Upcycling of manure with insects: current and future prospects

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Abstract

An unavoidable by-product of any animal production system, be it vertebrate- or invertebrate-based, is the manure generated by the animals themselves. In this review, we focus on the role that insects, particularly the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae), could play in managing the mass amount of manure produced through animal agriculture, and the subsequent commodities that could be generated by such a system. Although the focus of this review is on the black soldier fly, we postulate that other species, including the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) and the house fly *Musca domestica* L. (Diptera: Muscidae) are also well poised to help with the challenge of managing animal manure, while generating products of value.

Keywords: manure management, sustainable agriculture, lesser mealworm, house fly, black soldier fly, circular economy

1. Introduction

The insects as feed industry is positioned to alleviate many of the environmental burdens associated with livestock production, such as diverting plant protein to human consumption, reducing pressure on international fisheries for fish oil and fish meal, land use, and water required to generate protein. Although using insect products as components of livestock feed could allow for more sustainable (i.e. efficient and environmentally-conscious) meat production, one environmental issue that remains is manure management and its associated negative environmental impacts (e.g. runoff, eutrophication, groundwater contamination, greenhouse gas and volatile organic compound emissions, and proliferation of pest species and pathogens) (Adhikari *et al.*, 2005; Beskin *et al.*, 2018; Chang and Janzen, 1996; Cossé and Baker, 1996; FAO, 2006; Kyakuwaire *et al.*, 2019).

Over the past 25+ years, structural changes in animal production across the globe (i.e. reduction in total farm numbers and an increase in the number of animals per farm) have resulted in large quantities of manure in smaller areas, that must be properly managed. For example, from 1970 to 2006, the number of USA dairy farms decreased

by 88%, while average herd size increased six-fold, and milk production twelve-fold (MacDonald *et al.*, 2007). Traditionally, a majority (70% in 1994) of swine produced in the USA was from small operations (<5,000 head/operation). By 2014, large operations (5,000+ head/operation) accounted for 90% of annual production (United States Department of Agriculture, 2015). A similar trend has occurred in the broiler industry. From 1995 to 2010, the number of broiler firms decreased from 55 to 41 (Davis *et al.*, 2013), while chicken meat production increased by 47% (National Chicken Council, 2019b). By 2019, the number of USA broiler firms dropped to 30 (National Chicken Council, 2019a), but chicken meat production increased by almost 19% (National Chicken Council, 2019b). These trends are similar across the globe: in the European Union (EU), an estimated 90% of broilers were produced by large firms in 2018, with production increasing approximately 25% during the previous decade (Augère-Granier, 2019). From 1996-2006 in China, the number of poultry farms decreased by 67%, while production increased by over 75% (FAO, 2008).

The evolution of these industries to larger facilities has amplified concerns regarding waste management, as the volume of manure produced outweighs the amount that

may be utilised as fertiliser on nearby land. While manure is typically considered a valuable resource, like anything else, too much can be a major issue, and current methods of manure management (e.g. lagoons, composting, spreading, etc.) are inefficient, environmentally challenging, and not fully sustainable (Adhikari *et al.*, 2005; Chang and Janzen, 1996; Edmonds *et al.*, 2003; Grossman, 2014; Kyakuwaire *et al.*, 2019; Welch and O'Hagan, 2010). Novel methods to manage manure that decrease the environmental impact and increase the sustainability of animal production are desperately needed; insects are likely ideal candidates for tackling such a global challenge.

2. Insects that consume manure

Although thousands of arthropods feed on animal manures, these relationships have been investigated mainly in natural habitats (Miller, 1954; Mohr, 1943; Nichols *et al.*, 2017) with few species studied for manure management. Dung beetles (Coleoptera: Scarabaeidae) are widely studied for their impacts on manure recycling as related to pasture health (Bertone *et al.*, 2006; Nichols *et al.*, 2008), whereas most fly (Diptera) species associated with manure are typically regarded as pests, and research efforts are typically focused on their control (Axtell, 1986; Barth, 1986; Hall and Foehse, 1980; Sheppard, 1983). While the following three species discussed for manure management are by no means exhaustive, the knowledge-base of these species has them positioned as the most promising fits for this monumental task.

Alphitobius diaperinus

The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is a small, dark beetle, with global distribution. Eggs are deposited in larval resources such as grains, poultry litter, and manure (Rumbos *et al.*, 2019); resulting larvae complete development in 40 to 60 d, while pupae require 7–12 d to develop to the adult stage (Axtell and Arends, 1990). When one considers the insect species frequently recommended for recycling animal manure, or used as protein, the lesser mealworm is not typically highlighted. Worldwide, this species is considered a primary pest of poultry production. Its presence signifies the potential for vectored pathogens (Crippen *et al.*, 2009, 2012), reduced feed conversion by the birds, and damage to poultry houses (Lyons *et al.*, 2017; Tomberlin *et al.*, 2008). Thus, being labelled a pest is not a surprise, especially from the perspective of the poultry producer (Axtell and Arends, 1990).

However, the lesser mealworm might have more to offer than is appreciated (Rumbos *et al.*, 2019). This species is commonly associated with manure, can develop massive populations in poultry facilities, and has a similar nutritional

value to yellow mealworm (*Tenebrio molitor* (L.)), which is currently mass produced worldwide for food and feed (Rumbos *et al.*, 2019). If facilities were designed to manage the population, lesser mealworms could possess the same potential as the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) for recycling manure, while producing protein and associated compost.

Recall, the black soldier fly was considered a pest (Axtell and Edwards, 1970) long before it was recognised as the 'crown jewel' of the insects as feed industry (Tomberlin and Van Huis, 2020). Issues with this species ranged from destruction of poultry houses and inhibition of manure management, to causing myiasis (Tomberlin and Van Huis, 2020). While these are all accurate in terms of problems the black soldier fly can cause when present, harnessing its abilities eliminated these issues. Could the lesser mealworm be on the same path created by the industrialisation of the black soldier fly? Answering that question is challenging given its current pest status and lack of effort investigating its use to recycle waste and produce protein (Rumbos *et al.*, 2019). However, the stage has been set for the industrial development of this species, as the European Union has taken the first step by approving its use as a feed (Leni *et al.*, 2020). And, several studies have examined the nutritional value of this insect as a potential food and feed (Azzollini *et al.*, 2019; Roncolini *et al.*, 2020).

Musca domestica

Another insect known to feed on manure is the house fly, *Musca domestica* L. (Diptera: Muscidae). Owing up to its namesake, the house fly is commonly found in or around homes and is the most widely distributed insect, colonising every location where humans and domestic animals occur (Keiding, 1986). As such, its biology has been well described in an effort toward control. House flies fed milking calf, swine, or poultry manure can develop to pupae in as little as 6 d (Larrain and Salas, 2008), but manure type influences their performance and fitness (Khan *et al.*, 2012; Larrain and Salas, 2008). Still, the house fly can consume manure from a variety of animals, including those produced from concentrated animal facilities (e.g. swine, dairy, and poultry) (Miranda *et al.*, 2020a), which makes them a suitable candidate for manure management.

House flies can degrade large volumes of manure in a short amount of time. Compared to the lengthy time necessary for traditional composting, house fly larvae, depending on the density, have been demonstrated to digest over 100 kg of swine manure in one week (Zhang *et al.*, 2012) and over 400 kg of swine manure in two weeks (Čičková *et al.*, 2012). As larvae feed on manure, reductions in odours (Wang *et al.*, 2013) and moisture (Miller *et al.*, 1974; Wang *et al.*, 2013) occur. These reductions can be attributed to changes in

the microbial community, but the presence of house flies is also beneficial as they mechanically aerate the substrate, allowing the environment to remain aerobic for increased loss of gases and water (Beard and Sands, 1973). House flies also reduce antibiotic resistant genes in swine manure and in doing so, provide a biofertiliser that is less of a threat to the environment than untreated manure (Wang *et al.*, 2015, 2017). Additionally, the presence of house fly larvae can reduce swine manure mass (wet weight) by up to 70% (Wang *et al.*, 2013), dry matter by 31–35% (Miranda *et al.*, 2020a; Wang *et al.*, 2013) and nitrogen and phosphorous by up to 78% and up to 30%, respectively (Wang *et al.*, 2013). The extent of total mass reductions (wet weight) for dairy and poultry manure by house flies on a full scale is not known as it is for swine manure, but house flies can reduce dry matter in dairy and poultry manure on a small scale by up to approximately 20 and 40%, respectively (Miranda *et al.*, 2020a).

House flies reduce nitrogen and phosphorous in dairy and poultry manure as well. For example, Hussein *et al.* (2017) determined house flies reduce nitrogen in dairy manure by 25% and phosphorous by 6% on a dry matter basis. In poultry manure, house flies reduced nitrogen from 7.5 to 2.6% and phosphorous from 3.4 to 1.9% on a dry matter basis (Teotia and Miller, 1974). At the same rate, the biomass generated from house flies fed manure is valuable as lipids extracted from larvae can be processed for biofuel (Zi-zhe *et al.*, 2017).

Part of the challenge in using the house fly for waste management is that it is considered a pest, and like the lesser mealworm, is a vector of numerous pathogens (Keiding, 1986). There are multiple ways in which house flies harbour and disseminate pathogens. Since larvae partially rely on bacteria for development (Zurek *et al.*, 2000), all life stages are associated with microbe-rich habitats. As larvae develop, some harmful bacteria may remain within the gut and can be carried through subsequent life-stages (Zurek and Nayduch, 2016). As an adult, they must feed to mature reproductively (Morrison and Davies, 1964) and so pathogens may be spread via regurgitation and excretion. Sasaki *et al.* (2000) showed *Escherichia coli* O157: H7 remained in the adult house fly crop for 4 d and was excreted for 3 d after feeding on contaminated food (Sasaki *et al.*, 2000). Pathogens can also be disseminated by adults through mechanical contact via their tarsi (Wasala *et al.*, 2013). Adult house flies are opportunistic and will move indiscriminately among locations in search of food or a place to oviposit. Yet, despite their behaviour and pest status, house flies offer a promising solution to waste management because of their ability to digest manure in a short amount of time.

Hermetia illucens

Compared to the previous insects discussed, the black soldier fly is currently the best insect candidate for manure management. Found in temperate and tropical regions throughout the world, the black soldier fly is now viewed as a non-pest species and is highly regarded due to the wide range of wastes it can digest (Alyokhin *et al.*, 2019; Lalander *et al.*, 2019; Nguyen *et al.*, 2013). The life cycle of the black soldier fly takes approximately 40–43 d when fed an artificial diet, with the larval stage lasting 22–24 d (Cammack and Tomberlin, 2017; Tomberlin *et al.*, 2002), which is nearly twice as long as the entire house fly life cycle. However, black soldier fly larvae are larger, capable of consuming more resource (Čičková *et al.*, 2015), and possess a more diverse array of digestive enzymes when compared to house fly larvae (Kim *et al.*, 2011b).

Research regarding black soldier fly larvae fed manure has largely focused on dairy, swine, or poultry manures (Diener *et al.*, 2009; Miranda *et al.*, 2019; Oonincx *et al.*, 2015a; Zhou *et al.*, 2013). All three manure types are suitable for their development; however, poultry manure appears to be optimal (Miranda *et al.*, 2019; Zhou *et al.*, 2013), likely because it has higher nutrient concentrations than the other manure types (Chen *et al.*, 2003). Blending waste streams can increase the digestion and subsequent larval yield. This is particularly effective when feeding lower quality manure types, such as dairy manure. For example, feeding black soldier fly larvae a mixture of dairy and poultry manure (40 and 60%, respectively) decreased development time by 2 d, increased survivorship by 6%, and increased larval weight by ~75% when compared to larvae fed dairy manure alone (ur Rehman *et al.*, 2017a). Similarly, other wastes (e.g. kitchen wastes, poultry slaughterhouse waste, fish offal, and human excrement) can be mixed with cow manure to improve black soldier fly survivorship, weight gain, and dry matter reduction (Gold *et al.*, 2020; St-Hilaire *et al.*, 2007). Black soldier fly digestion of manure also results in reduced production of odorous volatile organic compounds (Beskin *et al.*, 2018), further contributing to their positive impact on the manure management system.

Currently, there is little information available on large-scale digestion of manure by black soldier fly larvae. Sheppard *et al.* (1994) developed a system where the innate behaviour of the black soldier fly larvae and prepupae could be harnessed to collect larvae produced from poultry layer manure that accumulated beneath the birds, and Newton *et al.* (2005) modified this system to manage manure generated from swine production (Newton *et al.*, 2005; Sheppard *et al.*, 1994). Industrialisation of the black soldier fly on different wastes certainly exists across the globe, but most research available on this species is studied on a small/ laboratory scale (hundreds of larvae fed grams of substrate). To our

knowledge, there is only one study that has examined the development of black soldier fly larvae fed manure on a large scale (10,000 larvae fed 7 kg of swine, dairy, or poultry manure) (Miranda *et al.*, 2020b). Despite the lack of information available on large-scale production of black soldier fly larvae fed manure, there is great potential for this species to serve as a waste management agent. Given their voracious appetite for all things decomposing, biomass accumulation, literature availability, and the current global focus of mass production, the remainder of this manuscript will focus on using the black soldier fly for manure management.

3. Microbiology, black soldier fly, and manure

The increasing human population has led to more intensive livestock farming, and with this comes concern about pathogens, antibiotics, and other pharmaceutical residues in animal manure shed into the environment with potential for increased accumulation of antibiotic resistant bacteria or human allergies from environmental exposure. Several pathogens comprising bacteria, viruses, and protozoan species, naturally occur in animal manure and under certain circumstances may pose a risk to human health. However, owing to differences in microbial characteristics between and within these groups of organisms, pathogen survival in the environment or manure depends on the characteristics of the particular organism, the source, and chemical composition of manure (e.g. ammonium content), pH, dry matter, temperature, oxygen, microbial competition, and moisture of these materials (Haruta and Kanno, 2015; Manyi-Loh *et al.*, 2016; Sheng *et al.*, 2019). Bacterial pathogens that are most often associated with manure with regard to human health include *Salmonella* spp., *E. coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Clostridium perfringens*, some of which may be widely distributed in the environment and include over 2000 species and strains (Manyi-Loh *et al.*, 2016; Sobsey *et al.*, 2006). These bacterial pathogens may persist for long periods in animal manures under typical farm conditions. This may be extended when the temperatures are low, moisture remains optimal, and aeration is not used. For instance, *E. coli* O157:H7 survived for 4–6 months in animal manures and slurries kept at 1–9 °C, 49 times longer than if kept at 40–60 °C (Kudva *et al.*, 1998). *Salmonella enterica* serovar Typhimurium was also found to survive best at 5 °C for up to 40 d, compared to 15 and 25 °C (Garcia *et al.*, 2010). Other studies have shown that aeration of solid manure decreased survival times for *E. coli* O157:H7 and *Salmonella* by as much as 88%. The persistence of *L. monocytogenes* for several weeks in manure-amended soil, suggests it could be transmitted through soil, especially during the cold months (Jiang *et al.*, 2004). Survival of protozoa in animal manures may also be related to temperature, but the trends are not as prominent

as those reported for bacterial pathogens (Olson, 2020; Olsen *et al.*, 1999).

Composting manure significantly reduces the risk of exposure to these pathogens, as the high temperatures (≥ 54 °C) achieved during the process kills many pathogens and parasites. However, because of the pathogen diversity in manure, composting may not kill all pathogens, particularly those capable of forming spores or surviving in biofilms (Manyi-Loh *et al.*, 2016; Stanford *et al.*, 2015; Usui *et al.*, 2017). Hence, multiple manure management practices should be used.

Insects, such as the lesser mealworm, house fly, or black soldier fly, may suppress many of these pathogens given their coprophagous nature. The larvae aerate the manure and also promote the growth of beneficial saprophytic microbes (Sarpong *et al.*, 2019). And, as previously mentioned, their larvae may reduce or eliminate bacteria through direct ingestion and digestion or through the generation of antimicrobial compounds.

Lab studies show black soldier fly larvae reduce pathogens in manure

One of the first studies determining the ability of black soldier larvae to reduce manure-associated pathogens was in 2004, when Erickson *et al.* inoculated gfp-labelled *E. coli* O157:H7 and gfp-*Salmonella enterica* serovar Enteritidis into dairy, swine, and poultry manure (Erickson *et al.*, 2004). They determined black soldier fly larvae significantly accelerated *E. coli* inactivation in poultry manure, while showing no effect in dairy manure. On the other hand, *E. coli* growth accelerated in swine manure. Storage conditions and pH likely impacted pathogen populations and black soldier fly antibacterial activity within the differing manure substrates. Larval feeding also reduced *Salmonella* in poultry manure, but the pathogen accumulated in the larvae. From these data, they speculated larval waste accumulation and increased moisture in older manure likely led to reduced larval feeding, and a decrease in *Salmonella* Enteritidis inactivation (Erickson *et al.*, 2004).

A study investigating the reduction of *E. coli* in manure was conducted in 2008 (Liu *et al.*, 2008), when gfp-*E. coli* was inoculated into 50–125 g sterile dairy manure with or without black soldier fly larvae for 72 h at 23, 27, 31 or 35 °C. Black soldier fly larvae significantly reduced *E. coli* counts in all manure weight treatments, but the capacity to reduce *E. coli* populations was temperature dependent, with greatest suppression occurring at 27 °C. Additionally, pathogen accumulation within the larvae was not determined.

Lalander *et al.* (2015) measured the effects of black soldier fly larvae feeding on the concentration of *Salmonella* spp.,

Enterococcus spp., the ΦX174 bacteriophage, and the parasite *Ascaris suum* in human faecal waste, and whether these accumulated in, or on, the prepupae (Lalander *et al.*, 2015). *Salmonella* spp. count in the waste was reduced by $6\log_{10}$ in eight days, compared with a $<2\log_{10}$ reduction in the control. However, they found no significant reduction of *Enterococcus* spp., bacteriophage ΦX174 or *A. suum* ova associated with larval feeding. In contrast to Erickson *et al.* (2004), the *Salmonella* spp. concentration was 10^5 cfu/ml at the start of the experiment but decreased to below the detection limit in both the waste and prepupae as the experiment progressed, even though freshly inoculated material was continuously added. Concentrations of *Enterococcus* and bacteriophage ΦX174 remained between 10^5 and 10^6 cfu/ml in larval guts. Also, there was an overall lower concentration of organisms found inside the gut of the prepupae compared with the gut of the larvae, suggesting that the prepupae empty their gut prior to dispersal (Lalander *et al.*, 2015). These studies of pathogen inoculation have led to data supporting the utilisation of black soldier fly larvae for waste management. However, more studies are needed to determine industrial scale applications, as well as the accumulation of these and other manure-associated pathogens throughout black soldier fly life stages, and whether transmission occurs between generations. Research into the means to achieve absolute pathogen removal from the system when using insects to digest waste should also be considered.

For instance, it is well known that insect endosymbionts can confer ecologically relevant traits and contribute to the fitness of their host through outcompeting pathogens (Eleftherianos *et al.*, 2013; Kaltenpoth, 2009; Kaltenpoth *et al.*, 2005; Paniagua Voirol *et al.*, 2018). Symbiotic bacteria often facilitate degradation of complex macromolecules for utilisation and increasing nutrient quality for the insect. Yu *et al.* (2011) measured black soldier fly larval growth and development when fed poultry manure inoculated with four *Bacillus subtilis* strains isolated from black soldier fly larval guts or from feed. They found that bacterial amendment increased larval growth while shortening the development time. Mazza *et al.* (2020) explored the effect of companion bacteria when poultry manure was converted to insect biomass. They isolated nine bacterial species from black soldier fly eggs and larval gut and inoculated them into poultry manure along with black soldier fly larvae. Results indicated that black soldier fly larvae reared in manure with *Kocuria marina*, *Lysinibacillus boronitolerans*, *Proteus mirabilis* and *B. subtilis*, alone or in combination, had higher weight gain and protein content and increased manure reduction rates compared to the control (Mazza *et al.*, 2020). Both of these studies showed that bacterial amendment into the manure was beneficial with respect to black soldier fly larval development and manure reduction. This nutrient provisioning with probiotics, along with

natural mechanisms of pathogen reduction within the black soldier fly can be exploited and represent a promising strategy for manure reduction, with potential for pathogen control.

Feeding black soldier flies to livestock reduces gut pathogens

Another avenue of research has been the investigation of insects used as feed to reduce pathogens in the digestive tract of traditional livestock through modulation of gut microbiota and their metabolites. For instance, Park *et al.* (2017) showed that laying hens fed black soldier fly larval meal had higher *Lactobacillus* counts, but coliforms, total aerobic bacteria, and *E. coli* were lower than in those not provided the insect-based diet. Another study by Kim *et al.* (2020) found that laying hens fed diets containing black soldier fly oil lowered *C. perfringens* counts in the ileum, but not cecum samples, by 13.7% at 30 d, indicating moderate, although nonsignificant, antimicrobial activity of black soldier fly larvae oil in broilers.

Borrelli *et al.* (2017) used 16s rRNA gene sequencing to investigate the effect of black soldier fly larval meal on gastrointestinal microbiomes and bacterial metabolite production in laying hens. Results showed strong differences between caecal microbiota of soybean and black soldier fly meal fed groups, both in type and relative abundance of microbial species. They found microbial DNA signatures with potential to degrade the insect meal that correlated with high levels of gut-associated short chain fatty acid (SCFA) production. The authors suggested the increase in SCFA concentrations may result from modulation in the microbial population induced by the insect-based diet and may be associated with SCFAs derived by microbial degradation of the insect meal. Because SCFAs regulate bacterial pathogenesis (Sun and O'Riordan, 2013, and references therein), they speculated the possible beneficial effects of a black soldier fly larval meal diet (Borrelli *et al.*, 2017). Though further studies are necessary to determine the impact of this feeding strategy on human and animal physiology and long-term health, these studies suggest that feeding animals with black soldier fly larvae meal or oil modulate gut microbes, and merits further investigation into the potential for pathogen reduction in resulting manure.

Larval black soldier fly production of antimicrobial peptides

Antimicrobial peptides (AMPs) are short and generally positively charged peptides with the ability to kill microbial pathogens directly, or indirectly by modulating host defences. These peptides are found in a wide variety of life forms from microorganisms to humans and are also part of

the innate immunity of insects (Sierra *et al.*, 2017; Wu *et al.*, 2018). These insect peptides exhibit antimicrobial activity through disruption of pathogen membranes and have been associated with lower levels of antimicrobial resistance (Wu *et al.*, 2018). As a result, insects have been surveyed for novel AMPs, and several studies have shown that black soldier fly larvae could be a rich source. Elhag *et al.* (2017) identified seven gene fragments of three types of AMPs in black soldier fly larvae. One gene, named *stomoxynZH1*, was shown to have diverse inhibitory activity on *Staphylococcus aureus*, *E. coli*, fungus *Rhizoctonia solani* Kühn (rice)-10, and fungus *Sclerotinia sclerotiorum* (Lib.) (Elhag *et al.*, 2017).

In another study, Vogel *et al.* (2018) fed black soldier fly larvae diets supplemented with four inoculated bacterial mixtures and variable organic plant waste and measured the immune related transcriptome. The authors identified 53 genes encoding putative AMPs, including 26 putative defensins, from the black soldier fly transcriptome. Larval protein and lipid extracts also showed a broad range of antibacterial activity. And, Alvarez *et al.* (2019) isolated peptides from *E. coli* challenged and control black soldier fly larvae, and tested their anti-*Helicobacter pylori* activity. The authors found over 90 peptides in their analysis, four of which they determined to have pronounced anti-*H. pylori* activity. But only larvae injected with *E. coli* were found to have anti-*H. pylori* antimicrobial peptides, suggesting that these peptides were produced in response to *E. coli* challenge (Alvarez *et al.*, 2019).

These studies showed that AMP activity was dependent on diet and associated with bacterial loads within the diet. Additionally, immune challenge was induced with a needle prick, with AMP activity measured between 24–72 h post infection (Alvarez *et al.*, 2019; Elhag *et al.*, 2017; Vogel *et al.*, 2018). It would thus be interesting to measure AMP activity of black soldier fly actively feeding on animal manures versus a control. This would allow measurement of black soldier fly AMPs under constant exposure to a wide array of microbial pathogens.

Black soldier fly larvae degrade pharmaceuticals and reduce antibiotic resistance genes in manure

Antibiotic residues are frequently detected in livestock wastes, where mechanisms of dissemination into the environment may be through the direct spreading of manure onto fields, processed in lagoon slurry, or spreading solids for livestock bedding (Wallace *et al.*, 2018). Often, antibiotic residues have been detected at levels much higher (as much as 28,000 times) than in background soil or water (Chee-Sanford *et al.*, 2009; Sim *et al.*, 2011). And, though the abundance of antibiotic residues in livestock waste varies among livestock farm types, and regional regulations, the most frequently detected antibiotic residues include those

from the fluoroquinolone, sulphonamide, and tetracycline drug classes, though other classes such as trimethoprim and macrolides have also been detected (Chee-Sanford *et al.*, 2009; Prosser and Sibley, 2015; Wallace *et al.*, 2018; Yuan *et al.*, 2019). While further research needs to be conducted to determine the full impact on human, animal, and ecosystem health, there is general agreement within public and private entities that mitigation strategies should be implemented to prolong the effectiveness of currently used antibiotics while lessening the dissemination of antibiotic resistant bacteria and genes (Cabajal *et al.*, 2017; CDC, 2015; EU, 2016; World Health Organization, 2015).

A recent and exciting finding is the black soldier fly larvae-mediated degradation of pharmaceuticals. A study showed black soldier fly larvae degraded three pharmaceuticals (e.g. carbamazepine, roxithromycin and trimethoprim) and two pesticides (e.g. azoxystrobin and propiconazole) in a composting system, with no bioaccumulation within the larvae detected (Lalander *et al.*, 2016). Recently, Cai *et al.* (2018b) showed that black soldier fly larvae can degrade tetracycline. Further, the authors found that intestinal bacterial and fungal communities were significantly modified. They also detected an increase in *tet* gene copy number (tetracycline resistance gene) in the gut microbial community, suggesting gut microbes increased tetracycline tolerance by black soldier fly and facilitated tetracycline biodegradation. Their analyses suggested synergistic mechanisms of tetracycline degradation between intestinal microbes and black soldier fly larvae including hydrolysis, oxygenation, deamination, demethylation, ring-cleavage and modification, where degradation products could serve as nutritional resources (Cai *et al.*, 2018b). As a follow-up to this work, Cai *et al.* (2018a) investigated the impact of larval sterility on the bioconversion of poultry manure and the persistence of associated antibiotic resistance genes (ARGs). The authors used qPCR targeting 11 tetracycline resistance genes and 2 integrase genes and found that non-sterile black soldier fly larvae reduced ARGs by 95%, versus 88.7 and 48.4% in sterile larvae and controls, respectively. This significant difference between treatments suggested a synergistic effect between intestinal microbes and black soldier fly in ARG reduction mechanisms. They also showed a shift in the microbial community, with decreases in human pathogens within the waste by 70–92%. Furthermore, they found that environmental factors that were altered by larval activity, such as pH and nitrogen content, influenced bacterial community composition, and thus the persistence of ARGs (Cai *et al.*, 2018a).

Overall, these results are promising for developing strategies for accelerating the degradation of antibiotics by adjusting the intestinal microbiota of black soldier fly larvae. But, though encouraging, these studies only targeted a few ARGs for detection, limiting elucidation of the impact of black soldier fly larvae and associated microbes on a broad

range of ARGs and mechanisms. And recently, Gao *et al.* (2019) measured life histories of 4-day old black soldier fly larvae fed with diet supplemented with sulphonamides (e.g. sulphamonomethoxine, sulfamethoxazole, sulfamethazine, and sulfadiazine) in 0.1, 1 to 10 mg/kg concentrations, comparing to unsupplemented controls. Results showed that 0.1 and 1 mg/kg concentrations had no effects on black soldier fly larval survival, pupation, or eclosion. However, 10 mg/kg sulphonamide concentrations had a significantly negative impact on larval and pupal survival, body weight, pupation, and eclosion. Sulfadiazine was also detected in prepupae originating from larvae with feed supplements of 1 and 10 mg/kg, though at lower than input supplement concentrations (0.4663 and 0.7814 mg/100 larvae, respectively). These data indicate that black soldier fly larvae can reduce some sulphonamides, but depending on the concentration, may be detrimental to black soldier fly growth and development, while harbouring residues through the prepupal stage. These data underscore the importance of caution when generalising data across all life stages and antibiotic classes (GAO *et al.*, 2019).

Research on larvae raised on a variety of animal manures with or without antibiotic supplementation, and identifying key pathogens (both naturally occurring and intentionally inoculated), their frequency of occurrence, and expression of ARGs and virulence factors in both the digested manure and black soldier fly larvae are valuable. Such approaches will allow for an assessment of the utility of black soldier

fly as a method to reduce antimicrobial agents and related compounds. Data may also allow for industrialisation of such processes that mitigate emergence, spread, or persistence of antibiotic resistant pathogens in the animal waste residue produced. Furthermore, these data would be useful for the federal government when reviewing current regulations on the use of insect protein as feed.

4. Black soldier fly bioproducts for the future: an example of the potential for other insect species

The black soldier fly system offers an innovative option for managing animal wastes that can be translated into widespread industrial use, transforming low value waste into high value, upcycled products (Figure 1), while also producing protein for use as livestock feed. Black soldier fly products for animal feeds (as whole insects, protein meals, fats, etc.) are currently the main (and most well-known) outputs when the black soldier fly is used for waste management. However, we recognise that until the biosafety of products generated from manure management can be ensured through continued research on the topic, in addition to the stigma associated with such a process, products generated from manure management should not enter the food stream and should be as purified as possible. This system would be useful worldwide to support circular economies in rural and urban locations worldwide, and even low-earth-orbit environments.

Figure 1. A diagram of the potential products and benefits that could be generated by processing animal manure with black soldier fly larvae.

Biodiesel

Black soldier fly larvae are rich in many nutrients, especially fats and fatty acids, making them ideal candidates for renewable sources of biodiesel (Zheng *et al.*, 2012). The high ratio of medium chain saturated to polyunsaturated fatty acids makes larval black soldier fly fats ideal for producing biodiesel (Surendra *et al.* 2016). Li *et al.* (2011) were the first to investigate the conversion of dairy manure into biodiesel. At their laboratory scale, approximately 1.25 kg of manure yielded approximately 16 g of biodiesel. Although this resulting amount (1.26%) might seem small, at an industrial scale, this translates to a massive amount of biodiesel. For example, the average dairy cow produces approximately 36 kg of manure per day per 450 kg of weight (United States Department of Agriculture, 1995). Consider, an average weight (680 kg) mature Holstein cow produced approximately 54 kg of manure per day. If industrialised, such waste could be transformed to 0.68 kg of biodiesel. A black soldier fly facility co-located with a dairy farm with 2,000 head of cattle could produce 1,360 kg of biodiesel/day. At a density of 0.88 kg/l (Alleman *et al.*, 2016), this equates to approximately 1,550 l of biodiesel/day. At Huazhong Agricultural University in Wuhan, China, a biogas plant mixes agricultural waste streams (i.e. corn cobs and swine manure) to optimise energy yield (Li *et al.*, 2015). A 3:2 ratio of volatile solids was found to yield the most biogas, and the resulting residue investigated for biodiesel production. Bioconversion of this residue by black soldier fly larvae yielded 0.79 g biodiesel per 100 g of residue. The authors extrapolated from their study that the amount of corn cob and manure needed to produce 1 metric ton of biodiesel could come from 16.78 hectares of corn and 387 head of swine per year. As another example, in June 2019, an estimated 75.5 million pigs were being raised in the USA (Barrett, 2019). If pig wastes were co-digested with corn residue for biogas production, and the biogas residue subsequently digested by black soldier fly larvae, the manure generated from US swine production could yield 195,090 metric tons (or approximately 172 million litres) of biodiesel annually. As of April 2020, China was estimated to produce approximately 2.6 times more pork than the USA, and 1.4 times more than the EU in 2020 (Shahbandeh, 2020). The amount of biodiesel that could result from swine production alone could be massive; the aforementioned volume of 172 million litres could replace approximately 1% of the total diesel used in the USA in 2019 (United States Energy Information Administration, 2020).

Microbiological products

Products from the black soldier fly larvae could result in a thriving industry to supply broad needs in waste management, agriculture and home garden protection, pharmaceuticals, and remediation of industrial biofouling. Resulting black soldier fly-derived products, such

as antimicrobial peptides, probiotics and prebiotics, represent promising strategies for selective microbial control in manure management. But these products may also offer additional practical benefits to society, such as antimicrobials in treating infections or in reducing biofouling. Some of these alternatives have been reported to embody most or all of the essential antibiotics' functions, while also being less likely to induce bacterial resistance (Alvarez *et al.*, 2019; Elhag *et al.*, 2017; Vogel *et al.*, 2018; Wu *et al.*, 2018). As discussed above, research has showed that a black soldier fly diet and associated bioproducts modify gut microbial communities, modulate immune systems, reduce or inhibit pathogens, and establish a favourable gut microbiome. Thus, the continued identification of novel black soldier fly-associated microbial strains could lead to the production of a microbial cocktail for use as a manure pretreatment for more efficient waste conversion to black soldier fly protein and lipids. And, as microbial amendment to manure reduced black soldier fly development time (Yu *et al.*, 2011), this application would have a direct impact on the productivity of black soldier fly in industrial applications of waste management. Furthermore, antimicrobial peptide testing will determine if any of the isolated AMPs have activity against other pathogens, or with microbial signalling or biofilm development. Indeed, research conducted thus far has yielded encouraging data that compel continuation into the investigation of black soldier fly bioconversion of animal manure, for optimisation aiming to ensure animal, food and environmental safety, while producing products of quality and environmental value.

The antimicrobial properties of insect haemolymph have been known and used for many years in traditional medicine (Seabrooks and Hu, 2017). It is not surprising, then, that the AMPs produced in black soldier fly larval haemolymph, excretions, and secretions are attractive options for antimicrobial therapeutics; in fact, they have been shown to have comparable, synergistic, or improved activity over traditional antibiotics against important human pathogens, as described above (Seabrooks and Hu, 2017; Wu *et al.*, 2018). Antimicrobial effects by black soldier fly larval extracts have also been demonstrated toward *E. coli*, *Micrococcus luteus*, *Pseudomonas fluorescens* and *B. subtilis* biofilms (Muller *et al.*, 2017; O'Toole and Kolter, 1998), and also against plant pathogens (Park *et al.*, 2015). These data are encouraging in that black soldier fly AMPs could be suitable as alternative or a combined therapy against a broad array of plant and animal infections, with potential to reduce antimicrobial resistance, with additional application for bioremediation and biofouling control. But, the concentrations of AMPs needed for clinical trials and other large-scale applications may be an obstacle for bringing black soldier fly AMPs to market. Other obstacles, such as potential cytotoxic, immunogenic, and allergenic reactions must also be considered. Nevertheless, continued investigations are merited in that black soldier fly mass

production may be beneficial to produce desired AMP concentrations, as would be synthesising AMPs and optimising AMP properties.

Fertiliser

Once digested by black soldier fly larvae, the material remaining (frass) is lower in moisture content, mass, and nutrient content, making it more suitable (both economically and environmentally) for land applications as a fertiliser than the raw manure itself. Black soldier fly larvae are capable of reducing manure mass and nutrients in manure (Matos *et al.*, 2020). In dairy, poultry, and swine manure, black soldier flies reduce dry matter by 26–78% (Li *et al.*, 2011; Myers *et al.*, 2014; ur Rehman *et al.*, 2017b), 36–60% (Lalander *et al.*, 2019; Xiao *et al.*, 2018), and 33–56% (Miranda *et al.*, 2019; Newton *et al.*, 2005), respectively. Furthermore, black soldier fly larvae also reduce numerous nutrients that could otherwise be dangerous to the environment if overapplied as fertiliser. In dairy, poultry, and swine manure, nitrogen is reduced by 6–50% (Liu *et al.*, 2019; Myers *et al.*, 2014), 14–82% (Liu *et al.*, 2019; Oonincx *et al.*, 2015a), and 13–37% (Lalander *et al.*, 2015; Liu *et al.*, 2019), respectively. In dairy manure, phosphorus content is reduced by 61–70% (Myers *et al.*, 2014), and when reared on swine manure, phosphorus is reduced by 44%, respectively (Newton *et al.*, 2005). Though, Oonincx *et al.* (2015a) and Liu *et al.* (2019) found phosphorous increased over time for all three manure types; therefore, variation across studies may be due to differences in methodologies. Metals such as zinc, iron, and copper are also reduced by 26–85% in dairy, swine, and poultry manure (C.D. Miranda, T.L. Crippen, J.A. Cammack, and J.K. Tomberlin, unpublished data). Unfortunately, few studies exist on the utility of black soldier fly larval frass as a fertiliser, and even fewer studies used animal manure as the starting material. Zhu *et al.* (2012) found that black soldier fly larval frass produced from swine manure resulted in faster germination of cucumber and Chinese cabbage seeds in comparison to traditionally composted swine manure. Although the data are promising, more research is needed to understand the full potential and limitations of black soldier fly digested animal manure as a fertiliser.

Other direct black soldier fly products

Bioactive products from the black soldier fly could also be used as a green technology in pest management. As many organisations and individuals are turning more toward integrated pest management and practices, and organic farming, the black soldier fly and its products may be an attractive resource. For instance, black soldier fly larvae produce allomones that are repellent to house flies, resulting in the reduction in house fly occurrence and subsequent oviposition (Bradley and Sheppard, 1984; Sheppard, 1983). Farming the insect for integrated pest management would

provide a means to aid in controlling house fly populations. However, the studies showed that the time black soldier fly larvae occupied the manure influenced house fly ovipositional responses (Bradley and Sheppard, 1984), suggesting that a constant black soldier fly population might need to be in place for efficient housefly reduction. Further, geographical ranges of black soldier flies and systematics of adaptation and genetics of the pests need to be considered for targeted or broad use. For these reasons, isolation of the allomones would seem to be a more beneficial approach.

Black soldier fly larvae also produce trypsin and chymotrypsin, digestive enzymes responsible for protein break down through interactions with targeted amino acids (Kim *et al.*, 2011a,b; Park *et al.*, 2012). These proteases are utilised commercially for scientific research to understand protein structure and function, but also in broad food processing to improve the functional and nutritional quality of food proteins, as ingredients in detergents, in the pharmaceutical industry as therapeutic agents, and for use in bioremediation (Shah and Mital, 2018; Yu and Ahmedna, 2012; Zhou *et al.*, 2011). Commercial sources for proteases, such as trypsin and chymotrypsin, include plants, animals, and microbes. But several factors such as the availability of plants and animals for protease retrieval, land for cultivation and growth, climatic conditions, and agricultural policies, could restrict commercial production of these resources. Furthermore, isolation from microbes would also require sophisticated equipment and skills to prevent contamination. Mass production of insects, such as the black soldier fly, that would result in protease isolation is an interesting alternative to the previously mentioned sources, due to the ease of rearing and reduced space and nutritional needs. Additionally, the wide range of substrates used by black soldier fly could be exploited for those diverse protease commercial applications. However, the full scope of proteases and other biologically active enzymes in black soldier fly larvae, their activities, substrate specificity, pH tolerance, and thermotolerance is lacking. These, along with bioengineering for a robust yield, will need to be solved before these black soldier fly enzymes could be utilised commercially.

Individual components

In contrast to the old adage, we speculate the sum of the parts is greater than the whole especially as related to the black soldier fly. Black soldier fly larvae are quite nutritionally rich, especially at the amino acid level. Compared to soybean meal, black soldier flies contain higher levels of alanine, methionine, histidine, and tryptophan (Barragan-Fonseca *et al.*, 2017). Black soldier fly larvae are also high in fatty acids (Oonincx *et al.*, 2015b) particularly lauric acid, which has known antiviral and antibacterial properties (Gasco *et al.*, 2018). However, as mentioned above, techniques for the isolation and extraction of these

compounds must be available and affordable before the full potential of insects and the associated products can be fully appreciated. Further research is needed to identify the processes (both the order/sequence and extraction/purification techniques) that could be implemented to yield the most benefits from the insect biomass. For example, once the insect biomass is dried, oil can be extracted for use as biodiesel, or further fractionated into specific fatty acids. Additionally, provided that the drying or subsequent extraction techniques do not damage the bioproducts of interest, amino acids, chitin, antimicrobial peptides, and/or enzymes could be subsequently extracted from the defatted biomass.

5. Conclusions

Using black soldier fly larvae for manure management is a win-win across the board. Their digestion reduces biomass, nutrients, odorous volatile organic compounds, and pathogens, while creating numerous products of value that can have both direct and indirect positive impacts on agriculture. Imagine a society where we could truly come full circle: brewery waste, post-consumer food waste, or food processing by products, are diverted to a black soldier fly farm, where it is upcycled by the larvae into feed for chickens. The resulting poultry manure is diverted to yet another black soldier fly farm, where it is upcycled by the larvae into purified proteins and amino acids for aquaculture feeds, and oil for biodiesel that powers the trucks transporting the materials. The frass from both black soldier fly farms is used as a soil amendment in crop production, and used to grow more corn and soybean, which can now be diverted to produce food for people. The possibilities of such a circular economy are endless. Regardless, black soldier fly data to date demonstrate what can be done with insects that digest manure. The question remains: will the lesser mealworm, house fly, and other insects be optimised and embraced for such abilities?

Conflict of interest

The authors declare no conflict of interest.

References

Adhikari, M., Paudel, K.P., Martin Jr, N.R. and Gauthier, W.M., 2005. Economics of dairy waste use as fertilizer in central Texas. *Waste Management* 25: 1067-1074. <https://doi.org/10.1016/j.wasman.2005.06.012>

Alleman, T.L., McCormick, R.L., Christensen, E.D., Fioroni, G. and Moriarty, K., 2016. Biodiesel handling and use guide. US Department of Energy, Washington, DC, USA.

Alvarez, D., Wilkinson, K.A., Treilhou, M., Tene, N., Castillo, D. and Sauvain, M., 2019. Prospecting peptides isolated from black soldier fly (Diptera: Stratiomyidae) with antimicrobial activity against *Helicobacter pylori* (Campylobacterales: Helicobacteraceae). *Journal of Insect Science* 19(6): 17. <https://doi.org/10.1093/jisesa/iez120>

Alyokhin, A., Buzza, A. and Beaulieu, J., 2019. Effects of food substrates and moxidectin on development of black soldier fly, *Hermetia illucens*. *Journal of Applied Entomology* 143: 137-143.

Augère-Granier, M.-L., 2019. The EU poultry meat and egg sector: main features, challenges and prospects. European Parliamentary Research Service, Brussels, Belgium.

Axtell, R.C. and Arends, J.J., 1990. Ecology and management of arthropod pests of poultry. *Annual Review of Entomology* 35: 101-126.

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

Axtell, R.C., 1986. Fly management in poultry production: cultural, biological, and chemical. *Poultry Science* 65: 657-667.

Azzollini, D., Wibisaphira, T., Lakemond, C.M.M. and Fogliano, V., 2019. Toward the design of insect-based meat analogue: the role of calcium and temperature in coagulation behavior of *Alphitobius diaperinus* proteins. *LWT* 100: 75-82.

Barragan-Fonseca, K.B., Dicke, M. and Van Loon, J.J.A., 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. *Journal of Insects as Food and Feed* 3: 105-120. <https://doi.org/10.3920/JIFF2016.0055>

Barrett, J., 2019. United States hog inventory up 4 percent. United States Department of Agriculture, National Agricultural Statistics Service, Washington, DC, USA.

Barth, C.L., 1986. Fly control through manure management. *Poultry Science* 65: 668-674.

Beard, R.L. and Sands, D.C., 1973. Factors affecting degradation of poultry manure by flies. *Environmental Entomology* 2: 801-806.

Bertone, M.A., Green, J.T., Washburn, S.P., Poore, M.H. and Watson, D.W., 2006. The contribution of tunneling dung beetles to pasture soil nutrition. *Forage and Grazinglands* 4: 1-12.

Beskin, K.V., Holcomb, C.D., Cammack, J.A., Crippen, T.L., Knap, A.H., Sweet, S.T. and Tomberlin, J.K., 2018. Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions. *Waste Management* 74: 213-220. <https://doi.org/10.1016/j.wasman.2018.01.019>

Borrelli, L., Coretti, L., Dipineto, L., Bovera, F., Menna, F., Chiariotti, L., Nizza, A., Lembo, F. and Fioretti, A., 2017. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. *Scientific Reports* 7: 16269. <https://doi.org/10.1038/s41598-017-16560-6>

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

Cai, M., Ma, S., Hu, R., Tomberlin, J.K., Thomashow, L.S., Zheng, L., Li, W., Yu, Z. and Zhang, J., 2018a. Rapidly mitigating antibiotic resistant risks in chicken manure by *Hermetia illucens* bioconversion with intestinal microflora. *Environmental Microbiology* 20: 4051-4062. <https://doi.org/10.1111/1462-2920.14450>

- Cai, M., Ma, S., Hu, R., Tomberlin, J.K., Yu, C., Huang, Y., Zhan, S., Li, W., Zheng, L., Yu, Z. and Zhang, J., 2018b. Systematic characterization and proposed pathway of tetracycline degradation in solid waste treatment by *Hermetia illucens* with intestinal microbiota. *Environmental Pollution* 242: 634-642. <https://doi.org/10.1016/j.envpol.2018.06.105>
- Cammack, J.A. and Tomberlin, J.K., 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. <https://doi.org/10.3390/insects8020056>
- Carbajal, C., Picazo, A., Gutierrez, S., Blackmon, S., Perez, G., Gonzlez, A., Fuentes, E., Wickham, C., Lopez, C., Johnson, D., and Kannan, S. 2017. Genesis of antibiotic resistance XXVII: action plan for Global Union for Antibiotics Research and Development (GUARD) to mitigate AR pandemic (ARP). *FASEB Journal* 31: 777.
- Centers for Disease Control and Prevention (CDC), 2015. National action plan for combating antibiotic-resistant bacteria (national action plan). CDC, Atlanta, GA, USA.
- Chang, C.C.Y. and Janzen, H.H., 1996. Long-term fate of nitrogen from annual feedlot manure applications. *Journal of Environmental Quality* 25: 785-790.
- Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.-F., Yannarell, A.C., Maxwell, S. and Aminov, R.I., 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of Environmental Quality* 38(3): 1086-1108.
- Chen, S., Liao, W., Liu, C., Elliott, D.C., Brown, M.D. and Solana, A.E., 2003. Value-added chemicals from animal manure. US Department of Energy, Oak Ridge, TX, USA. Available at: <https://www.osti.gov/biblio/15009485/>
- Čičková, H., Newton, G.L., Lacy, R.C. and Kozanek, M., 2015. The use of fly larvae for organic waste treatment. *Waste Management* 35: 68-80. <https://doi.org/10.1016/j.wasman.2014.09.026>
- Čičková, H., Pastor, B., Kozanek, M., Martinez-Sanchez, A., Rojo, S. and Takac, P., 2012. Biodegradation of pig manure by the housefly, *Musca domestica*: a viable ecological strategy for pig manure management. *PLoS ONE* 7: e32798. <https://doi.org/10.1371/journal.pone.0032798>
- Cosse, A.A. and Baker, T.C., 1996. House flies and pig manure volatiles: wind tunnel behavioral studies and electrophysiological evaluations. *Journal of Agricultural Entomology* 13: 301-317.
- Crippen, T.L., Sheffield, C.L., Esquivel, S.V., Droleskey, R.E. and Esquivel, J.F., 2009. The acquisition and internalization of *Salmonella* by the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Vector Borne Zoonotic Diseases* 9: 65-72. <https://doi.org/10.1089/vbz.2008.0103>
- Crippen, T.L., Zheng, L., Sheffield, C.L., Tomberlin, J.K., Beier, R.C. and Yu, Z., 2012. Transient gut retention and persistence of *Salmonella* through metamorphosis in the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Journal of Applied Microbiology* 112: 920-926. <https://doi.org/10.1111/j.1365-2672.2012.05265.x>
- Davis, C.G., Harvey, D., Zahniser, S., Gale, F. and Liefert, W., 2013. Assessing the growth of US broiler and poultry meat exports. A report from the Economic Research Service. USDA, Washington, DC, USA, pp. 1-28.
- Diener, S., Zurbrugg, C. and Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research* 27: 603-610. <https://doi.org/10.1177/0734242x09103838>
- Edmonds, L., Gollehon, N., Kellogg, R.L., Knight, L., Lander, C., Lemunyon, J., Meyer, D., Moffitt, D.C. and Schaefer, J., 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. United States Department of Resources Natural Resources Conservation Service, Washington, DC, USA.
- Eleftherianos, I., Atri, J., Accetta, J. and Castillo, J.C., 2013. Endosymbiotic bacteria in insects: guardians of the immune system? *Frontiers in Physiology* 4: 46. <https://doi.org/10.3389/fphys.2013.00046>
- Elhag, O., Zhou, D., Song, Q., Soomro, A.A., Cai, M., Zheng, L., Yu, Z. and Zhang, J., 2017. Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from *Hermetia illucens* (L.). *PLoS ONE* 12: e0169582. <https://doi.org/10.1371/journal.pone.0169582>
- Erickson, M.C., Islam, M., Sheppard, C., Liao, J. and Doyle, M.P., 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. <https://doi.org/10.4315/0362-028x-67.4.685>
- European Union (EU), 2016. Action on antimicrobial resistance. EU, Brussels, Belgium. Available at: https://ec.europa.eu/health/antimicrobial-resistance/eu-action-on-antimicrobial-resistance_en
- Food and Agriculture Organisation (FAO), 2006. Livestock's long shadow: environmental issues and options. FAO, Rome, Italy.
- Food and Agriculture Organisation (FAO), 2008. Poultry in the 21st century: avian influenza and beyond. In: Thieme, O. and Pilling, D. (eds.) *Proceedings of the International Poultry Conference*. November 5-7, 2007. FAO, Bangkok, Thailand.
- Gao, Q., Deng, W., Gao, Z., Li, M., Liu, W., Wang, X. and Zhu, F., 2019. Effect of sulfonamide pollution on the growth of manure management candidate *Hermetia illucens*. *PLOS ONE*. 14: e0216086. <https://doi.org/10.1371/journal.pone.0216086>
- Garcia, R., Baelum, J., Fredslund, L., Santorum, P. and Jacobsen, C.S., 2010. Influence of temperature and predation on survival of *Salmonella enterica* serovar Typhimurium and expression of invA in soil and manure-amended soil. *Applied and Environmental Microbiology* 76: 5025-5031. <https://doi.org/10.1128/AEM.00628-10>
- Gasco, L., Finke, M. and Van Huis, A., 2018. Can diets containing insects promote animal health? *Journal of Insects as Food and Feed* 4: 1-4. <https://doi.org/10.3920/JIFF2018.x001>
- Gold, M., Cassar, C.M., Zurbrugg, C., Kreuzer, M., Boulos, S., Diener, S. and Mathys, A., 2020. Biowaste treatment with black soldier fly larvae: increasing performance through the formulation of biowastes based on protein and carbohydrates. *Waste Management* 102: 319-329.
- Grossman, E., 2014. As dairy farms grow bigger, new concerns about pollution. *Yale Environment* 360. Available at: https://e360.yale.edu/features/as_dairy_farms_grow_bigger_new_concerns_about_pollution

- Hall, R.D. and Foehse, M.C., 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.
- Haruta, S. and Kanno, N., 2015. Survivability of microbes in natural environments and their ecological impacts. *Microbes and Environments* 30: 123-125. <https://doi.org/10.1264/jsme2.ME3002rh>
- Hussein, M., Pillai, V.V., Goddard, J.M., Park, H.G., Kothapalli, K.S., Ross, D.A., Ketterings, Q.M., Brenna, J.T., Milstein, M.B. and Marquis, H., 2017. Sustainable production of housefly (*Musca domestica*) larvae as a protein-rich feed ingredient by utilizing cattle manure. *PLoS ONE* 12: e0171708.
- Jiang, X., Islam, M., Morgan, J. and Doyle, M.P., 2004. Fate of *Listeria monocytogenes* in bovine manure-amended soil. *Journal of Food Protection* 67: 1676-1681. <https://doi.org/10.4315/0362-028x-67.8.1676>
- Kaltenpoth, M., 2009. Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology* 17: 529-535. <https://doi.org/10.1016/j.tim.2009.09.006>
- Kaltenpoth, M., Gottler, W., Herzner, G. and Strohm, E., 2005. Symbiotic bacteria protect wasp larvae from fungal infestation. *Current Biology* 15: 475-479. <https://doi.org/10.1016/j.cub.2004.12.084>
- Keiding, J., 1986. The house-fly: biology and control. World Health Organization, Geneva, Switzerland.
- Khan, H.A.A., Shad, S.A. and Akram, W., 2012. Effect of livestock manures on the fitness of house fly, *Musca domestica* L. (Diptera: Muscidae). *Parasitology Research* 111: 1165-1171. <https://doi.org/10.1007/s00436-012-2947-1>
- Kim, W., Bae, S., Kim, A., Park, K., Lee, S., Choi, Y., Han, S., Park, Y. and Koh, Y., 2011a. Characterization of the molecular features and expression patterns of two serine proteases in *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *BMB Reports* 44: 387-392.
- Kim, W., Bae, S., Park, K., Lee, S., Choi, Y., Han, S. and Koh, Y., 2011b. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology* 14: 11-14. <https://doi.org/10.1016/j.aspen.2010.11.003>
- Kim, Y.B., Kim, D., Jeong, S., Lee, J.K., T., Lee, H. and Lee, K., 2020. Black soldier fly larvae oil as an alternative fat source in broiler nutrition. *Poultry Science* 99: 3133-3143.
- Kudva, I.T., Blanch, K. and Hovde, C.J., 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64: 3166-3174. <https://doi.org/10.1128/AEM.64.9.3166-3174.1998>
- Kyakuwairu, M., Olupot, G., Amoding, A., Nkedi-kizza, P. and Ateenyi, B.T., 2019. How safe is chicken litter for land application as an organic fertilizer? A review. *International Journal of Environmental Research and Public Health* 16: 3521.
- Lalander, C., Diener, S., Zurbrugg, C. and Vinnerås, B., 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., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K. and Vinnerås, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of the Total Environment* 565: 279-286. <https://doi.org/10.1016/j.scitotenv.2016.04.147>
- Lalander, C.H., Fidjeland, J., Diener, S., Eriksson, S. and Vinnerås, B., 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. <https://doi.org/10.1007/s13593-014-0235-4>
- Larriain, P.S. and Salas, C.F., 2008. House fly (*Musca domestica* L.) (Diptera: Muscidae) development in different types of manure. *Chilean Journal of Agricultural Research* 68: 192-197.
- Leni, G., Soetemans, L., Jacobs, J., Depraetere, S., Ganotten, N., Bastiaens, L., Calgiani, A. and Sforza, S., 2020. Protein hydrolysates from *Alphitobius diaperinus* and *Hermetia illucens* larvae treated with commercial proteases. *Journal of Insects as Food and Feed* 6: 393-404. <https://doi.org/10.3920/JIFF2019.0037>
- Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J.K. and Yu, Z., 2011. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management* 31: 1316-1320.
- Li, W., Zheng, L., Wang, Y., Zhang, J., Yu, X. and Zhang, Y., 2015. Potential biodiesel and biogas production from corn cob by anaerobic fermentation and black soldier fly. *Biosource Technology* 194: 276-282.
- Liu, Q., Tomberlin, J.K., Brady, J.A., Sanford, M.R. and Yu, Z., 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. *Environmental Entomology* 37: 1525-1530. <https://doi.org/10.1603/0046-225x-37.6.1525>
- Liu, T., Awasthi, M.K., Chen, H., Duan, Y., Awasthi, S.K. and Zhang, Z., 2019. Performance of black soldier fly larvae (Diptera: Stratiomyidae) for manure composting and production of cleaner compost. *Journal of Environmental Management* 251: 109593.
- Lyons, B.N., Crippen, T.L., Zheng, L., Teel, P.D., Swiger, S.L. and Tomberlin, J.K., 2017. Susceptibility of *Alphitobius diaperinus* in Texas to permethrin- and beta-cyfluthrin-treated surfaces. *Pest Management Science* 73: 562-567. <https://doi.org/10.1002/ps.4327>
- MacDonald, J.M., O'Donoghue, E., McBride, W.D., Nehring, R., Sandretto, C. and Mosheim, R., 2007. Profits, costs, and the changing structure of dairy farming. USDA-ERS, Washington, DC, USA.
- Manyi-Loh, C.E., Mamphweli, S.N., Meyer, E.L., Makaka, G., Simon, M. and Okoh, A.I., 2016. An overview of the control of bacterial pathogens in cattle manure. *International Journal of Environmental Research and Public Health* 13(9): 843. <https://doi.org/10.3390/ijerph13090843>
- Matos, J.S., Barberino, A.T.M.S., De Araujo, L.P., Lôbo, I.P. and De Almeida Neto, J.A., 2020. Potentials and limitations of the bioconversion of animal manure using fly larvae. *Waste and Biomass Valorization*: 1-24.
- Mazza, L., Xiao, X., Ur Rehman, K., Cai, M., Zhang, D., Fasulo, S., Tomberlin, J.K., Zheng, L., Soomro, A.A., Yu, Z. and Zhang, J., 2020. Management of chicken manure using black soldier fly (Diptera: Stratiomyidae) larvae assisted by companion bacteria. *Waste Management* 102: 312-318. <https://doi.org/10.1016/j.wasman.2019.10.055>
- Miller, A., 1954. Dung beetles (Coleoptera: Scarabaeidae) and other insects in relation to human feces in a hookworm area of southern Georgia. *American Journal of Tropical Medicine and Hygiene* 3: 372-389. <https://doi.org/10.4269/ajtmh.1954.3.372>

- Miller, B., Teotia, J. and Thatcher, T., 1974. Digestion of poultry manure by *Musca domestica*. *Journal of British Poultry Science* 15: 231-234.
- Miranda, C., Cammack, J. and Tomberlin, J., 2020a. Life-history traits of house fly, *Musca domestica* L. (Diptera: Muscidae), reared on three manure types. *Journal of Insects as Food and Feed* 6: 81-90. <https://doi.org/10.3920/JIFF2019.0001>
- Miranda, C.D., Cammack, J.A. and Tomberlin, J.K., 2019. Life-history traits of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), reared on three manure types. *Animals* 9: 281.
- Miranda, C.D., Cammack, J.A. and Tomberlin, J.K., 2020b. Mass production of the black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae) reared on three manure types. *Animals* 20: 1243.
- Mohr, C.O., 1943. Cattle droppings as ecological units. *Ecological Monographs* 13: 275-298.
- Morrison, P.E. and Davies, D.M., 1964. Feeding of dry chemically defined diets + egg production in adult house-fly. *Nature* 201: 104-105. <https://doi.org/10.1038/201104a0>
- Muller, A., Wolf, D. and Gutzeit, H., 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., Tomberlin, J.K., Lambert, B.D. and Kattes, D., 2014. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental Entomology* 37: 11-15. <https://doi.org/10.1093/ee/37.1.11>
- National Chicken Council, 2019a. Broiler chicken industry key facts 2019. NCC, Washington, DC, USA. Available at: <https://www.nationalchickencouncil.org/about-the-industry/statistics/broiler-chicken-industry-key-facts/>
- National Chicken Council, 2019b. U.S. broiler production. NCC, Washington, DC, USA. Available at: <https://www.nationalchickencouncil.org/about-the-industry/statistics/u-s-broiler-production/>
- Newton, L., Sheppard, C., Watson, D., Burtle, G. and Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. North Carolina State University, Raleigh, NC, USA.
- Nguyen, T.T.X., Tomberlin, J.K. and Vanlaerhoven, S., 2013. Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. *Journal of Medical Entomology* 50: 898-906. <https://doi.org/10.1603/me12260>
- Nichols, E., Alarcon, V., Forgie, S., Gomez-Puerta, L.A. and Jones, M.S., 2017. Coprophagous insects and the ecology of infectious diseases of wildlife. *ILAR Journal* 58: 336-342. <https://doi.org/10.1093/ilar/ilx022>
- Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezcua, S., Favila, M.E. and The Scarabaeinae Research Network, 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation* 161: 1461-1474.
- Olsen, M.E., Goh, J., Phillips, M., Guselle, N. and McAllister, T.A., 1999. *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil, and cattle feces. *Journal of Environmental Quality* 28: 1991-1996.
- Olson, M., 2020. Human and animal pathogens in manure. Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Canada.
- Oonincx, D.G.A.B., Van Huis, A. and Van Loon, J.J.A., 2015a. Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. *Journal of Insects as Food and Feed* 1: 131-139. <https://doi.org/10.3920/JIFF2014.0023>
- Oonincx, D.G.A.B., Van Broekhoven, S., Van Huis, A. and Van Loon, J.J.A., 2015b. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE* 10: e0144601. <https://doi.org/10.1371/journal.pone.0144601>
- O'Toole, G.A. and Kolter, R., 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Molecular Microbiology* 28: 449-461. <https://doi.org/10.1046/j.1365-2958.1998.00797.x>
- Paniagua Voirol, L.R., Frago, E., Kaltenpoth, M., Hilker, M. and Fatouros, N.E., 2018. Bacterial symbionts in lepidoptera: their diversity, transmission, and impact on the host. *Frontiers in Microbiology* 9: 556. <https://doi.org/10.3389/fmicb.2018.00556>
- Park, B., Um, K., Choi, W. and Park, S., 2017. Effect of feeding black soldier fly pupa meal in the diet on egg production, egg quality, blood lipid profiles and faecal bacteria in laying hens. *European Poultry Science* 81.
- Park, K.H., Choi, Y.C., Nam, S.H. and Kim, W., 2012. Recombinant expression and enzyme activity of chymotrypsin-like protease from black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *International Journal of Industrial Entomology* 25(2): 181-185.
- Park, K.H., Kwak, K.W., Nam, S.H., Choi, J.Y., Lee, S.H., Kim, H.G. and Kim, S.H., 2015. Antibacterial activity of larval extract from the black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) against plant pathogens. *Journal of Entomology and Zoology Studies* 3: 176-179.
- Prosser, R.S. and Sibley, P.K., 2015. Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. *Environment International* 75: 223-233.
- Roncolini, A., Milanovic, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., Clementi, F., Belleggia, L., Pasquini, M., Mozzon, M., Foligni, R., Federica Trombetta, M., Haouet, M.N., Serena Altissimi, M., Di Bella, S., Piersanti, A., Griffoni, F., Reale, A., Niro, S. and Osimani, A., 2020. Lesser mealworm (*Alphitobius diaperinus*) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. *Food Research International* 131: 109031. <https://doi.org/10.1016/j.foodres.2020.109031>
- Rumbos, C.I., Karapanagiotidis, I.T., Mente, E. and Athanassiou, C.G., 2019. The lesser mealworm *Alphitobius diaperinus*; a noxious pest or a promising nutrient source? *Reviews in Aquaculture* 11: 1418-1437.
- Sarpong, D., Oduru-Kwarteng, S., Gyasi, S.F., Buamah, R., Donkor, E., Awuah, E. and Baah, M.K., 2019. Biodegradation by composting of municipal organic solid waste into organic fertilizer using the black soldier fly (*Hermetia illucens*) (Diptera: Stratiomyidae) larvae. *International Journal of Recycling of Organic Waste in Agriculture* 8: 45-54.
- Sasaki, T., Kobayashi, M. and Agui, N., 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. <https://doi.org/10.1603/0022-2585-37.6.945>

- Seabrooks, L. and Hu, L., 2017. Insects: an underrepresented resource for the discovery of biologically active natural products. *Acta Pharmaceutica Sinica B* 7: 409-426. <https://doi.org/10.1016/j.apsb.2017.05.001>
- Shah, D. and Mital, K., 2018. The role of trypsin:chymotrypsin in tissue repair. *Advances in Therapy* 35: 31-42. <https://doi.org/10.1007/s12325-017-0648-y>
- Shahbandeh, M., 2020. Global pork production, by country. Statista, New York, NY, USA. Available at: <https://www.statista.com/statistics/273232/net-pork-production-worldwide-by-country>
- Sheng, L., Shen, X., Benedict, C., Su, Y., Tsai, H.C., Schacht, E., Kruger, C.E., Drennan, M. and Zhu, M.J., 2019. Microbial safety of dairy manure fertilizer application in raspberry production. *Frontiers in Microbiology* 10: 2276. <https://doi.org/10.3389/fmicb.2019.02276>
- 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., Newton, G.L. and Savage, S., 1994. A value added manure management system using the black soldier fly. *Bioresource Technology* 50: 275-279.
- Sierra, J.M., Fuste, E., Rabanal, F., Vinuesa, T. and Vinas, M., 2017. An overview of antimicrobial peptides and the latest advances in their development. *Expert Opinion on Biological Therapy* 17: 663-676. <https://doi.org/10.1080/14712598.2017.1315402>
- Sim, W.J., Lee, J.W., Lee, E.S., Shin, S.K., Hwang, S.R. and Oh, J.E., 2011. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. *Chemosphere* 82(2): 179-186.
- Sobsey, M.D., Khatib, L.A., Hill, V.R., Alocilja, E. and Pillai, S., 2006. Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. In: Rice, J.M., Caldwell, D.F. and Humenik, F.J. (eds.) *Animal agriculture and the environment: national center for manure and animal waste management white papers*. ASABE, St. Joseph, MI, USA.
- Stanford, K., Reuter, T., Gilroyed, B.H. and McAllister, T.A., 2015. Impacts of sporulation temperature, exposure to compost matrix and temperature on survival of *Bacillus cereus* spores during livestock mortality composting. *Journal of Applied Microbiology* 118: 989-997. <https://doi.org/10.1111/jam.12749>
- St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, G.L., Sealey, W., Sheppard, C. and Irving, S., 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.
- Sun, Y. and O'Riordan, M.X., 2013. Regulation of bacterial pathogenesis by intestinal short-chain fatty acids. *Advances in Applied Microbiology* 85: 93-118. <https://doi.org/10.1016/B978-0-12-407672-3.00003-4>
- Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. and Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy* 98: 197-202. <https://doi.org/10.1016/j.renene.2016.03.022>
- Teotia, J.S. and Miller, B.F., 1974. Nutritive content of house fly pupae and manure residue. *British Poultry Science* 15: 177-182. <https://doi.org/10.1080/00071667408416093>
- Tomberlin, J.K. and Van Huis, A., 2020. Black soldier fly from pest to 'crown jewel' of the insects as feed industry: an historical perspective. *Journal of Insects as Food and Feed* 6: 1-4. <https://doi.org/10.3920/JIFF2020.0003>
- Tomberlin, J.K., Richman, D. and Myers, H.M., 2008. Susceptibility of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) from broiler facilities in Texas to four insecticides. *Journal of Economic Entomology* 101: 480-483.
- Tomberlin, J.K., Sheppard, D.C. and Joyce, J.A., 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. [https://doi.org/10.1603/0013-8746\(2002\)095\[0379:slhtob\]2.0.co;2](https://doi.org/10.1603/0013-8746(2002)095[0379:slhtob]2.0.co;2)
- United States Department of Agriculture, 2015. Overview of the United States hog industry: breeding herd efficiency continues to increase. USDA, Washington, DC, USA.
- United States Department of Agriculture, 1995. Animal manure management. USDA, Washington, DC, USA. Available at: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/null?cid=nrcs143_014211
- United States Energy Information Administration, 2020. Diesel fuel explained. US Energy Information Administration, Washington, DC, USA. Available at: <https://www.eia.gov/energyexplained/diesel-fuel/use-of-diesel.php>
- ur Rehman, K., Cai, M., Xiao, X., Zheng, L., Wang, H., Soomro, A.A., Zhou, Y., Li, W., Yu, Z. and Zhang, J., 2017a. 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.
- ur Rehman, K., Rehman, A., Cai, M., Zheng, L., Xiao, X., Somroo, A.A., Wang, H., Li, W., Yu, Z. and Zhang, J., 2017b. 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.
- Usui, M., Kawakura, M., Yoshizawa, N., San, L.L., Nakajima, C., Suzuki, Y. and Tamura, Y., 2017. Survival and prevalence of *Clostridium difficile* in manure compost derived from pigs. *Anaerobe* 43: 15-20. <https://doi.org/10.1016/j.anaerobe.2016.11.004>
- Vogel, H., Muller, A., Heckel, D.G., Gutzeit, H. and Vilcinskis, A., 2018. Nutritional immunology: diversification and diet-dependent expression of antimicrobial peptides in the black soldier fly *Hermetia illucens*. *Developmental and Comparative Immunology* 78: 141-148. <https://doi.org/10.1016/j.dci.2017.09.008>
- Wallace, J.S., Garner, E., Pruden, A. and Aga, D.S., 2018. Occurrence and transformation of veterinary antibiotics and antibiotic resistance genes in dairy manure treated by advanced anaerobic digestion and conventional treatment methods. *Environmental Pollution* 236: 764-772
- Wang, H., Li, H., Gilbert, J.A., Li, H., Wu, L., Liu, M., Wang, L., Zhou, Q., Yuan, J. and Zhang, Z., 2015. Housefly larva vermicomposting efficiently attenuates antibiotic resistance genes in swine manure, with concomitant bacterial population changes. *Applied and Environmental Microbiology* 81: 7668-7679.

- Wang, H., Sangwan, N., Li, H.-Y., Su, J.-Q., Oyang, W.-Y., Zhang, Z.-J., Gilbert, J.A., Zhu, Y.-G., Ping, F. and Zhang, H.-L., 2017. The antibiotic resistome of swine manure is significantly altered by association with the *Musca domestica* larvae gut microbiome. *The ISME Journal* 11: 100-111.
- Wang, H., Zhang, Z., Czapar, G.F., Winkler, M.K. and Zheng, J., 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. *Waste Management & Research* 31: 223-231.
- Wasala, L., Talley, J.L., Desilva, U., Fletcher, J. and Wayadande, A., 2013. Transfer of *Escherichia coli* O157:H7 to spinach by house flies, *Musca domestica* (Diptera: Muscidae). *Phytopathology* 103: 373-380. <https://doi.org/10.1094/PHYTO-09-12-0217-FI>
- Welch, C. and O'Hagan, M., 2010. Officials seek cause of Snohomish dairy-manure spill. *The Seattle Times*, April 14. Available at: <https://www.seattletimes.com/seattle-news/officials-seek-cause-of-snohomish-dairy-manure-spill/>
- World Health Organization, 2015. Global action plan on antimicrobial resistance. WHO, Geneva, Switzerland.
- Wu, Q., Patocka, J. and Kuca, K., 2018. Insect antimicrobial peptides, a mini review. *Toxins* 10: 461. <https://doi.org/10.3390/toxins10110461>
- Xiao, X., Mazza, L., Yu, Y., Cai, M., Zheng, L., Tomberlin, J.K., Yu, J., Van Huis, A., Yu, Z. and Fasulo, S., 2018. Efficient co-conversion process of chicken manure into protein feed and organic fertilizer by *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae and functional bacteria. *Journal of Environmental Management* 217: 668-676.
- Yu, G., Cheng, P., Chen, Y., Li, Y., Yang, Z., Chen, Y. and Tomberlin, J.K., 2011. Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology* 40: 30-35. <https://doi.org/10.1603/EN10126>
- Yu, J. and Ahmedna, M., 2012. Functions/applications of trypsin in food processing and food science research. In: Weaver, K. and Kelley, C. (eds.) *Trypsin: structure, biosynthesis and functions*. Nova Science Publishers, Hauppauge, NY, USA, pp. 75-96.
- Yuan, Q.B., Zhai, Y.F., Mao, B.Y., Schwarz, C. and Hu, N., 2019. Fates of antibiotic resistance genes in a distributed swine wastewater treatment plant. *Water Environmental Research* 91: 1565-1575.
- Zhang, Z., Wang, H., Zhu, J., Suneethi, S. and Zheng, J., 2012. Swine manure vermicomposting via housefly larvae (*Musca domestica*): the dynamics of biochemical and microbial features. *Bioresource Technology* 118: 563-571. <https://doi.org/10.1016/j.biortech.2012.05.048>
- Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q. and Yu, Z., 2012. Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. *Energy* 47: 225-229.
- Zhou, F., Tomberlin, J.K., Zheng, L., Yu, Z. and Zhang, J., 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.
- Zhou, L., Budge, S.M., Ghaly, A.E., Brooks, M.S. and Dave, D., 2011. Extraction, purification and characterization of fish chymotrypsin: a review. *American Journal of Biochemistry and Biotechnology* 7: 104-123.
- Zhu, F.X., Wang, W.P., Hong, C.L., Feng, M.G., Xue, Z.Y., Chen, Y., Yao, Y.L. and Yu, M., 2012. Rapid production of maggots as feed supplement and organic fertilizer by the two-stage composting of pig manure. *Bioresource Technology* 116: 485-491.
- Zi-zhe, C., De-po, Y., Sheng-qing, W., Yong, W., Reaney, M.J., Zhi-min, Z., Long-ping, Z., Guo, S., Yi, N. and Dong, Z., 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.
- Zurek, K. and Nayduch, D., 2016. Bacterial associations across house fly life history: evidence for transstadial carriage from managed manure. *Journal of Insect Science* 16: 2.
- Zurek, L., Schal, C. and Watson, D., 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.