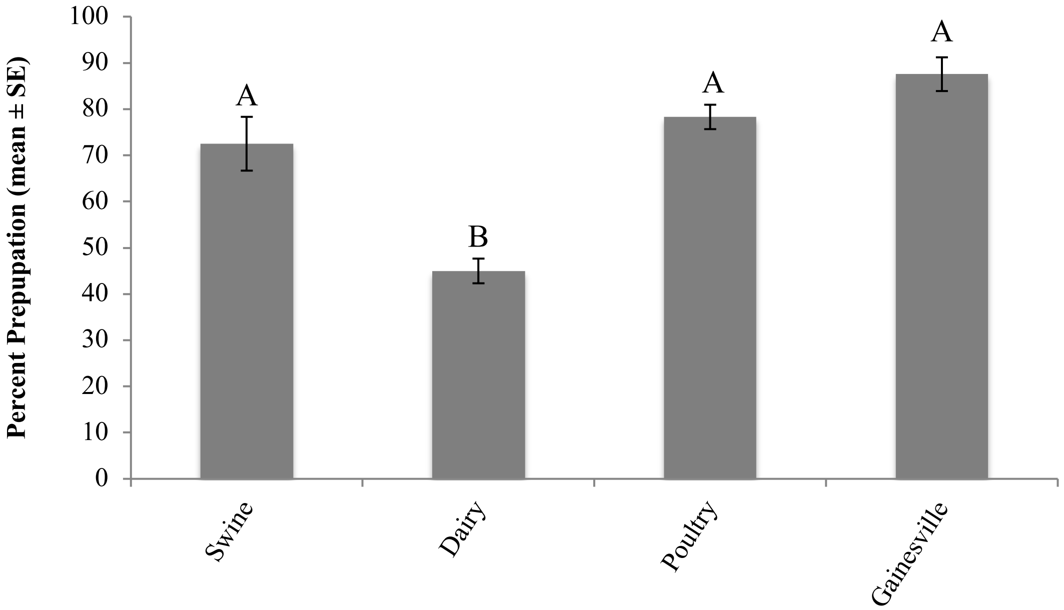


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Percent Survivorship to the Prepupal Stage

Percent survivorship to the prepupal stage (percent prepupation) was significantly different ($F_{3,16} = 22.2899$; $p < 0.0001$) across larval diets. No trial effect ($F_{1,16} = 0.6001$; $p = 0.4498$) or treatment by trial interaction ($F_{3,16} = 1.2535$; $p = 0.3235$) was found. The highest percent prepupation across all diets was found for those fed Gainesville diet (88%). In regard to larvae fed manure, the highest percent prepupation was found for those fed poultry manure (78%), followed by swine (73%) and dairy (45%) manures (Figure 4.3).

Figure 4.3 Percent prepupation (mean ± SE, ^{1}n = 6) of black soldier fly larvae fed 7 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 29˚C, 60% RH, and 16L:8D

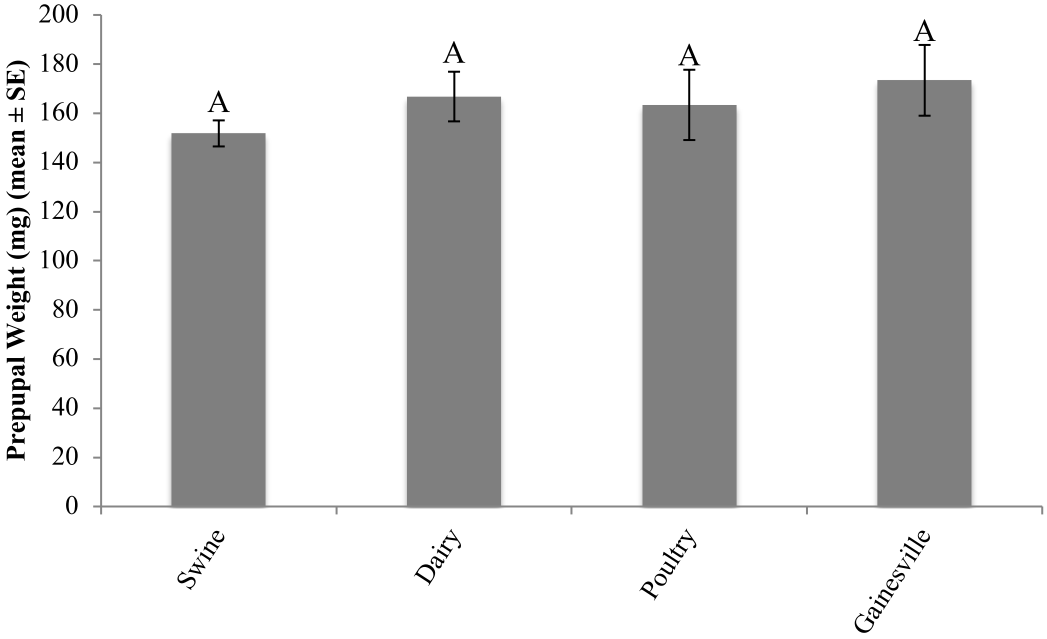


^{1}n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Prepupal Weight

Prepupal weight did not differ significantly ($F_{3,16} = 0.5997$; p = 0.6245) across larval diets. No trial effect ($F_{1,16} = 3.5717$; p = 0.0770) or treatment by trial interaction ($F_{3,16} = 0.0837$; p = 0.9679) was observed. Larvae fed Gainesville diet weighed approximately 173 mg and those fed poultry, swine and dairy weighed 163, 152, and 167 mg, respectfully (Figure 4.4).

Figure 4.4 Prepupal weight (mean ± SE, [1]n = 6) of black soldier fly larvae fed 7 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 29˚C, 60% RH, and 16L:8D.



[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Discussion

Results from this study show development and survivorship is significantly impacted by diet. Larvae fed the Gainesville diet (Hogsette 1992) had the shortest development time (13 d) (Figure 4.2), the highest survivorship to the prepupal stage (88%) (Figure 4.3) and resulted in the heaviest prepupae (173 mg) (Figure 4.4). In regard to those fed manure, variations in development time and survivorship were found across manure types. Although larval (Figure 4.1) or prepupal weight (Figure 4.4) did not differ significantly across treatments, more time (1 –2 d) was required for larvae to reach the prepupal stage and fewer larvae survived (45 %) when they were fed dairy manure. These differences are important in respect to production efficiencies and bioconversion and may be explained by variations in manure types.

Manure from different animals varies in chemical and physical composition (Li et al. 2014). Poultry manure is typically higher in nutrients and lower in fiber compared to dairy manure (Chen et al. 2003), and these differences may explain the impacts on development and survivorship seen in the current study. Variation in development time and survivorship could translate to variation in production efficiency and bioconversion yield. Although prepupal weight was not significantly different across treatments (Figure 4.4), slower development for larvae fed dairy manure (Figure 4.2) and lower survivorship (Figure 4.3) may negatively impact costs of production and resulting revenue. It is possible that larvae fed dairy manure were able to reach similar pupal weights to those fed swine or poultry manure because larvae fed on the resource longer and were subjected to reduced intraspecific competition because of high mortality (45% survivorship).

Based on a comparison between this study and others conducted at a smaller scale (i.e., lower number of larvae per replicate), it is clear that scale impacts production of the BSF. For example, Miranda et al. (accepted May 2019), which used larvae that originated from the same colony in the current study, found that 100 4-d-old BSF fed 27 g of swine, dairy or poultry manure every other day weighed less (30 –45%), had shorter development time (by 2 –6 d), and higher survivorship (6 –17%) compared to those in the current study. However, there were differences in methods that could have contributed to the variation observed across studies. Lower larval density (i.e., 100 –300 larvae; small-scale) with incremental feeding (daily or every other day) employed as the feeding regimen were utilized rather than a higher density (i.e., 10,000 larvae; large-scale) and a single feeding. Such methods employed in the small-scale study are not known to be utilized by mass-production facilities, and it is possible that results do not translate to a larger scale. Although, it is possible that scale is not the only influential factor as the amount of manure available to larvae differed (43 –50 mg vs. 135 mg/larvae/d) from the current study. Therefore, small-scale studies should include a large-scale component when possible. For additional comparative purposes, Lalander et al. (2019), which is a well-developed study, fed 200 10-d-old larvae poultry manure (40 mg/larvae/d) similar to the amount of food provided to larvae in the current study (43 – 50 mg/larvae/d). Individual prepupae in the above-mentioned study weighed 165 mg, and those from the current study weighed 163 mg, with the same development time to prepupation from placement on manure (14 d). However, the current study differs from Lalander et al. (2019) in survivorship (78% in the current study vs 92% in Lalander et al. (2019)) as well as time spent to condition larvae on a control diet (4 vs 10 d). It is possible that the higher survivorship reported by Lalander et al. (2019) was possible because larvae were conditioned on the control diet for 6 additional days. Consequently, total development time from neonate to prepupae differed with those from the current study reaching prepupation in less time (18 vs 24 d).

Other factors influence BSF development, including but not limited to, temperature (Tomberlin et al. 2009), diet composition (Nguyen et al. 2015, Cammack and Tomberlin 2017, Cheng et al. 2017), moisture (Cammack and Tomberlin 2017 Cheng et al. 2017), pH (Meneguz et al. 2018) and species strain (Zhou et al. 2013). However, because most studies are conducted with a few hundred larvae with incremental feeding, the impact of these factors is not well understood. It may also be difficult for individuals interested in mass-producing BSF to extrapolate and apply information with expectations similar to those reported from studies that utilize methods that differ from industrial standards. It should be noted that authors have also performed small-scale studies but recognize that the outcomes of these studies may differ at a larger scale. Additionally, there are facilities that produce larvae at higher densities greater than 10,000 larvae and provide larvae more than 7 kg of diet, so it is possible that the results from this study may not translate to a larger production scale. For these reasons, future studies should investigate BSF life-history parameters at larger scales than the current study and based on known industry methods.

The current study shows differences in development time and survivorship for BSF larvae fed different manure types. Although no significant difference was found across manure types for prepupal weight, differences were detected for development time and survivorship. Those provided dairy manure took 1–2 d longer to develop with fewer individuals surviving to the prepupal stage (45% vs >70%) compared to those provided swine or poultry manure. Additionally, this study highlights differences likely exist across different production scales and urges future studies to perform work on larger scales to advance industrialization of BSF.

References

Banks, I. J., W. T. Gibson, and M. M. Cameron. 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Tropical Medicine International Health 19: 14-22.

Barayovits, F. L. C. 1978. Cochineal carmine: An ancient dye with a modern role. Endeavour 2: 85-92.

Barragan-Fonseca, K. B., M. Dicke, and J. J. van Loon. 2018. Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). Entomologia Experimentalis et Applicata 166: 761-770.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Bryant, E. H., and R. R. Sokal. 1967. The fate of immature house fly populations at low and high densities. Population Ecology 9: 19-44.

Cammack, J. A., and J. K. Tomberlin. 2017. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). Insects 8: 56.

Chen, S., W. Liao, C. Liu, D. C. Elliott, M. D. Brown, and A. E. Solana. 2003. Value-added chemicals from animal manure, Pacific Northwest National Laboratory.

Cheng, J. Y., S. L. Chiu, and I. M. Lo. 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste Management 67: 315-323.

Chia, S. Y., C. M. Tanga, F. M. Khamis, S. A. Mohamed, D. Salifu, S. Sevgan, K. K. Fiaboe, S. Niassy, J. J. van Loon, and M. Dicke. 2018. Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. PloS ONE 13: e0206097.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. Journal of Food Protection 67: 685-690.

Franson, M. A. H. 1989. Total solids dried at 103-105C: Standard methods for the examination of water and wastewater., American Public Health Association, Washington, DC.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera: Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Hwangbo, J., E. Hong, A. Jang, H. Kang, J. Oh, B. Kim, and B. Park. 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. Journal of Environmental Biology 30: 609-614.

Kellert, S. R. 1993. Values and perceptions of invertebrates. Conservation Biology 7: 845-855.

Krammer, F., and R. Grabherr. 2010. Alternative influenza vaccines made by insect cells. Trends in Molecular Medicine 16: 313-320.

Lalander, C., S. Diener, C. Zurbrügg, and B. Vinnerås. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (*Hermetia illucens*). Journal of Cleaner Production. 208: 211-219.

Li, G., H. Li, P. A. Leffelaar, J. Shen, and F. Zhang. 2014. Characterization of phosphorus in animal manures collected from three (dairy, swine, and broiler) farms in China. PloS ONE 9.

Li, Q., L. Zheng, H. Cai, E. Garza, Z. Yu, and S. Zhou. 2011a. From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. Fuel 90: 1545-1548.

Li, Q., L. Zheng, N. Qiu, H. Cai, J. K. Tomberlin, and Z. Yu. 2011b. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Management 31: 1316-1320.

Liu, Q., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37: 1525-1530.

Meneguz, M., L. Gasco, and J. K. Tomberlin. 2018. Impact of pH and feeding system on black soldier fly (*Hermetia illucens*, L; Diptera: Stratiomyidae) larval development. PloS ONE 13: e0202591.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019. Life-History Traits of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), Reared on Three Manure Types. Animals 9: 281.

Morales-Ramos, J., M. Rojas, and A. Dossey. 2018. Age-dependent food utilisation of Acheta domesticus (Orthoptera: Gryllidae) in small groups at two temperatures. Journal of Insects as Food and Feed 4: 51-60.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Nguyen, T. T., J. K. Tomberlin, and S. Vanlaerhoven. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology 44: 406-410.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2013. Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. Journal of Medical Entomology 50: 898-906.

Oonincx, D. G., S. Van Broekhoven, A. Van Huis, and J. J. van Loon. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PloS ONE 10: e0144601.

Orr, D., and L. Sriyanka. 2014. Biological control of insect pests in crops. Integrated Pest Management: 531-548.

Peigler, R. S. 1993. Wild silks of the world. American Entomologist 39: 151-162.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera : Stratiomyidae). Journal of Medical Entomology 39: 695-698.

Sherman, R. A. 2003. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. Diabetes Care 26: 446-451.

Slone, D., and S. V. Gruner. 2007. Thermoregulation in larval aggregations of carrion-feeding blow flies (Diptera: Calliphoridae). Journal of Medical Entomology 44: 516-523.

Ssepuuya, G., C. Tanga, I. Yekko, F. Sengendo, C. Ndagire, K. Fiaboe, J. Karungi, and D. Nakimbugwe. 2018. Suitability of egg hatching conditions and commonly available food plants for rearing the long-horned grasshopper Ruspolia differens Serville (Orthoptera: Tettigoniidae). Journal of Insects as Food and Feed 4: 253-261.

Sullivan, R. L., and R. R. Sokal. 1963. The effects of larval density on several strains of the house fly. Ecology 44: 120-130.

Tomberlin, J. K., P. H. Adler, and H. M. Myers. 2009. Development of the Black Soldier Fly (Diptera: Stratiomyidae) in Relation to Temperature. Environmental Entomology 38: 930-934.

Van Broekhoven, S., D. G. Oonincx, A. Van Huis, and J. J. Van Loon. 2015. Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. Journal of Insect Physiology 73: 1-10.

van Huis, A., J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, and P. Vantomme. 2013. Edible insects: future prospects for food and feed security, Food and Agriculture Organization of the United nations (FAO).

Verma, R., E. Boleti, and A. George. 1998. Antibody engineering: comparison of bacterial, yeast, insect and mammalian expression systems. Journal of Immunological Methods 216: 165-181.

Zhao, Y., W. Wang, F. Zhu, X. Wang, X. Wang, and C. Lei. 2017. The gut microbiota in larvae of the house fly *Musca domestica* and their horizontal transfer through feeding. AMB Express 7: 147.

Zhou, F., J. K. Tomberlin, L. Zheng, Z. Yu, and J. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of Medical Entomology 50: 1224-1230.

Zumla, A., and A. Lulat. 1989. Honey-a remedy rediscovered. SAGE Publications Sage UK: London, England.

Zurbrügg, C., B. Dortmans, A. Fadhila, B. Verstappen, and S. Diener. 2018. From pilot to full scale operation of a waste-to-protein treatment facility. Detritus 1: 18.

5. LARGE-SCALE PRODUCTION OF THE HOUSE FLY, *Musca domestica* L., (DIPTERA: MUSCIDAE) REARED ON THREE MANURE TYPES

Overview

House flies (HF) are well-known pests of animal facilities; however, they can be used for biodegradation of manure. Utilizing HF to process animal manure offers a means to recycle nutrients and reduce contaminants (e.g., pathogens and heavy metals), while also producing multiple revenue streams (e.g., protein, biodiesel, fertilizer). This study determined HF larval performance fed swine, dairy, or poultry manure on a larger scale (thousands of larvae; single feeding) as a follow-up to previous experiments performed on a small-scale (hundreds of larvae; incremental feeding). Four thousand larvae were fed 1 kg of swine, dairy, or poultry manure or a control (Gainesville diet; 50% wheat bran, 30% alfalfa meal, and 20% corn meal). Peak larval weight occurred 4 d after inoculation and no significant difference ($p < 0.05$) in development time to first pupariation occurred across diets. However, percent pupariation varied with the highest occurring in Gainesville (74%), swine (73%), and poultry (67%) manure, whereas 50% survived when fed dairy manure. Pupal weight also varied with the highest found for those fed Gainesville (27 mg), but in regard to those fed manure, similar weights were found for those fed poultry (25mg) and dairy (24 mg), and swine (21 mg) manure. Although the idea of using HF to manage manure has received little consideration in western countries, other regions have such a practice in place. Results may provide insight on differences between small- and large-scale studies, which is valuable for industrialization of this species for waste management.

Introduction

Insects are a well-established form of agriculture globally (Kellert 1993, Zhang et al. 2008). Honey (Crittenden 2011) and silk (Cherry 1987) have been cultivated for centuries. Insects can also be used for producing vaccines (Cox and Karl Anderson 2007) or used maggot debridement therapy (Chan et al. 2007). A more common use is biological control (Orr and Sriyanka 2014) or for producing food and feed (DeFoliart 1989). Over the course of the past three decades, their used as a sustainable practice to recycle wastes, such as food (Jeon et al. 2011, Cheng et al. 2017), fish offal (St-Hilaire et al. 2007), brewer's waste (Kim et al. 2016, Meneguz et al. 2018), and manure (Sheppard et al. 1994, Newton et al. 2005)

Although little attention has been given to insects as manure management agents, they may be a solution to an impending concern, the growing global human population. As the global human population increases along with standard of living, confined animal production is following suit. Consequently, manure production is also increasing. Proper management of manure is essential as it can be a source of pollution and lead to eutrophic environments (Wright 1998). A large proportion of livestock and poultry in the United States are produced in geographically clustered areas, which can make dissemination of manure to surround land difficult and costly (Edmonds et al. 2003). By employing insects to manage manure, waste can be recycled while producing fertilizer as well as valuable biomass as feed. Such a circular economy is more sustainable than current methods (Jun and Xiang 2011).

The house fly (HF), *Musca domestica* L., (Diptera: Muscidae) is a global pest (Busvine 1951, Kaufman et al. 2006, Geden 2012, Bass et al. 2015). However, HF are utilized to degrade swine manure in China (personal communication, Tomberlin) as well as in other parts of the world (Čičková et al. 2012, Roffeis et al. 2015). As the global leader, China produced 433 million head of swine in 2018 (USDA 2018). Unfortunately, rapid industrialization has caused substantial pollution to arable land (Lee et al. 2018); thus, developing novel methods to sustainably recycle manure is essential.

The HF is an attractive means to manage animal wastes (Wang et al. 2017). Manure digested by HF yields biomass comprised of 55-63% protein and 15-23% fat (Calvert et al. 1969, Wang et al. 2013) while also reducing associated pathogens (Wang et al. 2013, Nordentoft et al. 2017) and odors (Wang et al. 2013). Larval HF can also be converted to biodiesel (Yang et al. 2014), while the digested manure can be used as fertilizer (Kováčik et al. 2014).

House fly development on manure can vary depending on manure type. Larrain and Salas (2008) investigated the development of HF fed swine, poultry, calf, cow, dog, goat, and horse manure and determined those fed swine, poultry and calf manure developed faster (>90% pupariation by 6 d) and weighed more as pupae (16-20mg) than those fed the other manure types. Similarly, Khan et al. (2012) determined HF developed best when fed poultry and swine manure rather than buffalo, cow, sheep, horse or goat manure. Yet, these studies were performed on a small-scale (30 larvae). When comparing these results to a large-scale (thousands of larvae) study on HF fed manure, Čičková et al. (2012) found 60% survivorship when 4 kg of manure was seeded with approximately 8800 eggs/kg. Similarly, Zi-zhe et al. (2017) showed that individual larval weight (from 28 mg/larva to 12 mg/larva) and survivorship (from 90% to <60%) decreased with increasing larval densities (2000-12,000 larvae/kg poultry manure).

The purpose of this study was to evaluate select life-history traits of HF larvae fed swine, dairy, or poultry manure on a different scale of production than previously evaluated (see chapter 3; Miranda et al. accepted May 2019). Research previously presented in chapter 3 was conducted on small-scale (i.e., bench-top), which consisted of 100 larvae fed 45 mg/larvae/d. Results demonstrated HF pupae weighed 12, 10, and 14 mg when reared on swine, dairy and poultry manure, respectfully (Miranda et al. accepted May 2019). Additionally, 58%, 46% and 63% reached pupariation, respectfully, in 6-7 d. However, because this work was performed on a small-scale (few hundred larvae; incremental feeding), translation of these results to a larger, more industrial scale, might not be possible.

Methods

Acquisition of HF

Methods for this experiment are based on those conducted by (Miranda et al. accepted May 2019). House flies used in this experiment came from a colony maintained at the Forensic Laboratory of Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University, College Station, TX, USA. This colony originated from adults collected in 2014 from swine and dairy facilities in Stephenville, TX, USA and is maintained by providing water as well as sugar mixed with milk powder (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA) (1:1 ratio by mass) *ad libitum*.

Acquisition of Manure

Manure less than 12-h-old was collected and used in this experiment. Swine manure was collected from a farm in Anderson, TX, USA., dairy manure was collected from a commercial dairy located in Stephenville, TX, USA, and poultry manure (from layer hens) was collected from the Poultry Science Research, Teaching and Extension Center (Texas A&M University, College Station, TX, USA). Once collected, manure was placed into 18.9 L buckets, covered with lids (Home Depot®, Leaktite™, Leominster, MA, USA), and transported to the F.L.I.E.S. Facility, where it was homogenized in the buckets (mixed vigorously by hand for approximately 5 minutes) and transferred to 3.76 L Ziploc® Freezer Bags (S.C. Johnson & Son, Racine, WI, USA) and stored at -20°C until used. Manure was allowed to thaw at room temperature for 24 h before initiation of the experiment. Three 10 g samples of thawed manure of each type were used to gravimetrically determine moisture content at the beginning of the experiment (Franson 1989). Initial moisture contents for swine, dairy, and poultry manure were 72%, 85%, and 76%, respectively.

Experiment Design

In order to collect eggs, a Kimwipe® (Kimberly-Clark Corp., Irving, TX) was balled up, saturated with Evaporated Milk (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA), and placed in an 88 ml bathroom cup (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA). The cup was placed inside a 30 x 30 x 30 cm cage (Bioquip Products, Rancho Dominquez, CA, USA) for 8 h, which housed gravid adult flies. The cup with the Kimwipe® was removed from the cage and the wipe was unfolded and rinsed with distilled water over a 100 ml beaker to collect eggs. Viable housefly eggs sank to the bottom of the beaker and were collected with a Pasteur pipette. The eggs were transferred to a 118 ml container (Glad® Brand, The Clorox Company, Oakland, CA, USA) filled with 10 g of Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% cornmeal) and moistened with water (1:1 ratio by volume) (Hogsette 1992) and stored in a Rheem Environmental Chamber (Asheville, NC, USA; at 26˚C, 60% RH, and 16L:8D until larval eclosion.

Treatments consisted of 4,000 1-d-old larvae fed 1 kg of swine, dairy or poultry manure. Density and feed amount (4 eggs/g manure) were suggested by Hussein et al. (2017) as the appropriate density for HF fed dairy manure. Larvae in control groups were fed 1 kg of Gainesville diet (70% moisture) and diets were placed in the center of a 6 Qt Sterilite® clear storage container (Townsend, MA, USA) with 100 g of dry Gainesville diet placed around the parameter of the manure or control diet to serve as a pupation substrate and to prevent developing larvae from escaping. Larvae were separated from the media at the first observation of pupariation via negative phototaxis, which included placing larvae and spent media on a SE GP2-18 metal sieve (1/8” screen) placed inside a 30 L Sterilite® ClearView latch storage container with a Dazor® Floating Fixture light (model m-1470; St. Louis, MO, USA) placed over the sieve. Larvae crawled through the sieve, away from the light, and were collected in the container. The total larval weight was measured and divided by the final larval weight to estimate percent survivorship.

Statistical Analyses

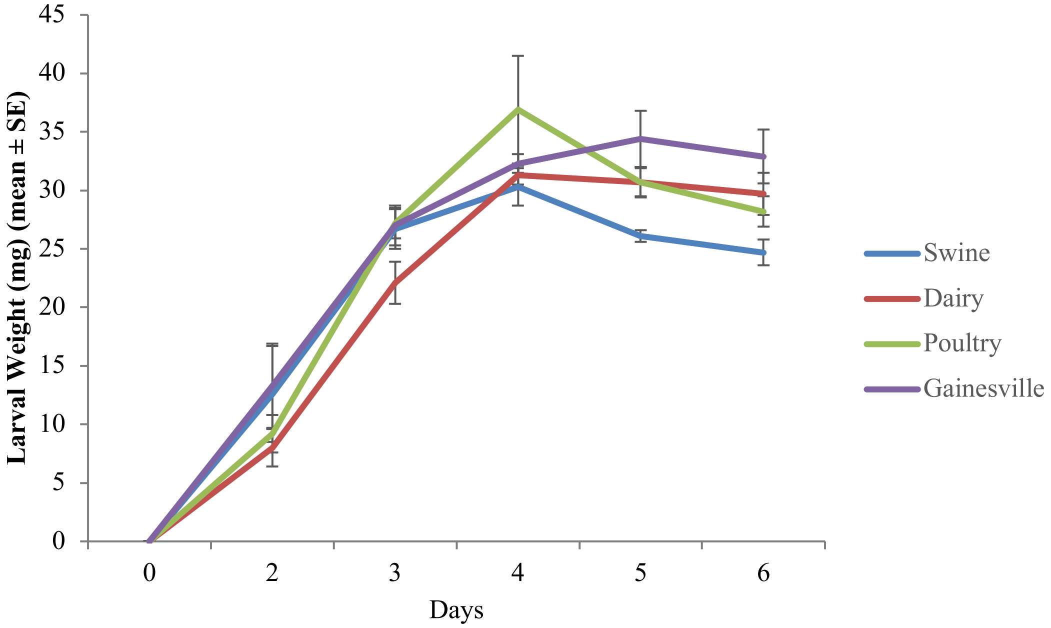
Larval development, development time to first pupariation, percent survivorship to the pupal stage (percent pupariation), and pupal weight were analyzed across treatments. An ANOVA was performed for each parameter above using JMP PRO 14 (SAS Institute Inc., Cary, NC, USA). Normality was checked using Shapiro Wilk Test and equal variances were checked using Bartlett’s test. Tukey’s HSD (honest significant difference) was used for mean separation ($p \leq 0.05$).

Results

Larval Development

Larval development differed significantly ($F_{3,59} = 3.2597$; $p = 0.0277$) across larval diets. A significant ($F_{1,59} = 8.8734$; $p = 0.0042$) trial effect was determined, but no treatment by trial interaction ($F_{3,59} = 0.8565$; $p = 0.4688$). In general, individuals in trial one weighed 11% more than those in trial two. Additionally, peak weights were found 4 d after the initiation of the experiment for all treatments with the highest weight recorded for those fed poultry manure, which was 12–18% more than those fed the other diets (Figure 5.1).

Figure 5.1 Mean house fly larval weight (mg) (mean ± SE[1]n = 6) of house flies fed 1 kg of standard diet (Hogsette 1992) or swine, dairy, or poultry at 26 °C, 60% RH, and 16L:8D**.**

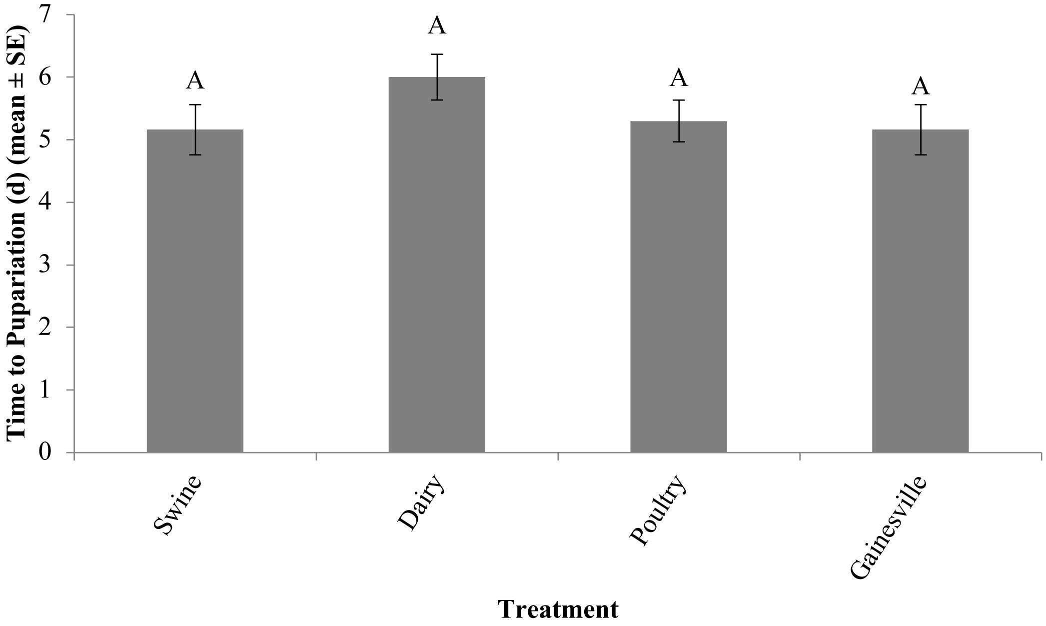


[1] n = number of replicates per treatment.

Development Time to First Pupariation

Development time to first pupariation did not differ significantly ($F_{3,16} = 1.8889$; $p = 0.1722$) across larval diets. No trial effect ($F_{1,16} = 16.3333$; $p = 0.0009$) or treatment by trial interaction ($F_{3,16} = 0.5556$; $p = 0.6519$) was determined. The shortest development time (~5 d) was found for those fed Gainesville diet, swine, and poultry manure. Those fed dairy manure took an additional day to reach pupariation (Figure 5.2).

Figure 5.2 House fly larval development time to first pupariation (mean ± SE, $^{1}n = 6$) fed 1 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 26˚C, 60% RH, and 16L:8D.

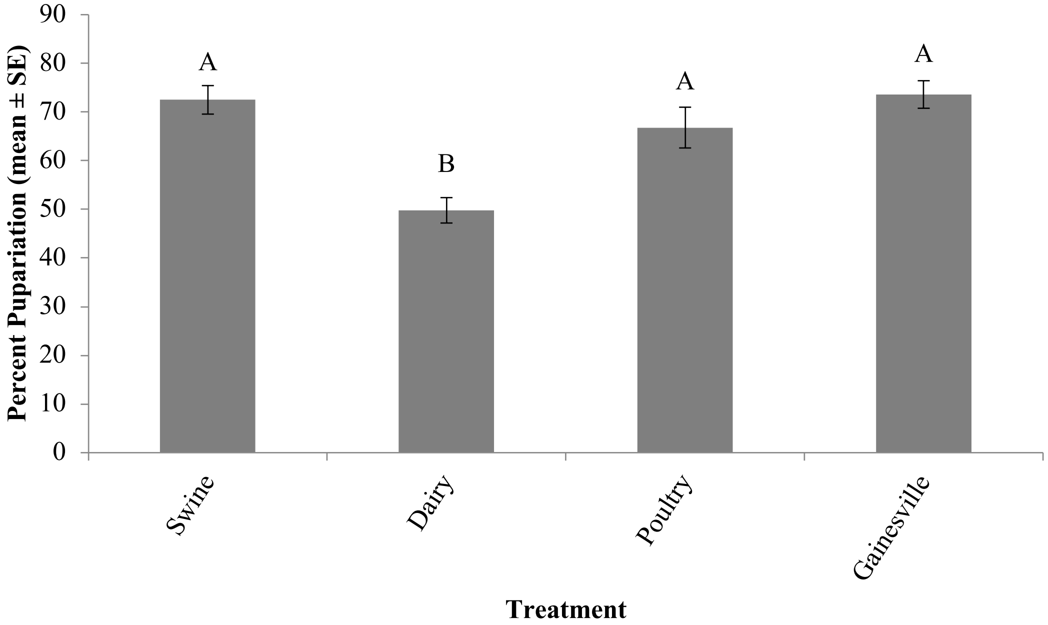


^{1}n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Percent Survivorship to the Pupal Stage

Percent survivorship to the pupal stage was significantly different ($F_{3,16} = 9.9903$; $p = 0.0006$) across larval diets. No trial effect ($p = 0.9295$) or treatment by trial interaction ($p = 0.8033$) was determined. The highest percent pupariation across all diets was found for those fed Gainesville diet (74%). In regard to larvae fed manure, the highest percent pupariation was found for those fed swine manure (73%), followed by poultry (67%) and dairy (50%) manures (Figure 5.3).

Figure 5.3 House fly percent pupariation (mean ± SE, [1]n = 6) when fed 1 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 26˚C, 60% RH, and 16L:8D.

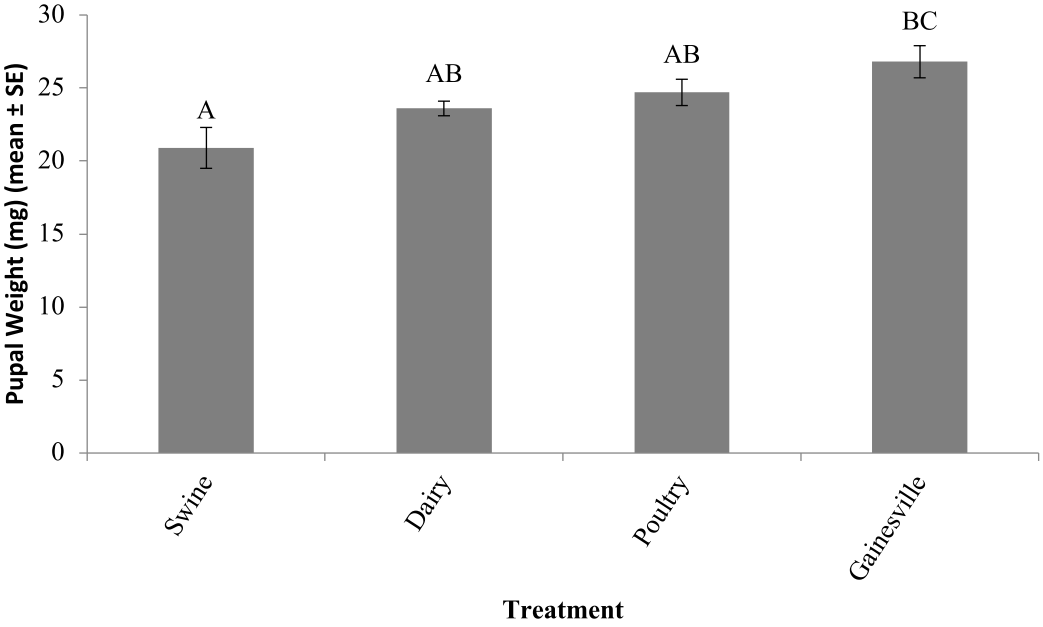


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Pupal Weight

Pupal weight differed significantly ($F_{3,16}$ = 6.0970; p = 0.0057) across larval diets. No trial effect (p = 0.7137) or treatment by trial interaction (p = 0.1835) was determined. Larvae fed Gainesville diet weighed approximately 27 mg and those fed swine, dairy or poultry manures weighed 21, 24, and 25 mg, respectfully (Figure 5.4).

Figure 5.4 House fly pupal weight (mean ± SE, [1]n = 6) when fed 1 kg of Gainesville diet (control) (Hogsette 1992) or swine, dairy, or poultry manure at 26˚C, 60% RH, and 16L:8D.



[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Discussion

Resource type provided to HF immatures impacted select-life history traits. House fly larvae fed the control diet had the highest survivorship (74%) and heaviest pupal weight (27 mg), but this was expected as this diet was specifically formulated for HF development (Hogsette 1992). In regard to those fed manure, more larvae reached pupariation (73%) when fed swine manure but weighed less as larvae (30 mg; peak larval weight) (Figure 5.1) and pupae (21 mg) compared to those fed poultry manure (67% survivorship, 37 mg/larvae, and 25 mg/pupae) (Figure 5.4). House flies reached pupariation in 5 –6 d regardless larval diet (Figure 5.2), but high mortality (50%) was found for those fed dairy manure (Figure 5.3).

Variation in life-history traits across manure types may be attributed to inherent differences. Results from Fatchurochim et al. (1989) show that the upper moisture threshold for HF reared on poultry manure lies somewhere between 70% and 80%. Moisture content of dairy manure was outside this range (i.e., 85%) and could explain the lower survivorship (50%) recorded. Moisture content of swine manure and the control diet (72% vs 70%) respectively were within the acceptable range, which could explain why the highest percent survivorship of HF fed these diets (73% in swine manure and 74% in control); however, those fed swine manure were smaller (21 mg) than those provided the control diet (27 mg).

No significant difference ($p < 0.05$) was found in development time to first pupariation across larval diets. Though those fed dairy manure took an and additional 1–2 d longer to reach pupariation. This is likely due to the fact that dairy manure has the lowest nutrient value in conjunction with high fiber content compared to the other manure types (Chen et al. 2003). Although not tested in the current study, HF possibly alter their consumption rate and metabolic synthesis of nutrients in response to lower quality diets. This physiological response has been shown in other insects, such as fruit flies, *Drosophila melanogaster* Meigen, (Diptera: Drosophilidae) (Fanson et al. 2012), monarch butterflies, *Danaus plexippus* (L.), (Lepidoptera: Nymphalidae) (Lavoie and Oberhauser 2004), and grasshoppers, *Melanoplus differentialis* Thomas (Orthoptera: Acrididae) (Yang and Joern 1994). However, it should be noted low survivorship (50%) in dairy manure reduced intraspecific competition allowing for greater resource consumption resulting in higher survivorship.

Results from the current study were quite different from those produced at a smaller scale (chapter 3; Miranda et al. accepted May 2019). Results from the previous study demonstrated significant differences ($p < 0.05$) in development time, survivorship, and weight of 100 1-d-old HF fed swine, dairy, and poultry manure at two different feed rates (9 g and 18 g/d^{-2}) were determined. Miranda et al. (accepted May 2019) determined HF larvae fed swine, dairy, or poultry manure at the lower feed rate weighed 12, 10, and 14 mg, respectfully, and 58%, 46% and 63% reached pupariation, respectfully, in 5 –7 d. While development time was similar between the aforementioned and current study, among those fed manure, pupal weight (10–14 mg vs. 21–25 mg in the current study) and survivorship (46% –58 % vs. 50% –73% in the current study) differed. When comparing the results of the current study to results reported by Hussein et al. (2017), which was a small-scale study (25–400 larvae fed 25 g dairy manure), similar findings of lower larval weight (~10 mg) and survivorship (~30%) were found. Collectively, this shows that different results in biomass (larval weight and survivorship) may be expected on a larger scale.

Composition of the manure and differences in methods employed (inoculating larvae in manure in the current study vs. eggs used by Hussein et al. (2017)) may explain differences between the current study and Hussein et al. (2017). Additionally, number of larvae (thousands of larvae vs hundreds used by Hussein et al. (2017)) may also be a factor. As previously discussed in other chapters, larvae feed in aggregates, and it is possible that larger aggregates are more efficient in conditioning the manure (Bryant and Sokal 1967, Zhao et al. 2017), which enhances HF performance. As such, future small-scale studies that intend to relay production estimates should include a large-scale component when possible. Additionally, HF development and survivorship on various manure types should be investigated on a larger scale than the current study in order to determine differences on a larger scale.

The current study showed variation in larval and pupal development, pupal survivorship and weight. Specifically, those fed dairy manure took 1 –2 d longer to develop with lower survivorship (50%). Lower survivorship was expected as dairy manure is typically considered the lowest quality manure among those tested in the current study; however, similar development time to first pupariation compared to other diets was not based on previous published work. It is possible that similarities in microbial communities across diets, modification of HF feeding behavior, or high mortality counteracted the negative effects of diet quality on HF performance. It is also possible that HF larvae were more efficient at conditioning the resource at higher densities, which allowed for faster development on a lower quality resource. Future studies should explore the impact of these factors as they may provide insight on how to optimize HF performance for waste management on a larger scale.

References

Bass, C., I. Denholm, M. S. Williamson, and R. Nauen. 2015. The global status of insect resistance to neonicotinoid insecticides. Pesticide Biochemistry and Physiology 121: 78-87.

Bryant, E. H., and R. R. Sokal. 1967. The fate of immature house fly populations at low and high densities. Population Ecology 9: 19-44.

Busvine, J. 1951. Mechanism of resistance to insecticide in houseflies. Nature: 193-195.

Calvert, C. C., R. D. Martin, and N. O. Morgan. 1969. House fly pupae as food for poultry. Journal of Economic Entomology 62: 938-939.

Chan, D. C., D. H. Fong, J. Y. Leung, N. Patil, and G. K. Leung. 2007. Maggot debridement therapy in chronic wound care. Hong Kong Medical Journal.

Chen, S., W. Liao, C. Liu, D. C. Elliott, M. D. Brown, and A. E. Solana. 2003. Value-added chemicals from animal manure, Pacific Northwest National Laboratory.

Cheng, J. Y., S. L. Chiu, and I. M. Lo. 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste Management 67: 315-323.

Cherry, R. H. 1987. History of sericulture. Bulletin of the Entomological Society of America 33: 83-85.

Čičková , H., B. Pastor, M. Kozanek, A. Martinez-Sanchez, S. Rojo, and P. Takac. 2012. Biodegradation of pig manure by the house fly, *Musca domestica*: A viable ecological strategy for pig manure management. PloS ONE 7.

Cox, M. M., and D. Karl Anderson. 2007. Production of a novel influenza vaccine using insect cells: protection against drifted strains. Influenza and Other Respiratory Viruses 1: 35-40.

Crittenden, A. N. 2011. The importance of honey consumption in human evolution. Food and Foodways 19: 257-273.

DeFoliart, G. R. 1989. The human use of insects as food and as animal feed. American Entomologist 35: 22-36.

Edmonds, L., N. Gollehon, R. L. Kellogg, B. Kintzer, L. Knight, C. Lander, J. Lemunyon, D. Meyer, D. C. Moffitt, and J. Schaeffer. 2003. Costs associated with development and implementation of comprehensive nutrient management plans. USDA Natural Resources Conservation Service.

Fanson, B. G., S. Yap, and P. W. Taylor. 2012. Geometry of compensatory feeding and water consumption in *Drosophila melanogaster*. Journal of Experimental Biology 215: 766-773.

Fatchurochim, S., C. Geden, and R. Axtell. 1989. Filth fly (Diptera) oviposition and larval development in poultry manure of various moisture levels. Journal of Entomological Science 24: 224-231.

Franson, M. A. H. 1989. Total solids dried at 103-105C: Standard methods for the examination of water and wastewater., American Public Health Association, Washington, DC.

Geden, C. J. 2012. Status of biopesticides for control of house flies. Journal of Biopesticides 5: 1.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Hussein, M., V. V. Pillai, J. M. Goddard, H. G. Park, K. S. Kothapalli, D. A. Ross, Q. M. Ketterings, J. T. Brenna, M. B. Milstein, and H. Marquis. 2017. Sustainable production of housefly (*Musca domestica*) larvae as a protein-rich feed ingredient by utilizing cattle manure. PloS ONE 12: e0171708.

Jeon, H., S. Park, J. Choi, G. Jeong, S.-B. Lee, Y. Choi, and S.-J. Lee. 2011. The intestinal bacterial community in the food waste-reducing larvae of *Hermetia illucens*. Current Microbiology 62: 1390-1399.

Jun, H., and H. Xiang. 2011. Development of circular economy is a fundamental way to achieve agriculture sustainable development in China. Energy Procedia 5: 1530-1534.

Kaufman, P. E., A. C. Gerry, D. A. Rutz, and J. G. Scott. 2006. Monitoring susceptibility of house flies (*Musca domestica* L.) in the United States to imidacloprid. Journal of Agriculture and Urban Entomology 23: 195-200.

Kellert, S. R. 1993. Values and perceptions of invertebrates. Conservation Biology 7: 845-855.

Khan, H. A. A., S. A. Shad, and W. Akram. 2012. Effect of livestock manures on the fitness of house fly, *Musca domestica* L. (Diptera: Muscidae). Parasitology Research 111: 1165-1171.

Kim, S. Y., H. G. Kim, K. Y. Lee, H. J. Yoon, and N. J. Kim. 2016. Effects of brewers spent grain (BSG) on larval growth of mealworms, *Tenebrio molitor* (Coleoptera: Tenebrionidae). International Journal of Industrial Entomology 32: 41-48.

Kováčik, P., M. Kozánek, P. Takáč, M. Galliková, and L. Varga. 2014. The effect of pig manure fermented by larvae of house flies on the yield parameters of sunflowers (*Helianthus annus* L.). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 58: 147-154.

Larrain, P. S., and C. F. Salas. 2008. House fly (*Musca domestica* L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Lavoie, B., and K. S. Oberhauser. 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environmental Entomology 33: 1062-1069.

Lee, K. S., D. S. Lee, S. W. Min, S. C. Kim, I.-H. Seo, and D. Y. Chung. 2018. Spatial changes and land use of arable land in China. Korean Journal of Soil Science and Fertilizer 51: 327-338.

Meneguz, M., A. Schiavone, F. Gai, A. Dama, C. Lussiana, M. Renna, and L. Gasco. 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. Journal of the Science of Food and Agriculture 98: 5776-5784.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019. Life-History Traits of Housefly, *Musca domestica* L. (Diptera: Muscidae), Reared on Three Manure Types. Journal of Insects as Food and Feed.

Newton, L., C. Sheppard, D. Watson, G. Burtle, and R. Dove. 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC 17.

Nordentoft, S., C. Fischer, L. Bjerrum, L. Heckmann, and B. Hald. 2017. Reduction of *Escherichia coli*, *Salmonella* Enteritidis and *Campylobacter jejuni* in poultry manure by rearing of *Musca domestica* fly larvae. Journal of Insects as Food and Feed 3: 145-153.

Orr, D., and L. Sriyanka. 2014. Biological control of insect pests in crops. Integrated Pest Management: 531-548.

Roffeis, M., B. Muys, J. Almeida, E. Mathijs, W. Achten, B. Pastor, Y. Velásquez, A. I. Martinez-Sanchez, and S. Rojo. 2015. Pig manure treatment with housefly (*Musca domestica*) rearing–an environmental life cycle assessment. Journal of Insects as Food and Feed 1: 195-214.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value-added manure management-system using the black soldier fly. Bioresource Technology 50: 275-279.

St-Hilaire, S., K. Cranfill, M. A. McGuire, E. E. Mosley, J. K. Tomberlin, L. Newton, W. Sealey, C. Sheppard, and S. Irving. 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38: 309-313.

USDA. 2018. Livestock and Poultry:World trade markets. Foreign Agrriculture Service, Office of Global Analysis.

Wang, H., Z. Zhang, G. F. Czapar, M. K. Winkler, and J. Zheng. 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. Waste Management & Research 31: 223-231.

Wang, W., W. Zhang, X. Wang, C. Lei, R. Tang, F. Zhang, Q. Yang, and F. Zhu. 2017. Tracing heavy metals in 'swine manure-maggot-chicken' production chain. Scientific Reports 7: 8417.

Wright, R. J. 1998. Agricultural uses of municipal, animal, and industrial byproducts.

Yang, S., Q. Li, Y. Gao, L. Zheng, and Z. Liu. 2014. Biodiesel production from swine manure via housefly larvae (*Musca domestica* L.). Renewable Energy 66: 222-227.

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

Zhang, C.-X., X.-D. Tamg, and J.-A. Cheng. 2008. The utilization and industrialization of insect resources in China. Entomological Research 38: S38-S47.

Zhao, Y., W. Wang, F. Zhu, X. Wang, X. Wang, and C. Lei. 2017. The gut microbiota in larvae of the housefly *Musca domestica* and their horizontal transfer through feeding. AMB Express 7: 147.

Zi-zhe, C., Y. De-po, W. Sheng-qing, W. Yong, M. J. Reaney, Z. Zhi-min, Z. Long-ping, S. Guo, N. Yi, and Z. Dong. 2017. Conversion of poultry manure to biodiesel, a practical method of producing fatty acid methyl esters via housefly (*Musca domestica* L.) larval lipid. Fuel 210: 463-471.

6. CHEMICAL COMPOSITION OF MANURE DEGRADED BY BLACK SOLDIER FLY, *Hermetia illucens* (L.) (DIPTERA: STRATIOMYIDAE) AND HOUSE FLY, *Musca domestica* L. (DIPTERA: MUSCIDAE) LARVAE AT TWO DIFFERENT SCALES

Overview

Large volumes of manure are produced by confined animal facilities around the globe. Insects offer a means to sustainably reduced dry matter and associated nutrients and heavy metals (HM). In the current study, the percent change in the chemical composition (nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), sulfur (S), boron (B), and neutral detergent fiber (NDF)) of swine, dairy, and poultry manure degraded by black soldier fly (BSF) and house fly (HF) larvae reared at two different scales (small: 100 larvae fed incrementally; large: thousands of larvae fed a single time) was examined. Results indicate that N is a key nutrient impacted by larval feeding of either species. Furthermore, greater reductions of nutrients (up to 80%) were found for dairy manure in small-scale BSF and HF studies but varied in the large-scale studies. Scale impacted nutrient and HM shifts in manure. In some instances, nutrient reduction was enhanced (small-scale studies), while for others (large scale-studies) it was not. Nitrogen was reduced regardless of scale. Data from this study demonstrate manure type and scale impact the ability of BSF and HF larvae to reduce nutrients and HM in manure.

Introduction

Animal production is a massive industry globally. In the United States alone, it is a multi-billion-dollar industry (USDA 2017). The US produces over 300 million tons of manure (dry weight) annually (USDA 2006) and approximately 65% of the manure produced comes from concentrated animal feeding operations (CAFOs) (Gurian-Sherman 2008). Operations that produce animals under high densities are geographically clustered, and due to the high output of manure in these regions and limited availability of land, the manure must be transported long distances in some cases, which can increase the cost of production (Edmonds et al. 2003).

Application of raw manure to land poses a threat to the environment due to nutrient surpluses and dissemination of pathogens (Venglovsky et al. 2009). One solution to mitigate transportation costs and minimize the risk of overapplication of nutrients and spread of pathogens is composting, which provides a means to reduce unfavorable attributes of manure, such as volume, nutrients, weed seeds, odors, and pathogens (Raabe 1981, Auvermann 2011). However, composting can take weeks to months to transform the manure a stable product (Lim et al. 2016), and factors such as pH (initial pH should be 6-7), moisture (50%), temperature (varies as the composting process progresses), and carbon to nitrogen ratio (30:1) must be maintained for successful and efficient composting (Raabe 1981, Cooperband 2000, Sweeten 2008). The material also needs to be turned so that the pile does not overheat (Raabe 1981, Sweeten 2008) and is provided with proper aeration for moisture control (Sweeten 2008). Compost piles can also spontaneously combust, which is why these factors must be carefully maintained (Cooperband 2000). Similarly, applying composted manure to soils may contaminate soil, as composted manure may serve as a reservoir for heavy metals (HM) such as Zn, Cu, and Mn (Hsu and Lo 1999).

Using insects to recycle manure is a sustainable solution; degradation of manure via insects is comparable to compost in that volume (Lim et al. 2016), nutrients (Dominguez et al. 1997), pathogens (Dominguez et al. 1997, Wang et al. 2013), and odors (Wang et al. 2013, Beskin et al. 2018) are reduced. However, insects can accelerate the process by altering the physical (moisture, temperature, and pH) and microbial enzyme activities (Dominguez et al. 1997, Zhu et al. 2012). Both the black soldier fly (BSF), *Hermetia illucens* (L.) and house fly, *Musca domestica* L. (Diptera: Muscidae) are widely distributed in tropical and subtropical regions throughout the world and are capable of digesting manure (Čičková et al. 2015, van Huis 2019). Black soldier fly larvae can reduce manure nutrients by over 50% (Myers et al. 2008) and DM by over 40 % (Zhou et al. 2013) as well as reduce HM in manure (Diener et al. 2015, Cai et al. 2018). Likewise, HF can reduce nutrients by 25-76% (Wang et al. 2013, Hussein et al. 2017), DM by up to 24% (Roffeis et al. 2015), as well as HM (Borowska et al. 2004, Wang et al. 2017). Though, the extent of the reduction may be depended on manure type and scale.

The purpose of this study was to evaluate the change in the chemical composition of dairy, swine, and poultry manure digested by BSF and HF, at two different scales. Initial and final manure samples were collected from previous experiments for BSF (Miranda et al. accepted May 2019a) and HF (Miranda et al. accepted May 2019b) based on a small- scale (100 larvae; incremental feeding) and large-scale (10,000 larvae; one single feeding). Scale was determined to be an important factor regulating select life-history traits of BSF an HF and has implications for industrialization of these two insects; results generated at a small-scale (i.e., benchtop) do not necessarily translate to large-scale (i.e., industrialization). Knowing such information is critical for efficient and optimal production of these insects as a means to recycle wastes and for use as livestock, poultry, and aquaculture feed. To explore the impact of scale and species on nutrient shifts in animal waste recycling, the following were quantified in pre- and post-digested manure: nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), sulfur (S), boron (B), and neutral detergent fiber (NDF).

Materials and Methods

Acquisition of BSF and HF

Methods for BSF and HF acquisition are based on those reported by Miranda et al. (accepted May 2019a) and Miranda et al. (accepted May 2019b). respectively. Both colonies are maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility in College Station, TX, USA.

Acquisition of Manure

Manure less than 12 h old was collected based on methods previously described by Miranda et al. (accepted May 2019a). Briefly, swine, dairy, and poultry manures were collected from a small local farm (Anderson, TX, USA), a commercial dairy (Stephenville, TX, USA), and a poultry research center (Texas A&M University Poultry Science Research, Teaching and Extension Center; College Station, TX, USA). Collected manure was homogenized (vigorously stirred by hand for 5 minutes) and stored at -20ºC until used. Manure was allowed to thaw at room temperature for 24 h prior to use.

Experiment Design

Initial and final manure samples (100 g) were collected for total N, P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn, S, B, and NDF from small- and- large scale studies previously published (references). The methods for these experiments are briefly described below.

Small-scale experiments

The methods for the small-scale experiments involved feeding 100 4-d old BSF and 100 1-d-old HF, dairy, swine or poultry manure at two feed rates. These studies were conducted independently and are described in more detail by Miranda et al. (accepted May 2019a) and Miranda et al. (accepted May 2019b). Briefly, BSF were fed 18 g or 27 g and HF were fed 9 or 18 g of manure every other day. Processed manure was collected when all apparent surviving insects reached the prepupal (BSF) or pupal (HF) stage and was stored at -20ºC until analysis.

Large-scale experiments

The methods for the large-scale experiments involved feeding 10,000 4-d old BSF 7 kg of dairy, swine or poultry manure. For the HF study, 4000 1-d-old larvae were fed 1 kg of dairy, swine or poultry manure. Similar to the small-scale studies, these experiments were conducted independently and are described in more detail in chapter 4 (BSF study) and chapter 5 (HF study) of this dissertation. Processed manure was collected when the first prepupae (BSF) or pupae (HF) was stored at -20ºC until analysis.

Chemical analysis. Initial and final manure samples were sent to Texas A&M AgriLife Extension Soil, Water and Forage Testing Laboratory in College station, TX, USA for nutrient (N, P, K, Ca, Mg, Na, S, and B), heavy metal (Zn, Fe, Cu, and Mn), and neutral detergent fiber analysis. Manure samples were oven dried at 65°C for 16 hours or until dry (Despatch LLB-15; Despatch Thermal Processing Technology, Minneapolis MN, USA), pulverized (Bico Ring and Puck Mill; Bico Braun International, Burbank CA, USA) and then filtered to remove particles > 2mm before analysis. Total nitrogen was determined via combustion (Elementar Rapid N; Elementar Americas Inc, Ronkonkoma, NY, USA) (Nelson and Sommers 1973) and minerals were determined by inductively coupled plasma spectrometry of a nitric acid digest (Havlin and Soltanpour 1980). Fiber was determined gravimetrically by liquid digestion (Komarek and Sirois 1993).

Percent change in chemical composition

The percent change in chemical composition was calculated by subtracting the final (F) composition from the initial (I) composition and then dividing by the initial composition and multiplying by 100.

$$\text{Percent change} = ((I - F)/ I) * 100$$

Statistical Analysis

A canonical discriminant analysis (CDA) was used to separate manure type (swine, dairy, or poultry) as the class level for the BSF small-scale study, BSF large-scale study, HF small-scale study, and HF large-scale study. All 13 parameters (N, P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn, S, B, and NDF) were included variables and the analyses were performed using JMP (version 14.0; SAS Institute, Inc., Cary, NC, USA). Canonical plots can be found under the Appendix A section of this document.

Results

Small-Scale BSF Experiment

The first two canonical variates described 99% of the variance among manure types (Table 6.1). Based on the values of canonical variate 1 ($r_{c1} = 0.9999$; $p = 0.0008$) and canonical variate 2 ($r_{c2} = 0.9988$; $p = 0.00133$), separation of manure type is most strongly influenced by changes in N, P, K, Mg, Na, Zn, Fe, Mn, and S (Figure 6.1a). In general, the greatest reductions occurred in dairy treatments (> 70%), and the lowest was in swine manure (> 50%) (Figure 6.2a).

Large-Scale BSF Experiment

The first two canonical variates described 100% of the variance among manure types (Table 6.1). Based on the values of canonical variate 1 ($r_{c1} = 0.9957$; $p < 0.0001$) and canonical variate 2 ($r_{c2} = 0.0.9865$; $p < 0.0001$), separation of manure type is most strongly influenced by changes in N, P, K, Ca, Zn, Fe, Cu, Mn and NDF (Figure 6.1b). In general, the greatest reductions occurred in swine treatments (27% –57%), whereas the lowest reductions occurred in poultry (2% –56%) (Figure 6.2b).

Table 6.1 Canonical loading coefficients of the first two canonical variates associated with the 13 parameters measured after black soldier fly and house fly larvae were fed dairy, poultry, or swine manure**.**

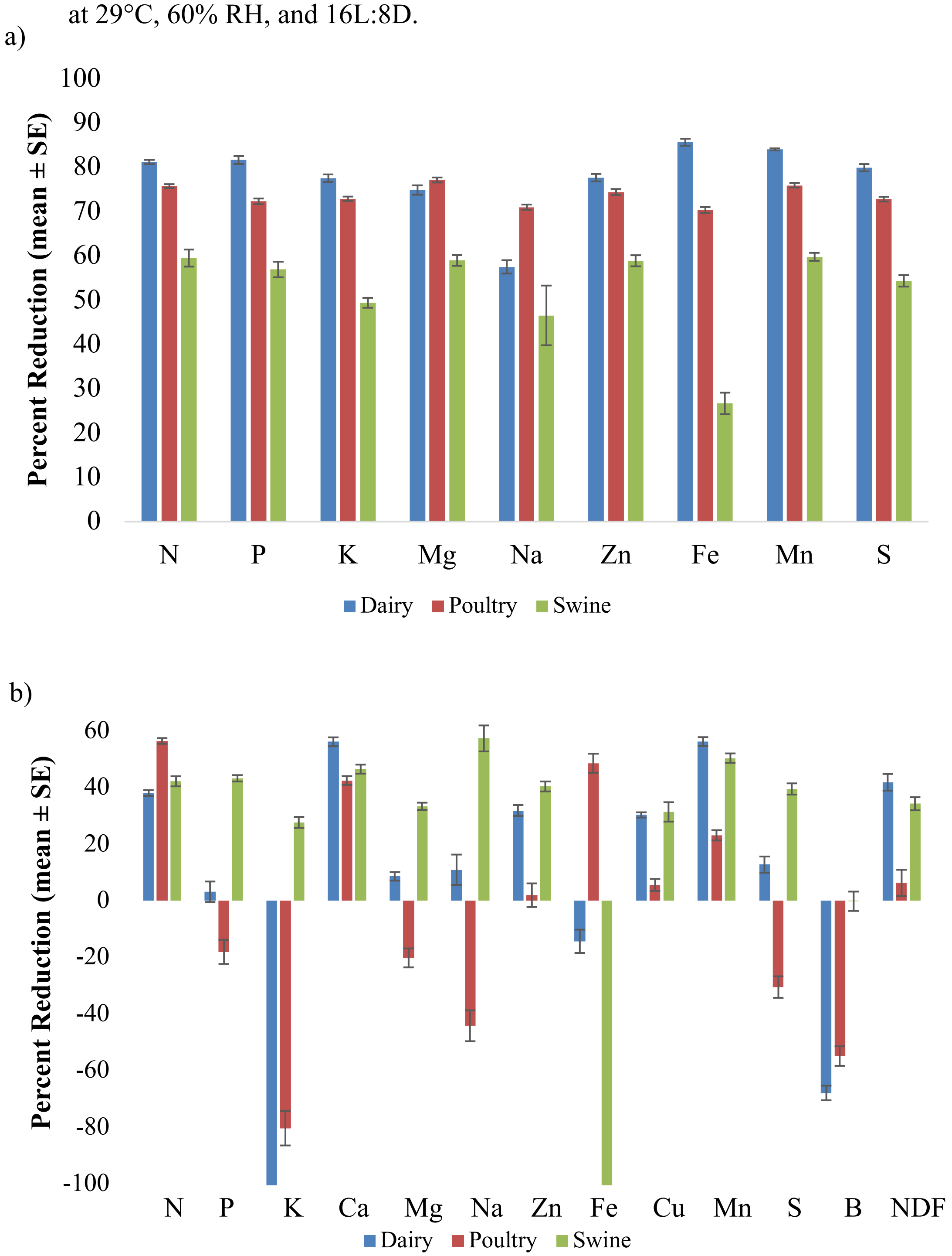
	BSF Small-scale[1]		BSF Large-scale[2]		HF Small-scale[3]		HF Large-scale[4]	
Parameter	Canonical Variate 1	Canonical Variate 2	Canonical Variate 1	Canonical Variate 2	Canonical Variate 1	Canonical Variate 2	Canonical Variate 1	Canonical Variate 2
N	**0.8868***	0.4057	**0.9655**	-0.1087	**-0.7271**	0.6024	**-0.5388248**	**-0.7497917**
P	**0.916**	0.3101	-0.4537	**0.8683**	-0.4594	**0.767**	0.08036926	0.13895256
K	**0.8315**	0.5016	0.1129	**0.9676**	-0.1358	**0.8792**	-0.3155464	**0.6284481**
Ca	0.6851	0.6665	**-0.8497**	-0.368	0.2158	0.3847	-0.454844	-0.4210375
Mg	0.6313	**0.7128**	-0.6539	0.7435	-0.2975	0.6808	0.20299412	0.219338
Na	0.0685	**0.9147**	-0.653	0.7331	0.1758	0.4264	0.10765583	0.1637101
Zn	**0.8139**	0.5062	**-0.8223**	0.5137	-0.3857	**0.766**	-0.0093937	-0.3650349
Fe	**0.8678**	0.3335	0.4835	**-0.8547**	**0.0732**	**0.9271**	-0.1144455	**-0.4793588**
Cu	0.7737	0.5197	**-0.8897**	0.3462	-0.4424	0.6098	0.15159015	**-0.5634808**
Mn	**0.913**	0.3585	**-0.9731**	0.1591	-0.4682	**0.7468**	0.18574903	-0.0256279
S	**0.89**	0.3898	-0.7236	0.6744	-0.2901	**0.7491**	**0.41214076**	-0.1023658
B	0.6652	0.6357	**0.0782**	0.9834	-0.0863	-0.1335	-0.3508499	-0.1264627
NDF	0.4045	-0.214	**-0.941**	0.0956	0.365	0.448	0.13757281	**-0.447116**
Variance Explained (%)	99.93	0.07	66.04	33.96	77.07	22.93	76.28	23.72
Canonical Correlation	0.9999	0.9989	0.9986	0.9973	0.9931	0.9774	0.0996	0.9865
p-value	0.0008	0.0133	< 0.0001	< 0.0001	0.0056	0.0362	< 0.0001	< 0.0001

Parameters with the largest influence are bolded

[1]Small-scale study followed methods described by Miranda et al. (accepted May 2019a) and [3]Miranda et al. (accepted May 2019b).

[2]Large-scale study followed methods described in chapter 4 and chapter 5

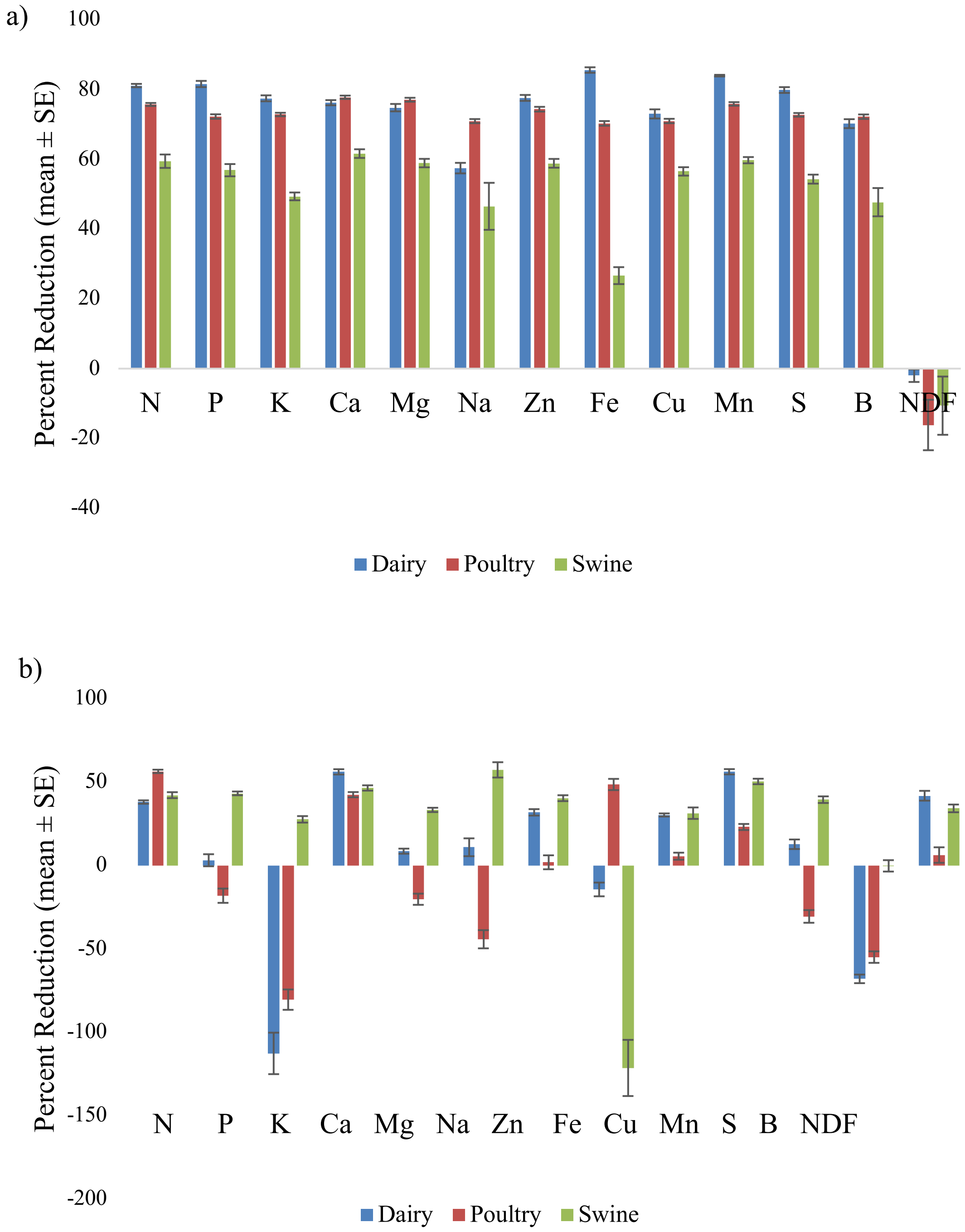
Figure 6.1 Percent reduction (mean ± SE, 1n = 6) of nutrients, heavy metals, and fiber in dairy, poultry, and swine manure after black soldier fly larval digestion on a) small-scale ([1]n=12) (Miranda et al. accepted May 2019a) and b) large-scale (n=6) (chapter 4) at 29°C, 60% RH, and 16L:8D.



---

[1] n = number of replicates per treatment.

Figure 6.2 Percent change (mean ± SE) of all nutrients, heavy metals, and fiber in dairy, poultry, and swine manure after black soldier fly larval digestion on a) small-scale ([1]n=12) (Miranda et al. accepted May 2019a) and b) large-scale (n=6) (chapter 4) at 29 °C , 60% RH, and 16L:8D.



[1] n = number of replicates per treatment.

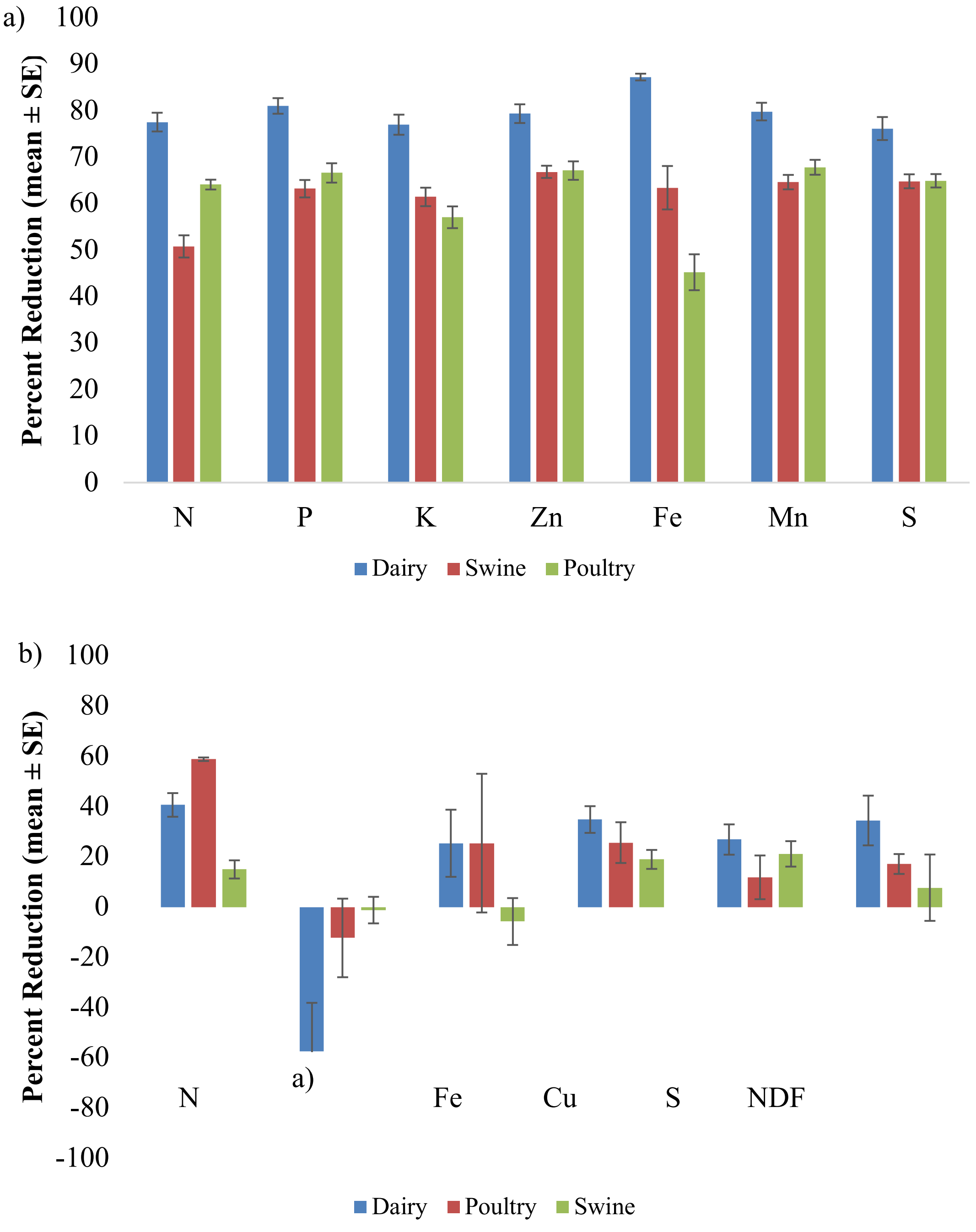
Small-Scale HF Experiment

The first two canonical variates (Table 1) described 100% of the variation among manure types (Table 6.1). Based on the values of canonical variate 1 ($r_{c1} = 0.9931$; $p = 0.0056$) and canonical variate 2 ($r_{c2} = 0.9774$; $p = 0.0362$), separation of manure type is most strongly influenced by changes in N, P, K, Zn, Fe, Mn, and S (Figure 6.3a). In general, the greatest reductions occurred in dairy treatments (70 –87%), whereas the lowest in swine manure (45 –68%) (Figure 6.4a)

Large-Scale HF Experiment

The first two canonical variates described 100% of the variance among manure types (Table 6.1). Based on the values of canonical variate 1 ($r_{c1} = 0.9957$; $p < 0.0001$) and canonical variate 2 ($r_{c2} = 0.0.9865$; $p < 0.0001$) separation of manure type is most strongly influenced by changes in N, K, Fe, Cu, S, and NDF (Figure 6.3b). In general, the highest reductions occurred in dairy manure (25% –34%) and the lowest in swine manure (up to 21%).

Figure 6.3 Percent reduction (mean ± SE) of nutrients, heavy metals, and fiber in dairy, poultry, and swine manure after house fly larval digestion on a) small-scale ([1]n=12) (Miranda et al. accepted May 2019b) at 33 °C, 60% RH, and 16L:8D and b) lar large-scale (n=6) (chapter 5) at 26 °C, 60% RH, and 16L:8D.

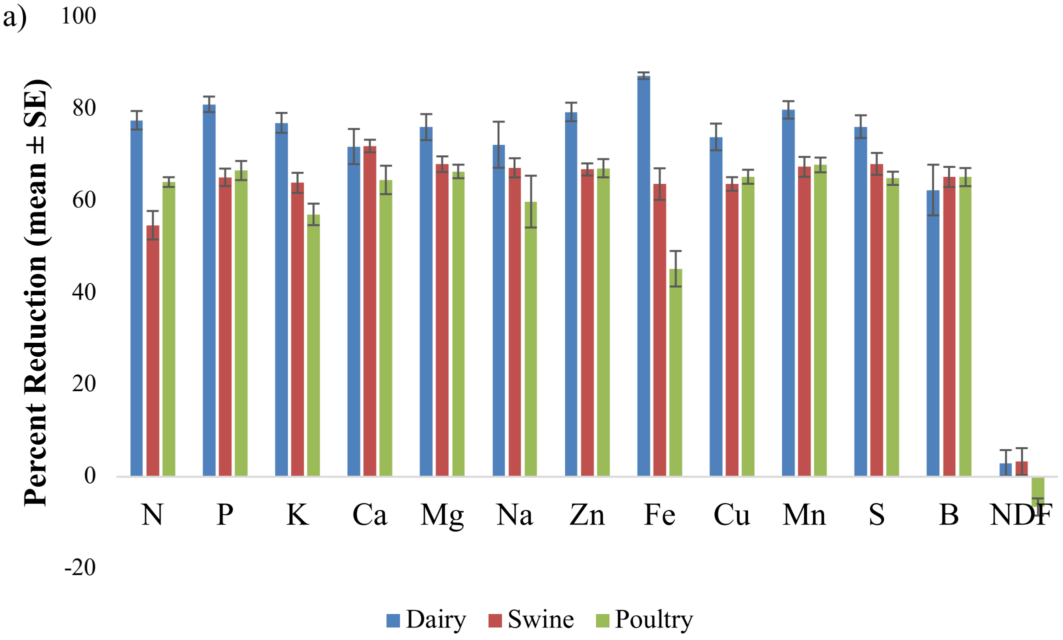


---

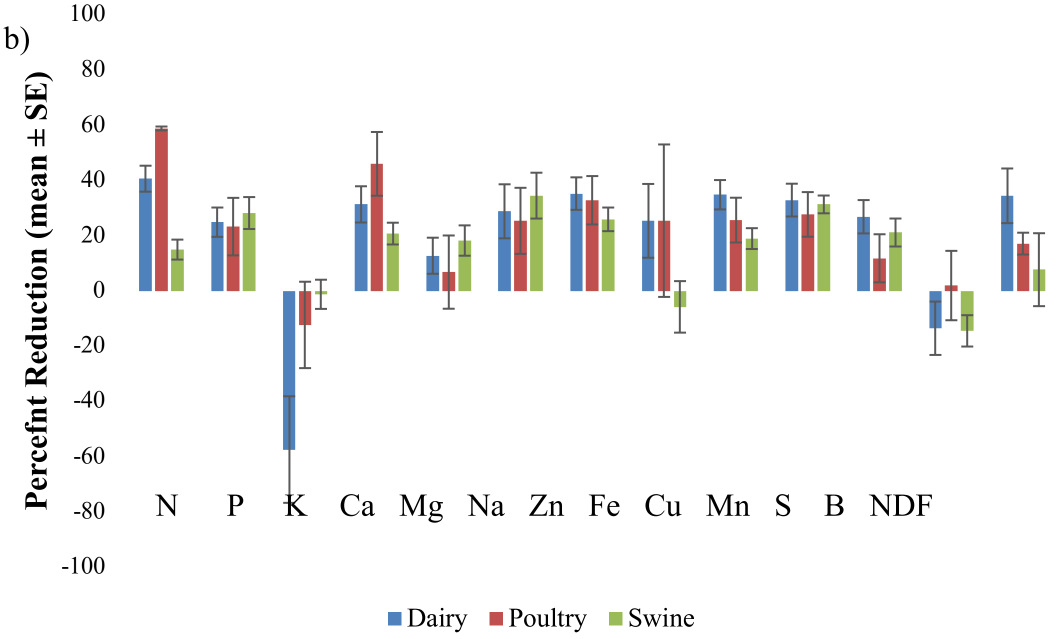
[1] n = number of replicates per treatment.

Figure 6.4 Percent change (mean ± SE) of all nutrients, heavy metals, and fiber in dairy, poultry, and swine manure after house fly larval digestion on a) small-scale ([1]n=12) (Miranda et al. accepted May 2019b) at 33 °C, 60% RH, and 16L:8D and b) large-scale (n=6) (chapter 5) at 26 °C, 60% RH, and 16L:8D.

a)



b)



[1]n = number of replicates per treatment.

Discussion

Nutrients

The impact of manure type and study scale on the ability of BSF and HF to reduce nutrients corresponded with impacts on insect development and production. Percent change in N, P, K, Mg, Na, and S influenced separation of manure types in the BSF small-scale study and N, P, K, Ca, and B were responsible for separation of manure types in the BSF large- scale study (Table 1; Figure 6.1). When comparing across scales for the BSF studies, changes in N, P, K were found to be responsible for separating manure types regardless of scale. Though, the extent of reduction differed across scales (Figures 6.1 and 6.2). For example, in the small-scale study, the highest N reduction was found in dairy manure (81%), followed by poultry manure (76%) and then swine (59%). Lower N reductions were found in the large-scale study, with the highest N reduction found in poultry manure (56%), followed by dairy (38%), and swine (42%) (Figure 6.1). A similar trend occurred for P and K in the small-scale study; however, in the large-scale, the highest reductions for P (43%) and K (27%) were found in swine manure, but this was still lower than P (56-82%) and K (49 –78%) reductions in the small-scale study. These nutrients are essential for insect growth (Ohmart et al. 1985, Athey and Connor 1989, Barnard et al. 1995, Ben-Yosef et al. 2014) and are components of essential amino acids. One explanation for lower nutrient reductions in swine manure compared to dairy and poultry in the small-scale study may be related to the lower prepupal weights found for those fed swine in previous small-scale studies (Oonincx et al. 2015, Miranda et al. accepted May 2019a), which may be governed by moisture content, as swine manure produced the smallest prepupae (72 –74%) compared to dairy (83 –85%) and poultry (76

–77%) manures. Though, it is important to note that other elements may have also influenced results, as elements function in concert, where uptake of one may be dependent on the presence of others (Busch and Phelan 1999, Wackett et al. 2004). Other nutrients that were responsible for separating manure types but varied across scale for the BSF studies were S, Ca, and B. These elements are important for various physiological functions in insects, such as membrane permeability and enzymatic activity (Dadd 1973). Differences in the significance of these nutrients across scale may be attributed to differences in microbial communities and metabolism (Inman 2011, Terrapon and Bernard 2014). Similar to the small-scale BSF study, N, P, K, and S separated manure types in the small-scale HF study (Figure 6.3a). However, the large-scale study differed; N, K, and S, but not P, influenced separation of manure types (Figure 6.3b). Also similar to BSF studies, higher N reduction was found in small-scale studies, with the highest reduction found in dairy (77%) and lowest in poultry (51%) manures (Figures 6.3 and 6.4). These results show variation in scales for reduction of nutrients across manure types.

Heavy Metals

The impact of manure type and study scale on the ability of BSF and HF to reduce HM corresponded with impacts on insect development and production. In both the small-scale and large-scale BSF studies, Zn, Fe, and Mn influenced separation of manure types (Table 1). Though, similar to nutrient reductions previously discussed, higher reductions were observed in the small-scale study. Black soldier flies bioaccumulate Zn (Diener et al. 2015, Cai et al. 2018); though there are conflicting opinions on the impact of Zn on BSF survivorship (Diener et al. 2015, Cai et al. 2018). In the HF studies, change in Zn, Fe, and Mn separated manure types in the small-scale studies, whereas changes in Fe and Cu separated manure types in the large-sale. Results for percent change in Zn and Fe from the current study (small-scale HF) differ from those reported by Hussein et al. (2017), which examined changes in Zn and Fe in dairy manure reported an 11% decrease in Zn and 4% increase in Fe. Our results show that these compounds are reduced by 79% and 87%, respectively. Though, this difference may be attributed to differences in methods employed, as Hussein et al. (2017) provided 25 g of manure to 100 larvae and we fed 100 larvae 18 or 27 every two days. Still, HF can bioaccumulate Zn and Cu in pupal casings (Borowska et al. 2004). Furthermore, it is known that these HM negatively impact survivorship and development of larvae via the innate immune system (Borowska and Pyza 2011); thus, for industrialization purposes, future research should examine changes in HM and the associated impacts on a larger scale than used in the current study.

Fiber

Parallel to nutrient and HM reduction, the ability of BSF and HF to reduce fiber is dependent on manure type and scale. Reduction in NDF was found to influence separation of manure types for the large-scale study, but not the small-scale. Specifically, BSF reduced 6-42% of NDF in the large –scale study, with the highest reduction found in dairy manure (42%) (Figure 6.1b), which is comparable to NDF reduction (46%) found by ur Rehman et al. (2017). Although NDF was not found to separate manure types in the small-scale BSF study (Table 6.1), the extent of BSF degradation of fiber in manure differed as NDF increased up to 16% in swine manure (Figure 6.2a). A similar pattern occurred for HF in that NDF was not reduced in the small-scale (up to 3%

increase) but was reduced 7 –37% in the large scale. It is likely that higher densities of larvae enhance fiber digestion via oral secretions as BSF possess digestive enzymes (Kim et al. 2011) and HF have gut bacteria (Zhao et al. 2017) that can break down cellulose.

Integration into Mass-Production

Manure varies in its chemical composition and shifts in scale correspond to various reductions in nutrients, HM, and fiber as previously discussed. As related, differences in BSF and HF ability to utilize, or reduce, manure components may impact life-history performance. Previous research has found nutritional variations in manure impact BSF (Zhou et al. 2013, Oonincx et al. 2015) and HF (Larrain and Salas 2008, Khan et al. 2012) performance. Though most of the data available was produced on a small-scale, which may not translate on a larger scale. For example, BSF from the small-scale study weighed up to 45% less, developed faster (by 5 –6 d), and had higher survivorship (up to 45% more) than those in the large-scale study (Miranda et al. accepted May 2019, chapter 4). Interestingly, when comparing changes in chemical composition across scale, higher reductions of nutrients and heavy metals occurred in small-scale studies. Thus, for the purpose of managing waste, small-scale practices (lower larval densities and incremental feeding) are suitable as the highest nutrient and HM reductions were found. Yet, a major focus of rearing larvae for the sole purpose of protein production (and disregarding utilizing insects for waste management purposes) is to maximize yield and efficiency (larval weight and development time), rather than maximizing manure reduction. Therefore, for mass-production purposes, large-scale practices may be more suitable for protein production. As such, the purpose of producing insects (waste management vs protein protein) should be considered as the outcome, in terms of nutrient reduction (waste management) or life-history performance (protein production), may differ. However, it is possible that the amount of manure provided as related to the larval density in the large-scale study is not optimized. Additionally, it is also possible that differences across scales may attributed to differences in methods employed. For example, manure was sampled later in the small-scale studies (after all larva reached the prepupal (BSF) or pupal stage (HF)) compared to the large-scale studies (after first sight of prepupation (BSF) or pupariation (HF)). Future research should explore optimizing mass-production of larvae for waste management and mass-production purposes to determine if time of sampling manure (at sight of first prepupae or pupae vs. sampling after majority reached the prepupal or pupal stage) impacts chemical composition.

Conclusion

This study reveals differential changes in nutrients, heavy metals, and fiber of swine, dairy, and poultry manure digested by BSF or HF, across two different scales. Such data may be explained by associated biological data (development time and weight), which highlights that manure type, scale, larval density, and feeding regimen can impact the ability of BSF and HF to reduce nutrients, heavy metals, and fiber in different manure types to various extents. Results also show that higher nutrient and HM reductions occurred in small-scale studies, but higher NDF reductions were found in the large-scale studies. Though it is possible that differences across scales may attributed to differences in methods employed. For example, manure was sampled later in the small-scale studies, as previously discussed, compared to the large-scale studies. Though, rearing BSF and HF at different scales may still elicit differences in chemical composition of digested manure and it is important to determine the purpose of production (waste management vs protein production). Future research should evaluate these parameters on a larger scale in order to optimize mass-production.

References

Athey, L. A., and E. F. Connor. 1989. The relationship between foliar nitrogen content and feeding by *Odontota dorsalis* Thun. on *Robinia pseudoacacia* L. Oecologia 79: 390-394.

Auvermann, B. W. 2011. Principles of feedyard manure quality. Texas A&M.

Barnard, D., R. Harms, and D. Sloan. 1995. Influence of nitrogen, phosphorus, and calcium in poultry manure on survival, growth, and reproduction in house fly (Diptera: Muscidae). Environmental Entomology 24: 1297-1301.

Ben-Yosef, M., Z. Pasternak, E. Jurkevitch, and B. Yuval. 2014. Symbiotic bacteria enable olive flies (*B actrocera oleae*) to exploit intractable sources of nitrogen. Journal of Evolutionary Biology 27: 2695-2705.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Borowska, J., and E. Pyza. 2011. Effects of heavy metals on insect immunocompetent cells. Journal of Insect Physiology 56: 760-770.

Borowska, J., B. Sulima, M. Niklinska, and E. Pyza. 2004. Heavy metal accumulation and its effects on development, survival and immuno-competent cells of the housefly *Musca domestica* from closed laboratory populations as model organism. Fresenius Environmental Bulletin 13: 1402-1409.

Busch, J. W., and P. L. Phelan. 1999. Mixture models of soybean growth and herbivore performance in response to nitrogen–sulphur–phosphorous nutrient interactions. Ecological Entomology 24: 132-145.

Cai, M., R. Hu, K. Zhang, S. Ma, L. Zheng, Z. Yu, and J. Zhang. 2018. Resistance of black soldier fly (Diptera: Stratiomyidae) larvae to combined heavy metals and potential application in municipal sewage sludge treatment. Environmental Science and Pollution Research 25: 1559-1567.

Čičková , H., G. L. Newton, R. C. Lacy, and M. Kozanek. 2015. The use of fly larvae for organic waste treatment. Waste Management 35: 68-80.

Cooperband, L. R. 2000. Composting: art and science of organic waste conversion to a valuable soil resource. Laboratory Medicine 31: 283-290.

Dadd, R. 1973. Insect nutrition: current developments and metabolic implications. Annual review of entomology 18: 381-420.

Diener, S., C. Zurbrügg, and K. Tockner. 2015. Bioaccumulation of heavy metals in the black soldier fly, Hermetia illucens and effects on its life cycle. Journal of Insects as Food and Feed 1: 261-270.

Dominguez, J., C. A. Edwards, and S. Subler. 1997. A comparison of vermicomposting and composting. Biocycle 38: 57-59.

Edmonds, L., R. Kellogg, B. Kintzer, L. Knight, C. Lander, J. Lemunyon, D. Meyer, D. Moffitt, and J. Schaefer. 2003. Costs associated with development and implementation of Comprehensive Nutrient Management Plans. Part I—Nutrient management, land treatment, manure and wastewater handling and storage, and recordkeeping. Natural Resources Conservation Service. US Department of Agriculture. .

Gurian-Sherman, D. 2008. CAFOs uncovered: The untold costs of confined animal feeding operations.

Havlin, J. L., and P. Soltanpour. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Communications in Soil Science and Plant Analysis 11: 969-980.

Hsu, J. H., and S. L. Lo. 1999. Recycling of separated pig manure: characterization of maturity and chemical fractionation of elements during composting. Water Science and Technology 40: 121-127.

Hussein, M., V. V. Pillai, J. M. Goddard, H. G. Park, K. S. Kothapalli, D. A. Ross, Q. M. Ketterings, J. T. Brenna, M. B. Milstein, and H. Marquis. 2017. Sustainable production of housefly (Musca domestica) larvae as a protein-rich feed ingredient by utilizing cattle manure. PloS ONE 12: e0171708.

Inman, M. 2011. How Bacteria Turn Fiber into Food. PLoS One 9.

Khan, H. A. A., S. A. Shad, and W. Akram. 2012. Effect of livestock manures on the fitness of house fly, Musca domestica L. (Diptera: Muscidae). Parasitology Research 111: 1165-1171.

Kim, W., S. Bae, K. Park, S. Lee, Y. Choi, S. Han, and Y. Koh. 2011. Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). Journal of Asia-Pacific Entomology 14: 11-14.

Komarek, A., and P. Sirois. 1993. An improved filtering technique for the analysis of neutral detergent fiber and acid detergent fiber utilizing the filter bag technique. Journal of Animal Science 71: 824-829.

Larrain, P. S., and C. F. Salas. 2008. House fly (Musca domestica L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Lim, S. L., L. H. Lee, and T. Y. Wu. 2016. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis Journal of Cleaner Production. 111: 262-278.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. accepted May 2019a. Life-History Traits of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), Reared on Three Manure Types. Animals 9: 281.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. accepted May 2019b. Life-History Traits of Housefly, *Musca domestica* L. (Diptera: Muscidae), Reared on Three Manure Types. Journal of Insects as Food and Feed.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Nelson, D., and L. Sommers. 1973. Determination of Total Nitrogen in Plant Material 1. Agronomy Journal 65: 109-112.

Ohmart, C., L. Stewart, and J. Thomas. 1985. Effects of food quality, particularly nitrogen concentrations, of *Eucalyptus blakelyi* foliage on the growth of *Paropsis atomaria* larvae (Coleoptera: Chrysomelidae). Oecologia 65: 543-549.

Oonincx, D., A. van Huis, and J. van Loon. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. Journal of Insects as Food and Feed 1: 131-139.

Raabe, R. D. 1981. The rapid composting method, Cooperative Extension, University of California.

Roffeis, M., B. Muys, J. Almeida, E. Mathijs, W. Achten, B. Pastor, Y. Velásquez, A. I. Martinez-Sanchez, and S. Rojo. 2015. Pig manure treatment with house fly (*Musca domestica*) rearing–an environmental life cycle assessment. Journal of Insects as Food and Feed 1: 195-214.

Sweeten, J. M. 2008. Composting manure and sludge. Texas FARMER Collection.

Terrapon, N., and H. Bernard. 2014. How do gut microbes break down dietary fiber? Trends in Biochemical Sciences 39: 156-158.

ur Rehman, K., M. Cai, X. Xiao, L. Zheng, H. Wang, A. A. Soomro, Y. Zhou, W. Li, Z. Yu, and J. Zhang. 2017. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). Journal of Environmental Management 196: 458-465.

USDA. 2006. Annual report: Manure and by-product utilization. United States Department of Agriculture—Agricultural Research Service. 2005.

USDA. 2017. Overview of U.S. livestock, poultry, and aquaculture production in 2017.

van Huis, A. 2019. Manure and flies: biodegradation and or bioconversion? Journal of Insects as Food and Feed 5: 55-58.

Venglovsky, J., N. Sasakova, and I. Placha. 2009. Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application. Bioresource technology 100: 5386-5391.

Wackett, L. P., A. G. Dodge, and L. B. Ellis. 2004. Microbial genomics and the periodic table. Appl. Environ. Microbiol. 70: 647-655.

Wang, H., Z. Zhang, G. F. Czapar, M. K. Winkler, and J. Zheng. 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. Waste Management & Research 31: 223-231.

Wang, W., W. Zhang, X. Wang, C. Lei, R. Tang, F. Zhang, Q. Yang, and F. Zhu. 2017. Tracing heavy metals in ‘swine manure-maggot-chicken’ production chain. Scientific Reports 7: 8417.

Zhao, Y., W. Wang, F. Zhu, X. Wang, X. Wang, and C. Lei. 2017. The gut microbiota in larvae of the housefly *Musca domestica* and their horizontal transfer through feeding. AMB Express 7: 147.

Zhou, F., J. K. Tomberlin, L. Zheng, Z. Yu, and J. Zhang. 2013. Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of Medical Entomology 50: 1224-1230.

Zhu, F. X., W. P. Wang, C. L. Hong, M. G. Feng, Z. Y. Xue, X. Y. Chen, Y. L. Yao, and M. Yu. 2012. Rapid production of maggots as feed supplement and organic fertilizer by the two-stage composting of pig manure. Bioresource Technology 116: 485-491.

7. INTERSPECIFIC COMPETITION BETWEEN THE HOUSE FLY, *Musca domestica* L. (DIPTERA: MUSCIDAE) AND BLACK SOLDIER FLY, *Hermetia illucens* (L.) (DIPTERA: STRATIOMYIDAE) WHEN REARED ON POULTRY MANURE

Overview

Few studies have examined the competitive interaction between the house fly (HF) and the black soldier fly (BSF). Yet, the fact that BSF deter HF oviposition is widely cited in BSF literature, but this interaction has not been assessed in over three decades. In this study, the competitive interaction of BSF and HF larvae was observed on fresh (day 0) and aged manure (day 2, 4, 6, or 8). Specifically, a priority effect study was conducted to determine if colonization sequence influences development time to the pupal (HF) or prepupal (BSF) stage, survivorship, and weight. Results show > 70% of HF reached pupariation in all treatments except when placed on manure 8 d after the initial inoculation with BSF. However, age of the resource negatively impacted pupal weight and days to first pupariation. These results differ from those found for BSF. No BSF prepupae were found in treatments in which HF were the pioneering species. BSF reached the highest percent prepupation when reared alone on fresh (0 d-old manure), but BSF may be more susceptible to the negative impacts of an aging resource, as no prepupae were observed when provided with 6 or 8 d-old manure. Similar to HF, age of the resource may have impacted development and survivorship, but other factors such as moisture content, chemical composition, and amount of resource provided may have also impacted our results. These data may be useful in implementing BSF as biological control agents of HF, as well provide valuable information for facilities mass-producing HF or BSF for food or feed.

Introduction

Competition is an interaction that occurs between individuals (of the same or different species) sharing a common resource potentially resulting in reduced growth, reproduction, and/or survivorship (Begon et al. 2006). Understanding how competition operates in nature or mass-rearing environments is important, as it may assist in reducing pest populations. In agriculture, pests of livestock can cause substantial economic loss (Taylor et al. 2012). Some of the arthropods associated with animal production rely on manure as a resource, and as a result, manure is often the site of intense competition. Two species that utilize manure as a resource for offspring development are the house fly (HF), *Musca domestica* L. (Diptera: Muscidae) and black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae).

House flies are known pests. They can develop to pupariation on calf, swine, or poultry manure in as little as 6 days (Larrain and Salas 2008). They are vectors of numerous pathogens, and may cause economic loss in animal production systems (Graczyk et al. 2001) via costs associated with fly control and associated lawsuits from residents of urban housing (Thomas and Skoda 1993) For these reasons, much of the research conducted on HF focuses on efforts to control them with pesticides (Afifi and Knutson 1956, Georghiou 1966, Sawicki and Lord 1970).Unfortunately, due to decades of pesticide use, they have been extensively studied for their resistance to pesticides (Busvine 1959, El Basheir 1967, Georghiou and Hawley 1971). Therefore, control of HF should involve an integrated approach, combining cultural, biological, and chemical methods. Of the available options for biological control, utilizing BSF is an attractive alternative; however, little is known about what governs HF control by BSF.

Black soldier flies are found in temperate and tropical regions throughout the world. Historically, they were considered a pest (Axtell and Edwards 1970), but due to their ability to reduce a variety of wastes (Nguyen et al. 2013) and convert them into valuable biomass of approximately 42% protein and 35% fat (Sheppard et al. 1994), as well as reduce dry matter and nutrients by 50% or more (Sheppard 1983, Myers et al. 2008) and odorous volatile compounds (Beskin et al. 2018), they are considered a beneficial species. Furthermore, BSF are beneficial because they inhibit house fly oviposition (Furman et al. 1959, Kilpatrick and Schoof 1959, Sheppard 1983, Bradley and Sheppard 1984). Thus, BSF offer a means to control HF, which may ultimately reduce pesticide dependence. However, the mechanism underlying how BSF deter HF is unknown, but appears to be related to changes in the microbial communities within the substrate. For example, BSF are known to reduce *Escherichia coli* (Erickson et al. 2004, Liu et al. 2008), which HF utilize for growth and development (Schmidtmann and Martin 1992). Despite the previous suppositions, the competitive interaction between HF and BSF has not been assessed in over three decades and additional work is needed to better understand the factors that govern this interaction.

Rising interest in production of BSF for food and feed, coupled with the fact that HF are often a pest in such facilities, calls for further investigation of the relationship between these two species. Therefore, this study was conducted to gain a better understanding of the competitive interactions between the larvae of HF and BSF. Specifically, we aimed to determine if colonization sequence influenced development time to the pupal (HF) or prepupal (BSF) stage, survivorship, and weight. This will provide insight on the mechanisms governing HF control when BSF are present and may be valuable for on-farm control of HF, or for industrial applications of BSF where eliminating pest persistence is the goal.

Materials and Methods

Acquisition of HF and BSF

Methods for this experiment are based on those conducted by Miranda et al. (accepted May 2019a) and Miranda et al. (accepted May 2019b). House fly larvae were obtained from a colony at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University, College Station, TX, USA, which is maintained by providing water, as well as a mixture of sugar and nonfat milk powder (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA) (1:1 ratio by mass) to adults ad libitum. The colony originated in 2014 from adults collected from dairy and swine facilities in Stephenville, TX, USA. Black soldier fly larvae in this experiment were from a colony established in 2014 maintained at the F.L.I.E.S. Facility at Texas A&M University, College Station, TX, USA using a modified version of the methods detailed by Sheppard et al. (2002). This colony originated from a colony at the Coastal Plain Experiment Station (University of Georgia) in Tifton, GA, USA.

Acquisition of Manure

Poultry manure less than 12-h-old was collected from layer hens at the Poultry Science Research, Teaching, and Extension Center (Texas A&M University) located in College Station, TX, USA. Manure was placed into 18.9 L buckets with lids (Home Depot®, LeaktiteTM, Leominster, MA, USA) and transported to the F.L.I.E.S Facility, where it was mixed vigorously by hand for approximately 5 minutes and transferred to 3.76 L Ziploc® Freezer Bags (S.C. Johnson & Son, Racine, WI, USA) and stored at -20°C until used. Manure was allowed to thaw at room temperature for 24 hours before initiation of the experiment and manure that was not used to initiate the experiment was placed in a 1.9 L Reditainer™ EXTREME FREEZE™ deli container with a lid (Clear Lake Enterprises, Port Richey, FL, USA) and stored in a refrigerator (4°C) until used. Moisture content was determined gravimetrically with three 10 g samples of thawed manure (Franson 1989). Final moisture content of manure was taken when all individuals within a treatment reached the pupal (for HF) or prepupal stage (for BSF), or after 5 d since the last individual reached the pupal or prepupal stage for treatments with larvae remaining.

Experiment Design

To collect HF eggs, a Kimwipe® (Kimberly-Clark Corp., Irving, TX, USA) saturated with Evaporated Milk (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA) was balled up, and placed in an 88 ml bathroom cup (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA). The cup was placed inside a 30 x 30 x 30 cm cage (BioQuip® Products, Rancho Dominquez, CA, USA) with gravid adult flies and checked for egg deposition every four hours. The Kimwipe® was unfolded and rinsed with distilled water over a 100 ml beaker to collect viable eggs that sank to the bottom of the beaker. Eggs were collected with a Pasteur glass pipette and transferred to a damp Kimwipe® that was placed inside an 88 ml bathroom cup (Great Value® Brand, Wal-Mart® Stores, Inc., Bentonville, AR, USA) covered with a Kimwipe® held in place with a rubber band. The cup was stored in a Rheem Environmental

Chamber (Asheville, NC, USA; at 26 ± 0.04 ˚C, 63 ± 8.72 % RH, and 16L:8D) until larval eclosion.

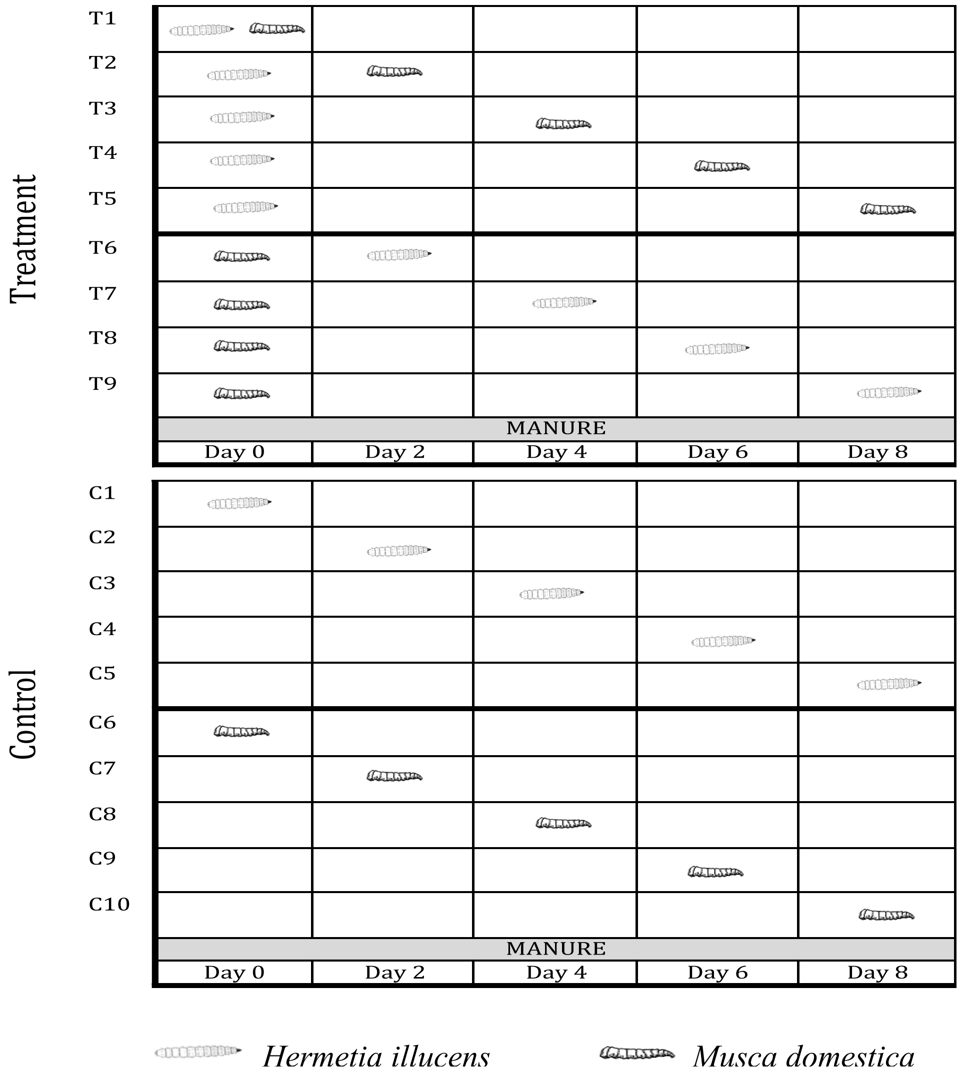
Black soldier fly adults were maintained in a 1.2 x 1.2 x 2.4 m wooden-framed cage with each side of the cage lined with fiberglass window screening (18x16 mesh size) sides in a greenhouse (25 –45°C, >50% RH). Corrugated cardboard was cut into 4.0 x 4.0 x 0.5 cm pieces and three pieces of cardboard were taped together to form a bundle, which was placed on the lid of 5.7 L Sterilite® container with a 15 x 7 cm hole covered with fiberglass window screen. Approximately 500 g Gainesville diet (50% wheat bran, 30% alfalfa meal, 20% corn meal) (Hogsette 1992) saturated with RO water was placed inside the Sterilite® container and served as an attraction medium. The cardboard was checked for egg clutches every four hours, after which the cardboard was dissected, and egg clutches removed. The eggs were placed in a 0.5 L plastic container, covered with a paper towel held in place with a rubber band, and stored in the Rheem Environmental Chamber described above, until larval eclosion.

Replicates consisted of 60 g of poultry manure placed inside 0.5 l deli containers. Manure placed inside the cups was inoculated with 100 newly-hatched (<12 hours old) first instar house flies and introduced into cups 0, 2, 4, 6 and 8 days after the introduction of 100 newly-hatched (< 12 hours old) first instar black soldier flies (Figure 1; T1-T5). Treatments with BSF as the pioneering species are referred to hereinafter as BSF0HF2, BSF0HF4, BSF0HF6, and BSF0HF8. For example, BSF0HF2 indicates BSF were placed on the manure on day 0 and HF were introduced on day 2. The introduction times were selected because they represent the beginning (0 day), middle (2 and 4 days), and end (6 and 8 days) of house fly development on poultry manure (El Boushy 1991). The study was reversed so that house flies represented the pioneering species and first instar black soldier flies were inoculated into the manure 0, 2, 4, 6 and 8 days after the introduction of first instar house flies (Figure 1; T6-T9). These treatments are referred to as HF0BSF2, HF0BSF4, HF0BSF6, and HF0BSF8. To ensure that results were not due to resource age, 100 newly hatched larvae of each species were placed on manure aged 0, 2, 4, 6 or 8 days without testing priority effects, and served as controls (Figure 1; C1-C10). These treatments are referred as HF0, HF2, HF 4, HF6, and HF8 for house flies and BSF0, BSF2, BSF4, BSF6, and BSF8 for black soldier flies. Cups with larvae were placed in the Rheem Environmental Chamber and maintained at the conditions described above. Cups were monitored daily for pupae (HF) or prepupae (BSF). Pupae and prepupae were removed daily from the cups, weighed on the scale described above, and percent pupariation (HF) or prepupation (BSF) as well as days to first pupariation (HF) or prepupation (BSF) were recorded.

Statistical Analyses

Development time to fist pupariation (HF), development time to first prepupation (BSF), percent pupariation (HF), percent prepupation (BSF), pupal (HF) and prepupal (BSF) weight across treatments were analyzed. An ANOVA was performed for each of the parameters listed above using JMP 14.0.0 (SAS Institute Inc., Cary, NC, USA). Tukey’s HSD (honest significant difference) was used for mean separation ($P \leq 0.05$)

Figure 7.1 Experiment design of black soldier fly (BSF) and house fly (HF) larvae fed poultry manure aged 0-8 d, at 26°C, 70% RH, 16L:8D.



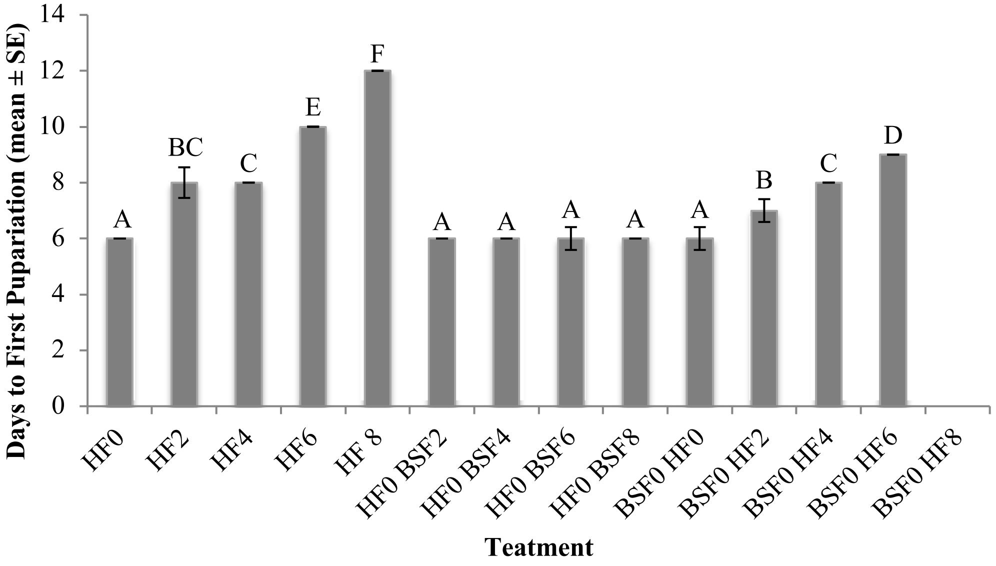
(a) In mixed treatments, the pioneer species, BSF was placed on fresh (0-day-old) manure, while the competing species, HF, was placed simultaneously (T1) or 2 days, (T2), 4 days (T3), 6 days (T4) or 8 days (T5) after the pioneering species. The study was reversed so that HF represented the pioneering species (T6-T9) and BSF were introduced 2, 4, 6, or 8 days after the initial introduction of HF. (b) controls for the effect of manure age on fitness. Pure (single species) cultures of larvae from each species (BSF and HF) were placed on fresh (C1, C6), 2-day-old (C2, C7), 4-day-old (C3, C8), 6-day-old (C4, C9) or 8-day-old (C5, C10) manure. This experiment design is based on the design in Brundage et al. (2014).

Results

Development Time to First Pupariation (HF)

Development time to first pupariation for HF was significantly different ($F_{12, 52} = 326.8$; $p < 0.0001$) across treatments. No significant treatment by trial interaction ($F_{12, 52} = 0.6000$; $p = 0.8321$) or trial effect were determined ($F_{1, 52} = 3.20$; $p = 0.0795$). In regard to larvae in the control groups, HF placed on manure on day 0 developed the fasted (6 d), whereas those placed on manure aged 2–8 d took 1.5 –10 d longer to develop (Figure 7.2). For those placed on manure in which HF were the pioneering species and BSF were introduced at 0 –8 d, all treatments developed to the pupal stage in approximately 6 d. In treatments where BSF were the pioneering species, the shortest development time (6 d) was found in treatments where HF were introduced on fresh manure, and this was 1–3 d faster than treatments where HF were introduced in manure aged 2–6 d. House fly larvae placed on manure 8 d after the initial introduction of BSF did not reach the pupal stage.

Figure 7.2 Time (d) to first pupariation (mean ± SE, [1]n= 6) for house fly (HF) larvae reared on poultry manure (aged 0-8 d), alone, or with black soldier fly (BSF) larvae, at 26°C, 70% RH, 16L:8D.



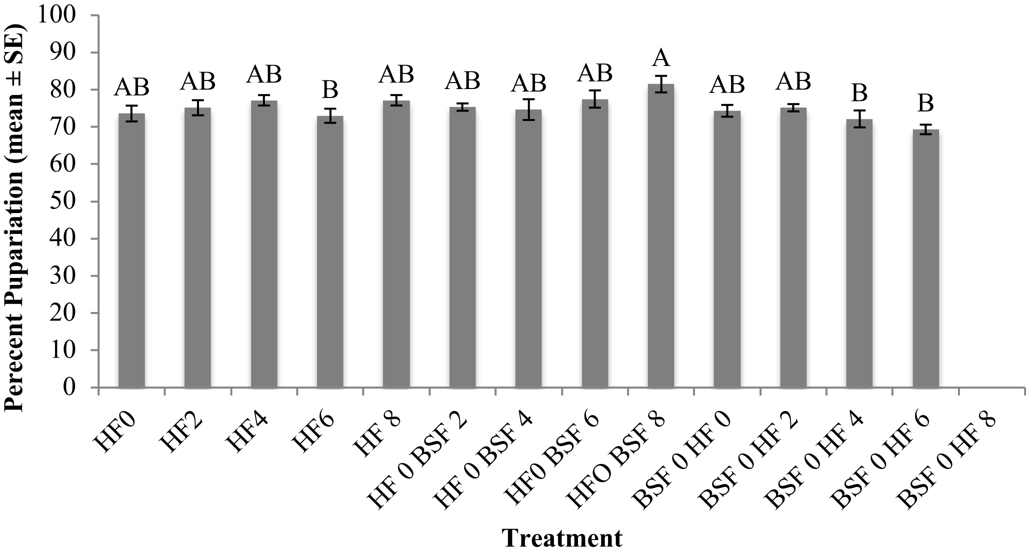
[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Percent Pupariation (HF)

Percent pupation for HF differed significantly across treatments ($F_{12, 52} = 2.9$; p = 0.0033). No treatment by trial interaction was found (p = 0.7069); however, a significant trial effect ($F_{1, 52} = 16.6$; p = 0.0002) was. In general, 4% more individuals reached pupariation in trial one than in trial two. The highest percent pupariation in the control groups was when HF were placed on manure aged 4 d (77%), which was up to 4% more than those placed on manure aged 0, 2, 6, or 8 days (Figure 7.3). In the mixed treatments in which HF were the pioneering species, the highest percent pupariation was found in treatments where BSF were introduced on 8-d-old manure (81%). This was 4–6% more than treatments in which BSF were placed on manure aged 2, 4, or 6

d. For treatments with BSF as the pioneering species, the highest percent pupariation was found in treatments in which HF were introduced on manure after 2 d (75%) and this was 1–6% more than those that had HF introduced on fresh manure, or manure aged 4 or 6 d, and 7 % more than those placed on manure with BSF at the same time. No house fly larvae placed on manure 8 d after the initial introduction of BSF were found to reach the pupal stage.

Figure 7.3 Percent pupariation (mean ± SE, [1]n=6) for house fly (HF) larvae when reared on poultry manure (aged 0-8 d), alone, or with black soldier fly (BSF) larvae, at 26°C, 70% RH, 16L:8D.



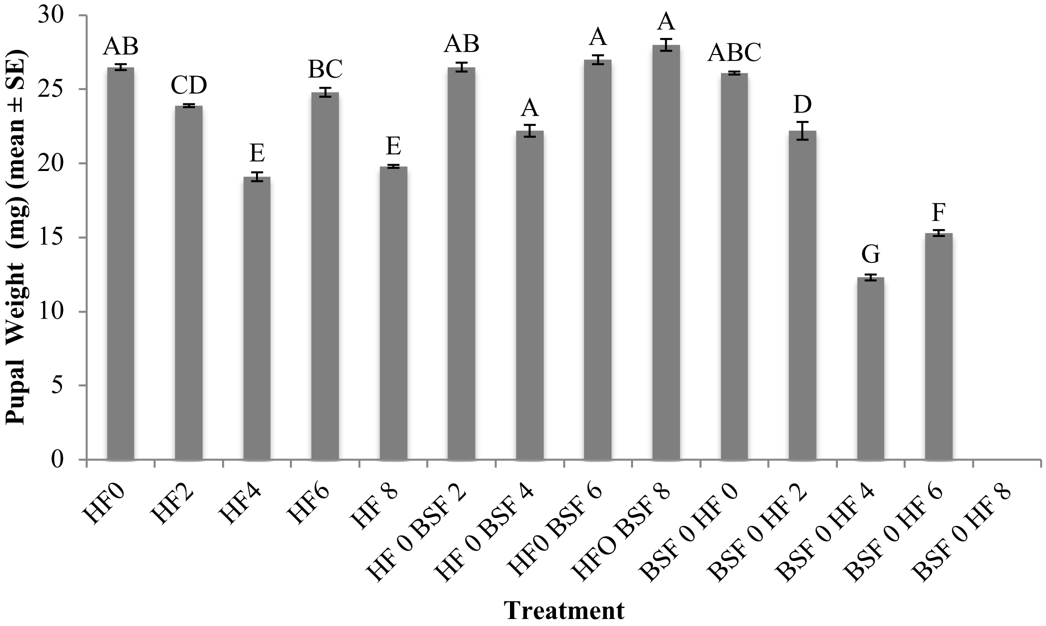
[1]n = number of replicates per treatment.

Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Pupal Weight (HF)

Pupal weight for HF was significantly different (F12, 52 = 124.8; $p < 0.0001$) across treatments. No significant treatment by trial interaction was found ($p = 0.9993$), but a significant trail effect was (F1, 52 = 4.72; $p = 0.0343$). In general, individuals in trial two weighed 2% more than those in trial one. In regard to larvae in the control groups, larvae placed on manure aged 0 d were the heaviest (26 mg) and weighed 6–28% more than those placed on manure aged 2–8 d (Figure 7.4). For those placed in mixed treatments where HF were the pioneering species, the heaviest weight was recorded in treatments in which BSF were placed on day 8 (28 mg), and they weighed 3–7% more than when black soldier flies were introduced to the manure on days 0–6. In treatments where HF were introduced with (on day 0) or after BSF (on days 2, 4, 6 days), HF placed on manure on day 0 weighed the most (26 mg) and this was 15–53% more than HF larvae placed on manure aged 0–6 days. House fly larvae placed on manure 8 days after the initial introduction of BSF did not survive to the pupal stage.

Figure 7.4 Pupal weight (mg) (mean ± SE, [1]n = 6) of house fly (HF) larvae when reared on poultry manure (aged 0-8 d), alone, or with black soldier fly (BSF) larvae, at 26°C, 70% RH, 16L:8D.

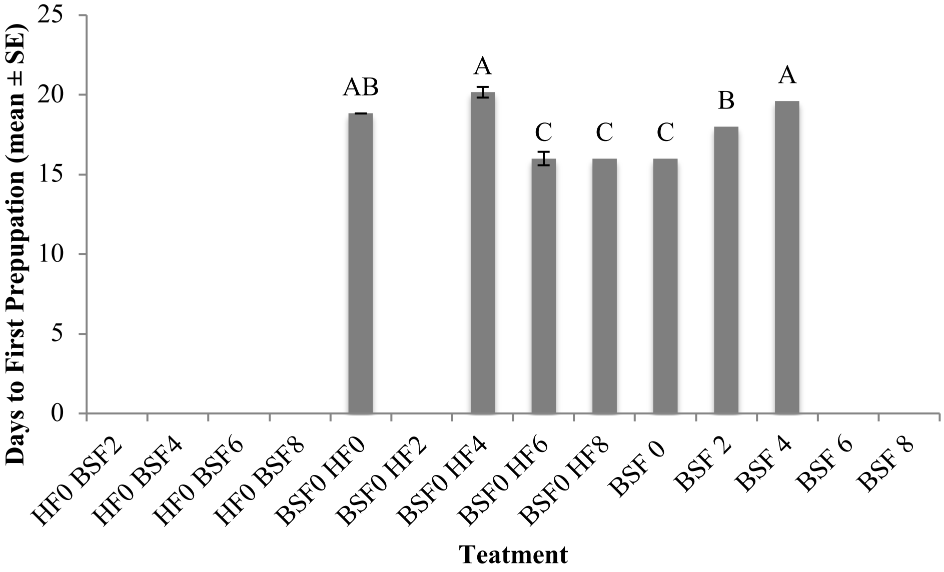


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Development Time to First Prepupation (BSF)

Development time to first prepupation of BSF was significantly different across treatments (F6, 27 = 37.9; p < 0.0001). No treatment by trial interaction (p = 0.9389) or trial effect (p = 0.6770) were found. In regard to larvae in the control groups, BSF placed on manure on day 0 developed the fastest (16 d), whereas those placed on manure aged 2 or 4 d took 2–4 d longer (Figure 7.5). No prepupae were found in treatments in which larvae were placed alone on manure aged 6 or 8 d. Additionally, no prepupae were found in mixed treatments in which HF were the pioneering species. However, in treatments in which BSF served as the pioneering species, the fastest development (16 d) was found in treatments with HF introduced on day 6, and this was 2–4 d less than those with HF introduced at the same time as BSF, or 4 d after BSF.

Figure 7.5 Time (d) to first prepupation (mean ± SE, [1]n= 6) of black soldier fly (BSF) larvae when reared on poultry manure (aged 0-8d), alone, or with house flies (HF), at 26°C, 70% RH, 16L:8D.



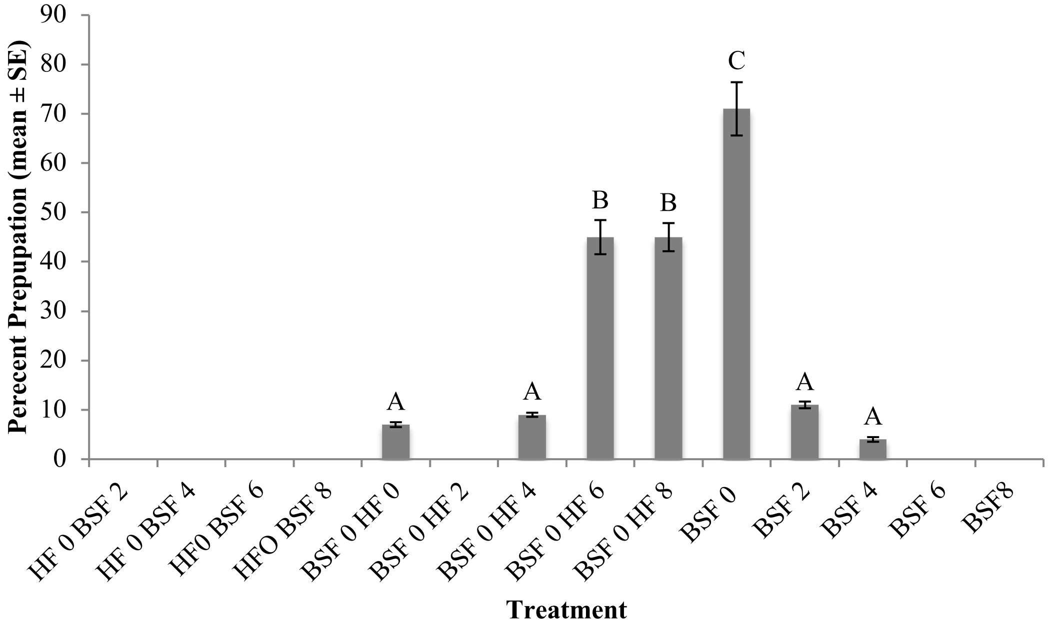
[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Percent Prepupation (BSF)

Percent prepupation of BSF was significantly different across treatments (F6, 27 = 83.3; p < 0.0001). No treatment by trial interaction (p = 0.9431) or trial effect (p = 0.5475) were found. In regard to larvae in the control groups, the highest percent prepupation (71 %) was found in treatments with BSF placed on manure on day 0, and this was 60–68% more than those inoculated into 2–4 d-old manure (Figure 7.6). In mixed treatments with BSF as the pioneering species, the highest percent prepupation

(45%) was found in treatments with HF introduced on day 6 or 8, and this was 34–41 % more than those with HF introduced on day 0, 2, or 4.

Figure 7.6 Percent prepupation (mean ± SE, [1]n=6) of black soldier flies (BSF) when reared on poultry manure (aged 0-8 d), alone, or with house flies (HF), at 26°C, 70% RH, 16L:8D.

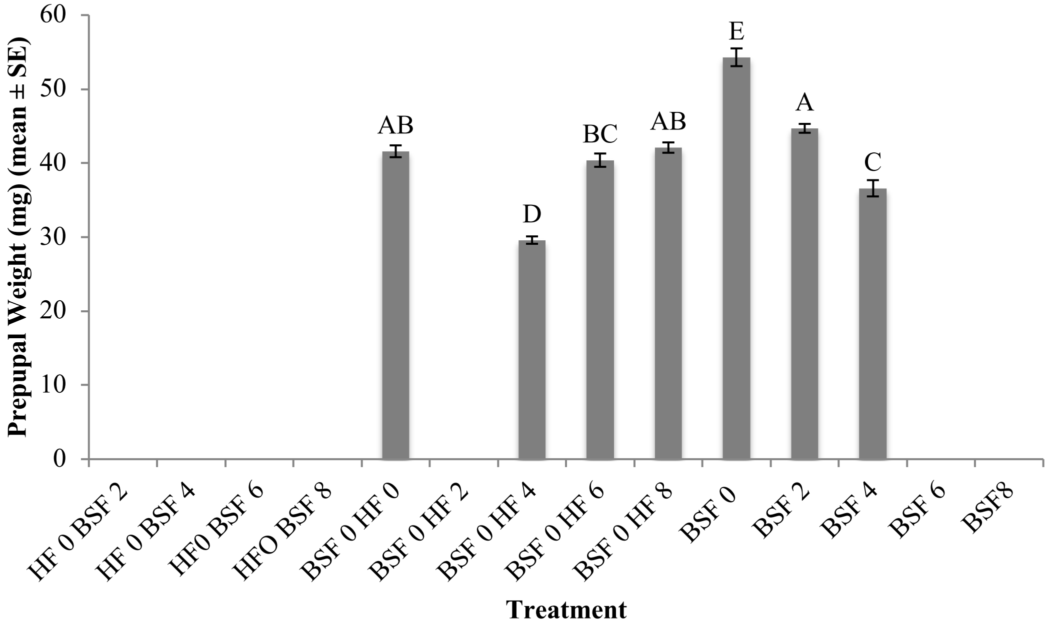


[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Prepupal Weight (BSF)

Prepupal weight was significantly different across treatments ($F_{6, 27}$ = 72.5; p < 0.0001). No treatment by trial interaction (p = 0.9832), but a significant trial effect ($F_{1, 56}$ = 12.6310; p = 0.0014) was found. In general, individuals in trial two weighed 6 % more than those in trial one. In regard to larvae in the control groups, the heaviest prepupae (53.1 mg) were found in treatments with BSF placed on manure on day 0 and they were 19–45% more than those inoculated into 2–4-d-old manure (Figure 7.7). In mixed treatments with BSF as the pioneering species, the heaviest prepupae (42.1 mg) were found in treatments with HF introduced on day 8, and this was 1–42 % more than those with HF introduced on day 0, 4, or 6.

Figure 7.7 Prepupal weight (mg) (mean ± SE, [1]n=6) of black soldier flies (BSF) when reared on poultry manure (aged 0-8 d), alone, or with house flies (HF)), at 26°C, 70% RH, 16L:8D.



[1]n = number of replicates per treatment.
Different letters indicate significant differences between treatments (α = 0.05), ANOVA followed by Tukey's HSD.

Discussion

Results from this study show colonization sequence impacts development time, survivorship, and weight of HF and BSF. More specifically, HF are able to successfully colonize the resource as a pioneer species, before any obvious negative impact due to the presence of the later colonizer, BSF, is apparent (Figure 7.2). In general, HF successfully reached > 70% pupariation in all but one treatment (BSF0HF8) regardless of whether they were placed on manure alone (control) or introduced before or after BSF (mixed treatments) (Figures 7.3). A likely explanation for poor performance of HF in BSF0HF8 may be due to age of the resource, as HF development time was extended by 2–6 d when reared alone on 8-d-old manure (Figure 7.2) and pupal weight was reduced by up to 25% (Figure 7.4) when compared to those placed on manure at earlier times. Age of manure, combined with the fact that BSF likely exhausted the majority of the nutrients available in 8 d, may explain why HF performed poorly when introduced 8 d after BSF.

When examining the same scenario from the BSF perspective, there are obvious negative impacts on BSF growth and survivorship, which may be due to the presence of HF, but may also be due to age of the resource or amount of resource provided during the experiment. Out of 14 treatments with BSF, prepupae were found in only seven treatments. Of those seven, three of them were controls (BSF 0, BSF2, and BSF4) in which development time was extended by up to 3 d (Figure 7.5) and 4–71% survived on manure aged 0–4 d (Figure 7.6). No BSF prepupae were found in treatments with manure aged 6 or 8 d (BSF 6 or BSF8). In mixed treatments in which HF were the pioneering species (HF0BSF2, HF0BSF4, HFOBSF6, and HFOBSF8), no BSF prepupae were found, although larvae were detected in all of the mixed treatments at the end of the experiment. When BSF was the pioneering species, survivorship increased from 7% in treatments with HF introduced at the same time as BSF (BSF0HF0), to 45% in treatments with later HF introductions (BSFOHF6 or BSF0HF8) (Figure 6). No BSF prepupae were found in treatments in which HF were introduced on day 2 (BSF0HF2). In regard to weight, BSF weighed 21–48% more when placed on fresh manure when compared to those placed on manure aged 2–4 d (BSF2 and BSF4) (Figure 7.7). In mixed treatments where HF were introduced on day 8 (BSF0HF8), BSF weighed 1% more than those placed with HF (BSF0HF0) and 20–42% more than those with HF placed on manure at later times (BSFOHF4 or BSF0HF6). Similar to the HF results, it cannot be disregarded that age of the resource influenced BSF performance.

Previous research has shown that colonization sequence may govern fitness among competitors. By definition, this is known as a priority effect, which has been observed in blow flies (Diptera: Calliphoridae) (Brundage et al. 2014), mosquitoes (Diptera: Muscidae) (Blaustein and Margalit 1996), beetles (Coleoptera: Cerambycidae, Scolytidae, and Trogossitidae) (Weslien et al. 2011), and ants (Hymenoptera: Formicidae) (Palmer et al. 2002), among others. However, few studies have examined the competitive interaction between HF and BSF, and none have incorporated colonization sequence as a factor. Initial research efforts on HF and BSF focused on how the presence or absence of BSF influenced abundance of HF. Furman et al. (1959) noted there was an absence of HF when BSF were present at poultry facilities. This observation led to an experiment in which gravid HF females were exposed to two containers: one with manure and 500 BSF larvae, and the other with only manure. The researchers found HF emerged from the container with manure only, but no HF were observed from the container with BSF. This study went further to suggest a density-dependent response for HF, such that as BSF larval density increased, percent emergence and survivorship for all HF life stages decreased. In a similar study, Bradley and Sheppard (1984) showed that BSF density as well as the amount of time that BSF occupy the resource negatively impacts oviposition of HF. Other studies showed that control of BSF with larvicides

(Axtell and Edwards 1970) or by clean manure basins (Sheppard 1983) reduce BSF populations and subsequently increase HF populations. Yet despite all of these findings, facilities that mass-produce BSF are still threatened by HF infestations. As such, it is possible that colonization sequence is a factor that governs successful HF infestations.

Development and survivorship for both species were impacted by the age of the resource. Although not tested in this study, it is possible that variations in moisture contents of aged manure may explain why no BSF prepupae were found in treatments with HF as the pioneering species (HF0BSF2, HF0BSF4, HF0BSF6, and HF0BSF8). As larvae consume the resource, they also mechanically aerate it, reducing the moisture content. Past research has shown that BSF cannot develop on resources with low moisture contents, and although lower threshold is not known, it likely is between 40–55% moisture (Cammack and Tomberlin 2017). Fatchurochim et al. (1989) found similar results for BSF and HF, and this may also be why no HF pupae were found in BSF0HF8 treatments. However, moisture may not be the only explanation for variation in our results, as the nutrient content of manure might change with age, and therefore could have impacted development.

The chemical composition of manure changes with age. Microbes break down nutrients in manure, and over time, these nutrients are volatilized as secondary metabolites (Maeda et al. 2011). Although HF development time and weight were reduced when fed aged manure, puparia were found in all controls, suggesting there were sufficient nutrients available to support growth on an aging resource. However, none were found in the mixed treatment BSF0HF8, and this may be due to depletion of nutrients, but could also be the result of impacts of BSF on bacteria. Black soldier flies are known to reduce *Escherichia coli* in poultry manure in as little as 3 d (Erickson et al. 2004), and house flies depend on *E. coli* to obtain essential nutrients for proper growth and development (Schmidtmann and Martin 1992). Although not tested in this study, it is possible that BSF reduced the nutrient content as well as crucial bacteria, such as *E. coli*, before HF were introduced, and thus, limited HF performance in these treatments. From an industrial standpoint, this insight is valuable in controlling HF infestation, as overfeeding BSF may hinder their ability to reduce essential microbes for HF development (Liu et al. 2008) and could result frequent pest infestations. Consequently, optimizing feed rates may be influential in HF control, but more importantly, the amount of resource provided may also be responsible for poor BSF performance in this experiment.

The amount of food provided may have impacted BSF performance. Black soldier flies are larger individuals with longer development time to the prepupal stage (16–20 d), and consequently require more resource than HF. Larval nutrition is critical to BSF, as they are acquiring all of the nutrients needed to sustain adult livelihood. House flies differ from BSF in that they require less resource because of their short development time to the puparium stage (6 d). They may also invest less in nutrient acquisition during larval development because they have fewer digestive enzymes compared to BSF (Kim et al. 2011), or because they must feed as adults to mature reproductively (Morrison and Davies 1964). Black soldier flies, on the other hand, do not necessarily have to feed as adults (Sheppard et al. 2002), and so there is an adaptive value for BSF to invest in more digestive enzymes to feed more efficiently and acquire the all of the nutrients needed to sustain adult livelihood, and avoid obligatory adult feeding.

The amount provided was determined by preliminary experiments that resulted in low survivorship (<20%) when newly-hatched BSF larvae were placed on ≥ 100 g of poultry manure. It appeared that the young larvae were not able to process the manure before unfavorable microbes proliferated; therefore, in an effort to avoid overfeeding and reduce the effect of competition, the minimum amount of fresh poultry manure for BSF was determined to be 60 g (>70% survivorship). However, as previously discussed, moisture and nutrient contents are reduced in aging manure, and manure with HF. The amount of manure selected for this experiment may not have been an adequate amount to support BSF development on aged manure or manure previously colonized by HF.

Recent interest in mass-producing BSF for food and feed production calls for further investigation of the competitive interactions between HF and BSF. These species are ecologically similar in regard to larval diet, which can prompt HF infestations in such facilities. The presence of HF may cause a reduction in BSF weight, development time, and survivorship, which in turn, may impact bioconversion and production efficiency. Within a mass production facility, there are few pest control methods that are available, but employing pesticides is not an option as they may kill or contaminate the insects that are intended for food and feed. Therefore, it is important to understand the mechanisms that govern HF control by BSF in order to avoid or reduce HF infestations.

This study is the first to evaluate the impact of colonization sequence on development time, survivorship, and weight of HF and BSF. Results demonstrate resource age negatively impacts HF and BSF development and survivorship; however, there appears to be a greater impact on BSF as those fed manure aged 6 or 8 d did not complete development to the prepupal stage; whereas HF developed to the pupal stage in all treatments, except when they were introduced 8 days after BSF. Findings from this study also reveal in mixed treatments, HF development and survivorship are not significantly impacted if they colonize poultry manure first, or within 2 d after the initial introduction of BSF. Conversely, BSF development and survivorship were negatively impacted when HF are introduced first or within 4 d after BSF. However, poor performance of BSF in single and mixed treatments may be a result of reduced moisture and nutrients contents in aged manure, as well as amount of resource provided, as previously discussed. Different results may be obtained if this study was performed with more resource. Thus, future studies should investigate this interaction when BSF are fed optimal amounts of manure

References

Afifi, S. E., and H. Knutson. 1956. Reproductive potential, longevity, and weight of house flies which survived one insecticidal treatment. Journal of Economic Entomology 49.

Axtell, R., and T. Edwards. 1970. *Hermetia illucens* control in poultry manure by larviciding. Journal of Economic Entomology 63: 1786-1787.

Begon, M., C. R. Townsend, and J. L. Harper. 2006. Ecology: From individuals to ecosystems.

Beskin, K. V., C. D. Holcomb, J. A. Cammack, T. L. Crippen, A. H. Knap, S. T. Sweet, and J. K. Tomberlin. 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. Waste Management 74: 213-220.

Blaustein, L., and J. Margalit. 1996. Priority effects in temporary pools: nature and outcome of mosquito larva-toad tadpole interactions depend on order of entrance. Journal of Animal Ecology: 77-84.

Bradley, S. W., and D. C. Sheppard. 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. Journal of Chemical Ecology 10: 853-859.

Brundage, A., M. E. Benbow, and J. K. Tomberlin. 2014. Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. Ecological Entomology 39: 539-547.

Busvine, J. R. 1959. Patterns of insecticide resistance to organo-phosphorus compounds in strains of houseflies from various sources. Entomologia Experimentalis et Applicata 2: 58-67.

Cammack, J. A., and J. K. Tomberlin. 2017. The impact of diet protein and carbohydrate on select life-history traits of the black soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). Insects 8: 56.

El Basheir, S. 1967. Causes of resistence to DDT in Diazinon-selected and DDT-selected strain of house flies. Entomologia Experimentalis et Applicata 10: 111-126.

El Boushy, A. 1991. House-fly pupae as poultry manure converters for animal feed: a review. Bioresource Technology 38: 45-49.

Erickson, M. C., M. Islam, C. Sheppard, J. Liao, and M. P. Doyle. 2004. Reduction of *Escherichia col*i O157: H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. Journal of Food Protection 67: 685-690.

Fatchurochim, S., C. Geden, and R. Axtell. 1989. Filth fly (Diptera) oviposition and larval development in poultry manure of various moisture levels. Journal of Entomological Science 24: 224-231.

Franson, M. A. H. 1989. Total solids dried at 103-105C: Standard methods for the examination of water and wastewater., American Public Health Association, Washington, DC.

Furman, D. P., R. D. Young, and E. P. Catts. 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. Journal of Economic Entomology 52: 917-921.

Georghiou, G. 1966. Distribution of insecticide-resistant house flies on neighboring farms. Journal of Economic Entomology 59: 341-346.

Georghiou, G. P., and M. K. Hawley. 1971. Insecticide resistance resulting from sequential selection of houseflies in the field by organophosphorus compounds. Bulletin of the World Health Organization 45: 43.

Graczyk, T. K., R. Knight, R. H. Gilman, M. R. J. M. Cranfield, and infection. 2001. The role of non-biting flies in the epidemiology of human infectious diseases. Microbes and Infection 3: 231-235.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Kilpatrick, J. W., and H. F. Schoof. 1959. Interrelationship of water and *Hermetia illucens* breeding to *Musca domestica* production in human excrement. The American Journal of Tropical Medicine Hygiene 8: 597-602.

Kim, W., S. Bae, K. Park, S. Lee, Y. Choi, S. Han, and Y. Koh. 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Journal of Asia-Pacific Entomology 14: 11-14.

Larrain, P. S., and C. F. Salas. 2008. House fly (*Musca domestica* L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Liu, Q., J. K. Tomberlin, J. A. Brady, M. R. Sanford, and Z. Yu. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. Environmental Entomology 37: 1525-1530.

Maeda, K., D. Hanajima, S. Toyoda, N. Yoshida, R. Morioka, and T. Osada. 2011. Microbiology of nitrogen cycle in animal manure compost. Microbial Biotechnology 4: 700-709.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019a. Life-history traits of house fly, *Musca domestica* L. (Diptera: Muscidae), reared on three manure types. Journal of Insects as Food and Feed.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. Accepted May 2019b. Life-history traits of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), reared on three manure types. Animals.

Morrison, P. E., and D. M. Davies. 1964. Feeding of dry chemically defined diets + egg production in adult house-fly. Nature 201: 104-105.

Myers, H. M., J. K. Tomberlin, B. D. Lambert, and D. Kattes. 2008. Development of black soldier fly (Diptera : Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2013. Influence of Resources on *Hermetia illucens* (Diptera: Stratiomyidae) Larval Development. Journal of Medical Entomology 50: 898-906.

Palmer, T. M., T. P. Young, and M. L. Stanton. 2002. Burning bridges: Priority effects and the persistence of a competitively subordinate acacia-ant in Laikipia, Kenya. Oecologia 133: 372-379.

Sawicki, R., and K. Lord. 1970. Some properties of a mechanism delaying penetration of insecticides into houseflies. Journal of Pesticide Science 1: 213-217.

Schmidtmann, E., and P. Martin. 1992. Relationship between selected bacteria and the growth of immature house flies, *Musca domestica*, in an axenic test system. Journal of Medical Entomology 29: 232-235.

Sheppard, C. 1983. House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environmental Entomology 12: 1439-1442.

Sheppard, D. C., G. L. Newton, S. A. Thompson, and S. Savage. 1994. A value-added manure management-system using the black soldier fly. Bioresource Technology 50: 275-279.

Sheppard, D. C., J. K. Tomberlin, J. A. Joyce, B. C. Kiser, and S. M. Sumner. 2002. Rearing methods for the black soldier fly (Diptera : Stratiomyidae). Journal of Medical Entomology 39: 695-698.

Taylor, D. B., R. D. Moon, and D. R. J. J. o. M. E. Mark. 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. Journal of Medical Entomology 49: 198-209.

Thomas, G. D., and S. R. Skoda. 1993. Rural flies in the urban environment?, University of Nebraska-Lincoln, Institute of Agriculture and Natural Resources.

Weslien, J., L. B. Djupström, M. Schroeder, and O. Widenfalk. 2011. Long-term priority effects among insects and fungi colonizing decaying wood. Journal of Animal Ecology 80: 1155-1162.

8. CONCLUSIONS

Summary of Findings

I conducted a series of experiments examining select-life history traits of the black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae) and the house fly (HF), *Musca domestica* L. (Diptera: Muscidae) on three manure types (swine, poultry, and dairy) at different feed rates and scales. I also examined corresponding nutrient shifts in these resources due to digestion by these insects. In order to explore the ecological interactions between these species, I also examined how immatures of each species responded to one another at given time points after introduction on a select manure type.

In the small-scale studies (chapter 2, Miranda et al. accepted May 2019a) where each species was reared independent of one another on the different manure types, I determined that BSF fed poultry manure resulted in the heaviest prepupae (0.0866-0.1137 g) and adults (> 0.037 g), with the shortest development time to prepupation (11-12 d), highest percent prepupation (>94%), and longest adult longevity (7-8 d) compared to those fed swine and dairy manures. Conversely for HF, those fed swine manure had the shortest development (5-6 d) and had the highest percent pupariation (>58%), with comparable pupal and adult weights as we all adult longevity to those fed poultry (except the high for the high feed rate for females) or dairy manure (chapter 3, Miranda et al. accepted May 2019b).

In the large-scale BSF study, I found that those fed poultry manure had the shortest development (14 d) and highest percent prepupation (78%) with comparable prepupal weight to those fed swine and dairy (chapter 4). Conversely, I found in the large-scale HF study that larvae fed swine manure had the highest percent survivorship (73%), but comparable development time (5 d) and pupal weight to those fed poultry manure (chapter 5). Though, when comparing across studies, I found higher survivorship, but lower prepupal weights in BSF small-scale studies, with similar findings for HF pupal weight, but the reverse was true for HF survivorship. I also found differences in the chemical composition of manure across these studies (chapter 6 and 7), with higher nutrient and heavy metal reductions in the small-scale studies and higher fiber reductions in the large-scale studies.

Lastly, when comparing the competitive interaction of BSF and HF on poultry manure (chapter 8) with colonization sequence as a factor, greater than 70% of HF reached pupariation regardless of whether or not they colonized the resource before or after BSF, except in treatments in which BSF colonized the manure 8 d prior to HF introduction. There was no effect on HF pupal weight when BSF were introduced after HF, but there was when BSF colonized the resource before HF. Conversely, no BSF reached prepupation in treatments where HF colonized first, and only 7% reached prepupation when HF were introduced at the same time as BSF. Later HF introductions (6 and 8 d) increased BSF prepupation, but only to 45%, but also negatively impacted prepupal weight. I also found that age of the resource strongly impacted BSF development, as only 11% reached prepupation when reared alone on manure aged 2 d.

Limitations

Shared limitations across the research presented include the use of flies maintained in colony on a grain-based diet, the use of frozen manure, and the fact that the large-scale studies are not truly representative of industrial conditions that rear thousands to hundreds of thousands of larvae at a time. Both BSF and HF colonies have been maintained in a laboratory on a standard diet (Gainesville diet) (Hogsette 1992) for multiple generations and it is possible that flies have been selected for optimal performance on the standard diet. Flies were not reared for successive generations prior to use in experiments, which may have limited their performance on the manures tested. The nutritional environment of parental generation can influence offspring life-history traits (Rossiter 1991, Vijendravarma et al. 2009); thus, rearing BSF and HF on Gainesville diet and then subjecting larvae to a different diet may have impacted results, as flies were conditioned on Gainesville diet for multiple generations. Furthermore, the manure was frozen (and refrigerated in the small-scale studies) prior to use, which may have impacted results. Speshock et al. (2019) showed that stable flies, *Stomoxys calcitrans* L. (Diptera: Muscidae), fed frozen dairy manure weighed 70% more as pupae, and 20% more reached pupariation compared to those fed refrigerated manure, which is likely due to microbial shifts in the manure. Furthermore, Pratt et al. (2014) concluded that freezing (-20ºC) or refrigerating (4ºC) poultry manure did not impact the physiochemical properties or microbe viability of the substrate; yet the impacts of storage are not known for swine manure. Lastly, the methods employed in the large-scale study are not truly representative of industrial practices, especially, in the HF studies, where 4000 larvae were fed 1 kg of manure. Unfortunately, the number of larvae that could be utilized in this study was limited by the size of the colony and this was the maximum number of eggs per treatment that could be obtained. Future studies should be conducted on a larger scale in order to determine the differences across small- scale and large-scale studies for industrialization purposes.

Future Studies on Food and Feed Safety

A major challenge that must be resolved to advance the idea of utilizing insects in animal diets involves concerns about food and feed safety (van Huis et al. 2015). Some concerns that impede the development of incorporating insects fed manure into animals diets are accumulation of heavy metals, veterinary medications, and pesticide residues (Charlton et al. 2015). Additionally, there are concerns about pathogens in the manure. For example, Schuster et al. (2013) fed house flies bovine manure inoculated with *Escherichia coli* at various concentrations (0, 3, 5, 8 $\log_{10}$ CFU/mL) and demonstrated the pathogen persisted through metamorphosis to the adult stage. Results determined that the concentration decreased with each successive life stage; however, flies emerged with up to 2.2 $\log_{10}$ CFU/mL demonstrating bacterial carriage across life stages (Schuster et al. 2016). Similar trends were reported by Su et al. (2010) and Zurek and Nayduch (2016) for house flies fed manure. Wang et al. (2017) showed that house fly larvae reduce heavy metals (zinc, copper, chromium, selenium, cadmium, and lead) in swine manure, and the concentration in the larvae decreased with age, as well as met the standard limits established by several countries for food and feed safety. Likewise, house flies are known to reduce antimicrobials in manure in as little as 6 days, compared to traditional composting methods that take 30-40 days (Zhang et al. 2014). Still, there are concerns about transstadial transmission of pathogens in larvae (Schuster et al. 2013, Zurek and Nayduch 2016), but this may be resolved by fermenting the manure, which has been shown to eliminate pathogenic bacteria (Marchaim et al. 2003). Additionally, manure may be pre-treated by oven drying, as previous research on HF development on different manure types showed that oven-dried (105°C for 15 minutes) manure (cow, calf, goat, horse, swine, dog, and chicken) that is subsequently re-hydrated with distilled water supports HF development (Larrain and Salas 2008), but the carriage of pathogens was not investigated. Conversely, for BSF, development time was extended 144-214 d when larvae were fed dried poultry, swine, or dairy manure (Oonincx et al. 2015); however, manure was dried at 60°C until constant weight, which may have been excessive and eliminated beneficial bacteria. Pre-treatment of the manure (via oven-drying) may reduce pathogens but will likely need to be inoculated with beneficial microbes to support BSF development (Yu et al. 2011) and may not be economical. Future studies should investigate development of BSF and HF on pre-treated (fermentation, dried and then inoculated with beneficial microbes) manure.

Future Studies with Black Soldier Flies

Black soldier flies did not perform as well (low prepupal weights and survivorship to prepupal stage) as expected in the priority effect study (objective 7) and one of the reasons may be due to the amount of available food. Black soldier flies are larger individuals and take more time to develop compared to HF, so it is likely that a single BSF larva consumes more manure (Čičková et al. 2015). Additionally, results from objective 3 (mass production of BSF) and 5 (chemical composition of manure) showed differences in life-history traits and nutrient, heavy metal, and fiber reductions across studies, which highlights the importance of scale in research conducted for industrialization purposes. Therefore, future studies that focus on the competitive interactions of immature BSF and HF should be conducted on a large scale with optimal feed rates for BSF as this may provide more valuable information on ways to enhance production while also managing HF as a pest.

Majority of the research conducted on BSF focuses on the immature life stages and little is known about adult behavior. One of the original objectives of this research was to determine the attraction and oviposition preference of BSF to undigested and BSF- and HF-digested manure. However, the focus of the research changed course and this objective was eliminated and others were added. Therefore, a study on adult behavior is necessary, as it may compliment the priority effect study in situations where BSF may be considered a pest in facilities that mass-produce HF. For these reasons, future BSF studies should examine adult BSF attraction and oviposition behaviors to undigested and digested manure.

Future Studies with House Flies

House flies are typically overlooked as candidates for waste management because of their pest status (Busvine 1951, Busvine 1959, Sawicki and Lord 1970, Malik et al. 2007, Zurek and Nayduch 2016). However, HF may be deemed beneficial because they are able to convert waste into valuable biomass (Ocio et al. 1979, El Boushy 1991, Hwangbo et al. 2009, van Zanten et al. 2015), an attribute that should not be ignored. Instead, we should harness this ability, but in ways that circumvent the factors that contribute to pathogen dispersal by HF. One way to manipulate the ability of HF to disperse pathogens is to utilize mutant strains that cannot fly. Studies as early as the 1960's have investigated several strains with malformed wings, such as curly winged, stubby wings, and perpendicular wings (Hoyer 1966), which limit HF ability to fly. By constraining flight, mechanical dispersal of pathogens is reduced; thus, future studies should explore utilizing mutant strains for waste management and protein production.

House flies are also capable of utilizing degraded manure (Barnard et al. 1998). This is an interesting attribute as HF may be able to survive on manure digested by BSF, which may resemble immature compost and need further processing (Zurbrügg et al. 2018). Furthermore, BSF and HF differ in their nutritional composition (Khan 2018). For example, BSF are typically high in calcium (5-8% dry matter), and essential amino acids (EAA) such as valine and leucine, but low in cysteine and tryptophan; whereas HF are high protein (60%) and tryptophan, and low in the EAA that BSF are not (Khan 2018). Therefore, feeding the waste to BSF, and then recycling it to be fed to HF may offer a way to transform manure into a sustainable substrate, while also potentially producing another species that is valuable for food or feed purposes.

Future Work

This coming summer (Summer 2019), I would like to evaluate the co-digestion of poultry manure by BSF and HF. I think the ability of HF to develop on degraded manure is an interesting attribute, but I am also interested in post-processing of manure. Another project that I recently became interested in is rearing BSF on cricket frass. This came about as a happenstance in a study I was conducting to determine if cricket frass was a suitable growing media for tomato seedlings. The experiment was conducted in the greenhouse where BSF adults are housed, and to my surprise, BSF colonized treatments with 100% frass. I currently have a small pilot scale underway to determine if cricket frass digested by BSF is a suitable growing media for tomato seedlings and hope to conduct a full-scale experiment if the pilot is successful. Lastly, this past semester I was granted the opportunity to work with an undergraduate interested in rearing BSF on brewer’s waste mixed with orange and coffee waste from a local café. We completed the first trial and I would like to perform a second trial.

References

Barnard, D., R. Harms, and D. Sloan. 1998. Biodegradation of poultry manure by house fly (Diptera: Muscidae). Environmental Entomology 27: 600-605.

Busvine, J. 1951. Mechanism of resistance to insecticide in houseflies. Nature: 193-195.

Busvine, J. R. 1959. Patterns of insecticide resistance to organo-phosphorus compounds in strains of houseflies from various sources. Entomologia Experimentalis et Applicata 2: 58-67.

Charlton, A., M. Dickinson, M. Wakefield, E. Fitches, M. Kenis, R. Han, F. Zhu, N. Kone, M. Grant, and E. Devic. 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1: 7-16.

Čičková , H., G. L. Newton, R. C. Lacy, and M. Kozanek. 2015. The use of fly larvae for organic waste treatment. Waste Management 35: 68-80.

El Boushy, A. 1991. House-fly pupae as poultry manure converters for animal feed: a review. Bioresource Technology 38: 45-49.

Hogsette, J. A. 1992. New diets for production of house flies and stable flies (Diptera, Muscidae) in the laboratory. Journal of Economic Entomology 85: 2291-2294.

Hoyer, R. F. 1966. Some new mutants of the house fly, *Musca domestica*, with notations of related phenomena. Journal of Economic Entomology 59: 133-137.

Hwangbo, J., E. Hong, A. Jang, H. Kang, J. Oh, B. Kim, and B. Park. 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. Journal of Environmental Biology 30: 609-614.

Khan, S. H. 2018. Recent advances in role of insects as alternative protein source in poultry nutrition. Journal of Applied Animal Research 46: 1144-1157.

Larrain, P. S., and C. F. Salas. 2008. House fly (*Musca domestica* L.) (Diptera : Muscidae) development in different types of manure. Chilean Journal of Agricultural Research 68: 192-197.

Malik, A., N. Singh, and S. Satya. 2007. House fly (*Musca domestica*): A review of control strategies for a challenging pest. Journal of Environmental Science and Health Part B 42: 453-469.

Marchaim, U., A. Gelman, and Y. Braverman. 2003. Reducing waste contamination from animal-processing plants by anaerobic thermophilic fermentation and by flesh fly digestion. Applied Biochemistry and Biotechnology 109: 107-115.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. accepted May 2019a. Life-History Traits of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), Reared on Three Manure Types. Animals 9: 281.

Miranda, C. D., J. A. Cammack, and J. K. Tomberlin. accepted May 2019b. Life-History Traits of Housefly, *Musca domestica* L. (Diptera: Muscidae), Reared on Three Manure Types. Journal of Insects as Food and Feed.

Ocio, E., R. Viñaras, and J. M. Rey. 1979. House fly larvae meal grown on municipal organic waste as a source of protein in poultry diets. Animal Feed Science and Technology 4: 227-231.

Oonincx, D., A. van Huis, and J. van Loon. 2015. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. Journal of Insects as Food and Feed 1: 131-139.

Pratt, C., M. Redding, J. Hill, S. R. Mudge, M. Westermann, C. Paungfoo-Lonhienne, and S. Schmidt. 2014. Assessing refrigerating and freezing effects on the biological/chemical composition of two livestock manures. Agriculture, Ecosystems & Environment 197: 288-292.

Rossiter, M. 1991. Environmentally-based maternal effects: a hidden force in insect population dynamics? Oecologia 87: 288-294.

Sawicki, R., and K. Lord. 1970. Some properties of a mechanism delaying penetration of insecticides into houseflies. Journal of Pesticide Science 1: 213-217.

Schuster, G. L., J. R. Donaldson, J. O. Buntyn, H. A. Duoss, T. R. Callaway, J. A. Carroll, S. M. Falkenberg, and T. B. Schmidt. 2013. Use of bioluminescent *Escherichia coli* to determine retention during the life cycle of the house fly, Musca domestica (Diptera: Muscidae, L). Foodborne Pathogens and Disease 10: 442-447.

Speshock, J. L., J. A. Brady, J. Eastman, T. Roach, S. Hays, and D. Kattes. 2019. Impact of Manure Storage Time and Temperature on Microbial Composition and Stable Fly (Diptera: Muscidae) Development. Advances in Microbiology: 248-265.

Su, Z., M. Zhang, X. Liu, L. Tong, Y. Huang, G. Li, and Y. Pang. 2010. Comparison of bacterial diversity in wheat bran and in the gut of larvae and newly emerged adult of *Musca domestica* (Diptera: Muscidae) by use of ethidium monoazide reveals bacterial colonization. Journal of economic entomology 103: 1832-1841.

van Huis, A., M. Dicke, and J. Van Loon. 2015. Insects to feed the world. Wageningen Academic Publishers.

van Zanten, H. H., H. Mollenhorst, D. G. Oonincx, P. Bikker, B. G. Meerburg, and I. J. de Boer. 2015. From environmental nuisance to environmental opportunity: house fly larvae convert waste to livestock feed. Journal of Cleaner Production 102: 362-369.

Vijendravarma, R. K., S. Narasimha, and T. J. Kawecki. 2009. Effects of parental larval diet on egg size and offspring traits in Drosophila. Biology Letters 6: 238-241.

Wang, W., W. Zhang, X. Wang, C. Lei, R. Tang, F. Zhang, Q. Yang, and F. Zhu. 2017. Tracing heavy metals in 'swine manure-maggot-chicken'production chain. Scientific Reports 7: 8417.

Yu, G., P. Cheng, Y. Chen, Y. Li, Z. Yang, Y. Chen, and J. K. Tomberlin. 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environmental Entomology 40: 30-35.

Zhang, Z., J. Shen, H. Wang, M. Liu, L. Wu, F. Ping, Q. He, H. Li, C. Zheng, and X. Xu. 2014. Attenuation of veterinary antibiotics in full-scale vermicomposting of swine manure via the house fly larvae (*Musca domestica*). Scientific Reports 4: 6844.

Zurbrügg, C., B. Dortmans, A. Fadhila, B. Verstappen, and S. Diener. 2018. From pilot to full scale operation of a waste-to-protein treatment facility. Detritus 1: 18.

Zurek, K., and D. Nayduch. 2016. Bacterial associations across house fly life history: Evidence for transstadial carriage from managed manure. Journal of Insect Science 16: 2.

APPENDIX A

Figure S.8.1 Canonical discriminant plot for black soldier fly small-scale digestion of dairy, poultry, and swine manure at 29 °C, 60% RH, and 16L:8D.

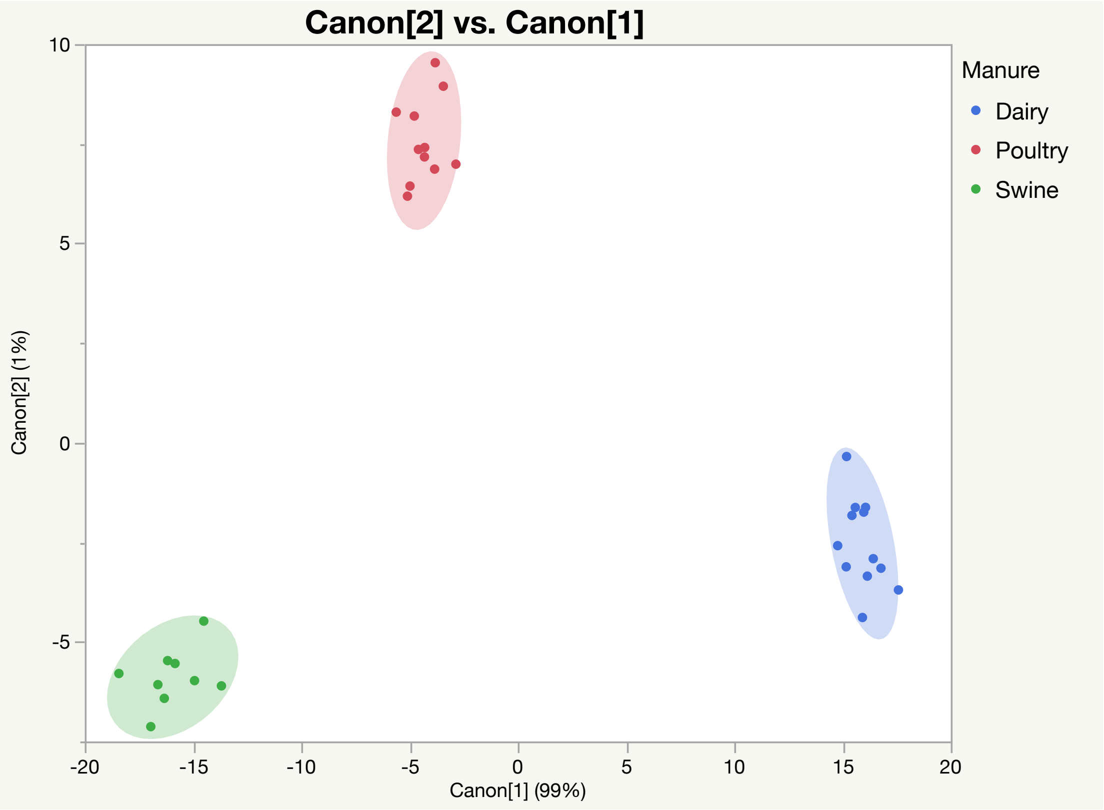


Figure S.8.2 Canonical discriminate plot for black soldier fly large-scale digestion of dairy, poultry, and swine manure at 29 °C, 60% RH, and 16L:8D.

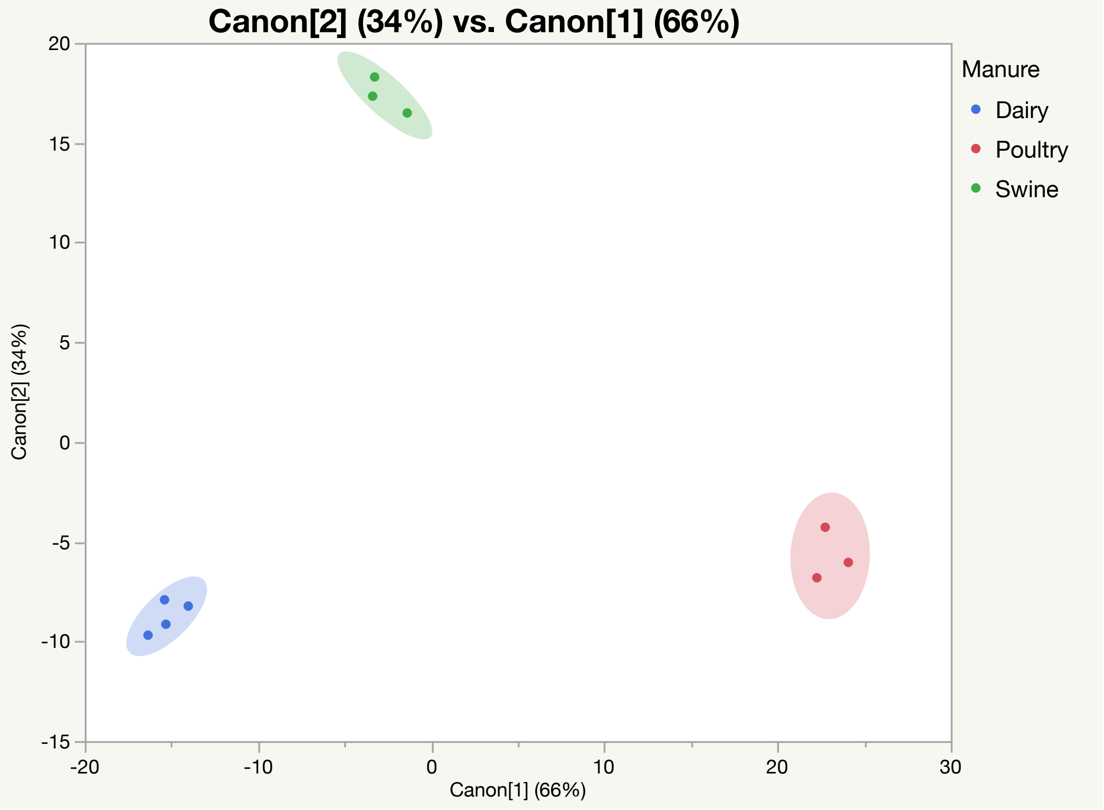


Figure S.8.3 Canonical discriminant plot for house fly small-scale digestion of dairy, poultry, and swine manure at 33 °C, 60% RH, and 16L:8D.

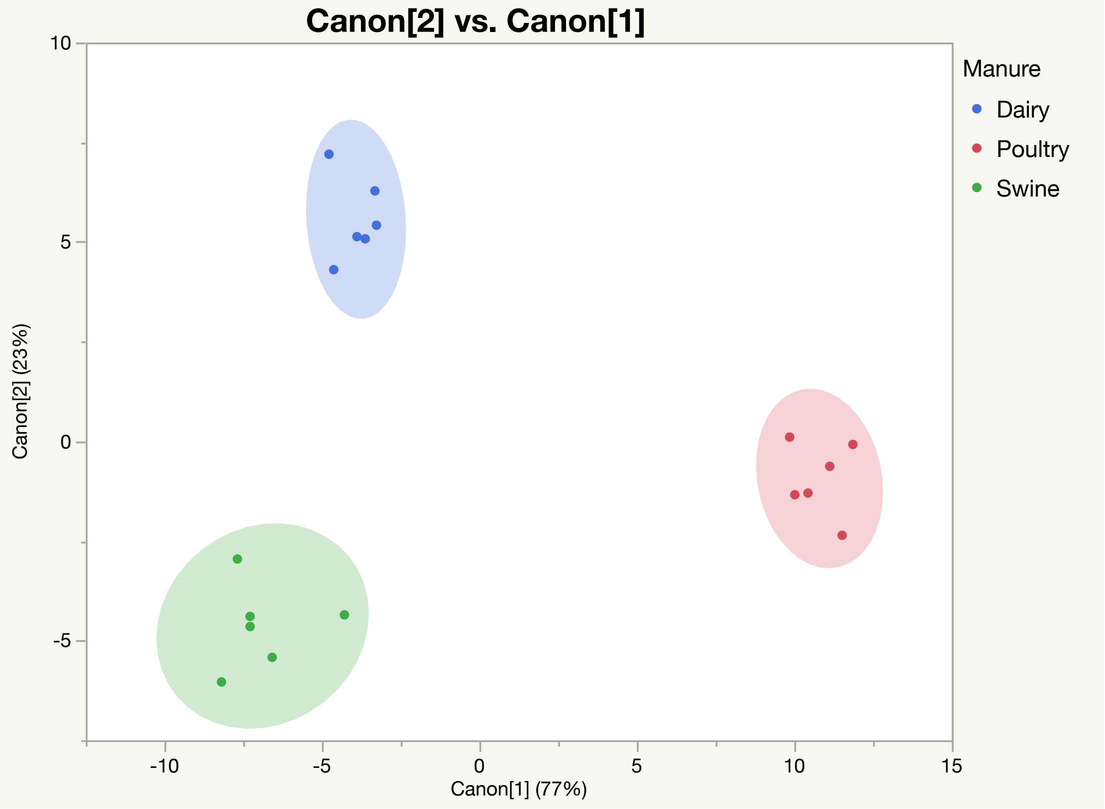


Figure S.8.4 Canonical discriminant plot for house fly large-scale digestion of dairy, poultry, and swine manure at 26 °C, 60% RH, and 16L:8D.