Larval digestion of different manure types by the black soldier fly (Diptera: Stratiomyidae) impacts associated volatile emissions


Keywords: Hermetia illucens Manure management Sustainable agriculture Indole

1. Introduction

The decomposition of manure is responsible for environmental emissions, such as greenhouse gases, ammonia and other volatile organic compounds (VOCs), which are pollutants and pose potential health risks (FAO, 2009). Between 100 and 330 VOCs and volatile fatty acids are generated by concentrated animal feeding operations (CAFOs) depending on management practices and the species of animal involved (Cai et al., 2015; Powers and Bastyr, 2004; Schiffman et al., 2001). The compounds most associated with or responsible for the odor of manure are phenols, indoles, alcohols, organic sulphides, and volatile fatty acids (Hales et al., 2012; Kuroda et al., 1996; El-Mashad et al., 2011). For example, Hales et al. (2012) found that 4-methylphenol was responsible for 67.3% of odor activity in dairy manure. The key odorous VOCs in poultry manure were butanoic acid, 3-methylbutanoic acid, dimethyl trisulphide, indole and skatole (Yasuhara, 1987). These VOCs, which are noxious odors, can also negatively affect humans by posing potential health risks to those living in communities surrounding animal farming facilities (PEW, 2008). VOCs responsible for strong odors contribute to higher levels of tension, depression, and anger experiences by those working or living in close proximity to areas with heavy animal farming (Barrett, 2006).

With the increasing amount of manure and the need for a sustainable method of management, fly (Diptera) larvae have become an alternative means to process this resource. Black soldier fly, Hermetia illucens (L.) (Diptera: Stratiomyidae) larvae (BSFL) have been studied as a means of manure management because of...
several beneficial abilities. First, BSFL reduce organic matter, such as livestock manure. Sheppard (1994) observed significant reductions in poultry manure (~50%) in one study, and in another study reported 56 and 42% reductions in dry weight, depending on whether manure had water added to it or not (Sheppard, 1983). Newton et al. (2005) observed a 39% reduction in the dry weight of swine manure processed by BSFL. Myers et al. (2008) documented a 58% and 33% reduction in dry matter of manure from BSFL fed 27 g and 70 g of dairy manure daily, respectively. In a study comparing poultry, swine, and dairy manure, the dry matter of all three manure types was reduced by ~37% (Oonincx et al., 2015).

In addition to the reduction of dry matter of manure, nutrients, which in excess can be detrimental to the environment, are decreased. BSFL reduce the nitrogen content of poultry manure by 62% (Sheppard, 1983). During the digestion of dairy manure, BSFL reduced concentrations of nitrogen and phosphorus by 30–50% and 61–70%, respectively (Myers et al., 2008). When BSFL were allowed to feed on poultry, swine, and dairy manure, nitrogen content was reduced by 80, 37, and 30%, respectively (Oonincx et al., 2015). Importantly, adult black soldier flies do not need to feed and their non-synanthropic nature has earned them the label of a non-pest species (Furman et al., 1959). However, to date, the impact of black soldier fly digestion of manure on VOC production has not been examined. The purpose of this study was to assess how the digestion of poultry, swine, and dairy manure by BSFL impacts select odorous VOCs.

2. Materials and methods

2.1. Acquisition of flies

The H. illucens larvae were from a colony established in 2014 from eggs from a colony maintained at the Coastal Plains Experiment Station, University of Georgia, (Tifton, GA) and are now maintained at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University (College Station, TX). Adult flies were maintained in a 2.6 × 1.3 × 1.3 m wooden cage fitted with metal screening, in a greenhouse maintained at approximately 29°C. Adults were allowed to oviposit in three 7.0 × 5.0 × 0.3 cm pieces of corrugated cardboard (Booth and Sheppard, 1984) held together with masking tape and placed on the lid of a 30.0 × 15.0 × 11.0 cm plastic shoe box containing one kilogram of Gainesville diet (Hogsette, 1992) saturated with water. A 13.0 × 5.0 cm portion of the lid was removed and replaced with metal screening on which the cardboard pieces were placed; this approach allowed volatiles to escape from the wet Gainesville diet and attract gravid flies, but prevented the flies from contacting and/or ovipositing directly into the media instead of the cardboard. The cardboard was removed from the cage after eight hours, and eggs were removed from cardboard using a sterile plastic spatula and weighed. One gram of eggs was then placed in a 0.5 L plastic container, covered with a paper towel secured with a rubber band, stored in a walk-in environmental chamber (29 ± 0.3 °C with 60 ± 5.1% relative humidity and 14:10 L:D cycle) and checked every 12 h until hatch. Two hundred grams of Gainesville diet at 70% moisture was added to the container once larvae eclosed. Newly-emerged larvae were allowed to feed for four days in the environmental chamber prior to use in the experiment.

2.2. Acquisition of manure

Three different livestock manure types were used in this study. 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. The hens were fed a mixture of corn and soybean meal that is considered typical layer diet, consisting of 18.5% crude protein, 2.5% crude fat and 2.4% crude fiber. Swine manure was collected from sows raised by Schroeder Genetics in Anderson, TX, USA. The sows were maintained on cubes containing 14.0% crude protein, 2.8% crude fat, and 6.5% crude fiber formulated for gilts, sows and adult boars. Dairy manure was collected from cows maintained at the Southwest Regional Dairy Center in Stephenville, TX, USA. The diet for these animals consisted of 16.1% crude protein, 5.0% crude fat and 28.1% crude fiber. The majority of this diet is composed of a mixture of corn silage (32.0%), ground corn (22.5%), and concentrate pre-mix (19.4%) composed of canola and soybean meal.

Each manure type was collected on site within 12 h of excretion, using a shovel and two 19 L buckets with lids, sterilized prior to use for manure collection. The manure was transported to the F.L.I.E.S. Facility where it was homogenized in the buckets and transferred to individual 3.78 L self-sealing plastic freezer bags and frozen at −20 °C until used. Manure was removed from the freezer and allowed to thaw for 24 h at room temperature before being used. Thawed manure was stored in a refrigerator at 4 °C.

2.3. Experiment design

One hundred 4-d-old larvae were placed in 88.7 ml plastic bathroom cups (Georgia-Pacific LLC, Atlanta, GA, USA) and assigned to one of the three manure types (poultry, swine, or dairy) and one of two feed rates (18.0 or 27.0 g every other day). Feed rates used were based off the methods of Myers et al. (2008) who used 300 larvae and feed rates of 27, 40, 54 and 70 g of manure per day. The feed rates used in this study are therefore modified from this due to only 100 larvae being used and feeding occurring every other day. Preliminary experiments were conducted to confirm the scalability of these feed rates (unpublished data).

Containers without larvae were used as controls and subsequently referred to as non-digested manure. These containers received manure assigned at a given feed rate in similar fashion to those with larvae. Three replicates for each feed rate and manure type with and without larvae were used. Containers were placed in a randomized block design among three levels of a shelving unit in the environmental chamber maintained at 29 ± 0.3 °C with 60 ± 5.1% relative humidity and 14:10 L:D cycle The experiment was replicated twice (Fig. 1).

Initially, larvae in each replicate were provided manure at the assigned amount and allowed to feed for four days. Larvae and contents of the cup were then transferred to a 1.89 L Reditainer™ EXTREME FREEZE™ deli container (Clear Lake Enterprises, Port Richey, FL, USA) and fed every other day. Manure was weighed directly into the containers using a Scout™ Pro Balance (Ohaus, Parsippany, NJ, USA). Containers were then covered with a 25.4 × 25.4 cm piece of tulle to prevent contamination.

Containers were checked daily for post-feeding larvae (i.e., prepupa, Sheppard et al. 1994), which were removed and identified by the cuticular color shifting from opaque to black (May 1961). Additionally, to monitor the progress of larval feeding, manure in each container was shifted using forceps that had been sterilized with 70% ethanol. Separate forceps were used for each manure type to prevent cross contamination. Feeding of larvae terminated when 40% of the larvae reached the prepupal stage (Sheppard et al. 2002). VOCs were sampled from the digested group and a matching randomly selected sample from the non-digested group of the same manure type and feed rate, when approximately 90% of the larvae reached the prepupal stage. This level of pupation was selected as it would also represent industrialized production of BSFL.
2.4. Moisture content

Moisture content of the freshly-thawed manure was assessed at the beginning of the experiment and compared to the treatment at 90% prepupation and its corresponding control. To do so, 10 g of manure in three replicates was weighed out into an aluminum pie dish and dried for 24 h at 55°C in a Precision Scientific Thelco Oven (Thermo Fisher Scientific, Waltham, MA, USA). Moisture content was determined gravimetrically using a Scout Pro Balance (Ohaus, Parsippany, NJ).

2.5. Volatile sampling

Larvae were removed from the manure when approximately 90% prepupation was reached and the volatile organic VOCs were collected from the following manure samples: (1) freshly-thawed (initial), (2) replicates of each treatment when approximately 90% of the larvae had reached the prepupal stage (BSFL digested), and (3) a corresponding replicate without larvae (non-digested). After removal of larvae the remaining waste homogenized and a 20 g sample was transferred to a 236.5 ml Ball mason jar (Ball Corporation, Broomfield, CO, USA) and covered with a metal lid with two center holes equidistant from each other. One hole held a 14.6 cm glass Labcraft Pasteur pipet (Curtin Matheson Scientific, Inc., Morris Plains, NJ, USA) filled with 0.75 g Black Diamond activated carbon (Marineland, Cincinnati, OH, USA) to purify incoming air. The second hole was fitted with a volatile trap packed with 30.0 mg of Hayesep Q porous polymer (Volatile Assay Systems, Rensselaer, NY, USA) to collect VOCs. The volatile traps were attached to an intake port on a flow meter (Dwyer Instruments, Inc., Michigan City, IN, USA) with 6.4 mm diameter Tygon tubing (Saint-Gobain S.A., Malvern, PA, USA). Zero-grade helium was used as the carrier gas at a flow rate was 1.29 ml min⁻¹. Electron impact ionization was at 70 eV and mass range was from m/z 45–450. VOCs were identified using standards where available in addition to comparing their mass spectra fragmentation patterns with those stored in the NIST05 mass spectra library. Upon identification, nine compounds were selected for relative quantification. These compounds included: propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, indole, 3-methylindole, phenol and 4-methylphenol. These compounds were processed in a GC-MS and two to be used in subsequent oviposition and attraction assays.

2.6. GC-MS analysis

VOCs from the Hayesep Q were eluted with 150 μl of dichloromethane (Thermo Fisher Scientific, Waltham, MA, USA) into a 1.5 ml SureStop GC vial (Thermo Fisher Scientific, Waltham, MA, USA) containing a 9.0 mm 300 μl insert (Thermo Fisher Scientific, Waltham, MA, USA) using N₂. An additional 5.0 μl of 80 ng/μl n-Octane (Sigma-Aldrich, St. Louis, MO, USA) was added to each sample as an internal standard. Samples were capped and stored at −20°C until analysis at the Geochemical and Environmental Research Group at Texas A&M University, College Station, Texas, using a Hewlett-Packard 6890 gas chromatograph with a Hewlett-Packard 5973 mass selective detector (Hewlett-Packard Company, Palo Alto, CA, USA). The column employed for the separation of VOCs was a fused silica DB-5MS capillary column (30 m x 0.25 mm ID, 0.50 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA).

Injections of 1 μl were performed in splitless mode with an injection temperature of 250°C. The column temperature program was as follows: an initial temperature of 35°C was held for 8 min then increased at a rate of 4°C min⁻¹ until 60°C was attained. This temperature was maintained for one minute followed by an increase at a rate of 6°C min⁻¹ until 160°C was reached and maintained for 1 min. Finally the temperature was increased to 300°C at a rate of 20°C min⁻¹ and held for 10 min. Zero-grade helium was used as the carrier gas at a flow rate was 1.29 ml min⁻¹. Electromagnetic ionization was at 70 eV and mass range was from m/z 45–450. VOCs were identified using standards where available in addition to comparing their mass spectra fragmentation patterns with those stored in the NIST05 mass spectra library. Upon identification, nine compounds were selected for relative quantification. These compounds included: propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, indole, 3-methylindole, phenol and 4-methylphenol. These compounds

Fig. 1. Experiment design for larval digestion experiment. Manure with and without black soldier fly larvae was each separated into two feed rates with each feed rate present for poultry, swine, and dairy manure. Three replicates (n = 3) for each manure were carried out. The experiment design was run twice.
were selected on the basis of contributing the greatest to the offensive odors associated with poultry (Yasuhara, 1987), swine (Yasuhara and Fuwa, 1983) and dairy manure (El-Mashad et al., 2011) according to previous studies.

2.7. Chemical standards

Acetic acid (99% purity), propanoic acid (99.5%), 2-methylpropanoic acid (99%), butyric acid (99%), 3-methylbutanoic acid (99%), pentanoic acid (99%), 4-methyl pentanoic acid, (99%), hexanoic acid (99.5%), heptanoic acid (97%), 3-methylindole (98%), 4-methylphenol (99%), benzaldehyde (99.5%), indole (99%), phenol (99%) were purchased from Absolute Standards, Inc. (Hamden, CT, USA). Octane (99%), dichloromethane (99.8%) and dimethyl disulfide (99%) were purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA). Beyond octane, the internal standard, and dichloromethane, the elution solvent, the additional chemicals herein were used to confirm the identifications of select VOCs of interest, which were further examined.

2.8. Statistical analyses

Numbers of VOCs and moisture present in treatments (initial, BSFL digested and non-digested manure) were compared using a two-way analysis of variance (ANOVA) using JMP Pro 12 statistical software (SAS Institute, Cary, NC, USA). Significant differences (P ≤ 0.05) in means were further separated using Tukey-Kramer Honest Significant Difference (HSD). Identified VOCs were quantified using peak areas obtained from each chromatogram. The peak area of the identified VOC was divided by the peak area of the internal standard, n-Octane, to obtain relative areas for use in analyses of the nine select odorous compounds. Relative peak areas were also compared using a two-way ANOVA using JMP Pro 12 statistical software with significant differences further separated using Tukey-Kramer Honest Significant Difference (HSD) (P ≤ 0.05).

3. Results

3.1. Moisture content

The experimental trial was not significant for moisture content levels in any manure type, treatment, or feed rate. The initial poultry, swine and dairy manure had significantly different moisture contents of 77.25% 73.77% and 84.16%, respectively (Fig. 2). For all manure types and feed rates, the moisture content of BSFL digested after development of 90% prepupae and non-digested manure was significantly lower than in the initial manure. No significant difference for BSFL digested or non-digested was found between manure types. Overall the effect of manure type and feed rate significantly impacted the associated final moisture content of BSFL digested manure and non-digested manure.

3.1.1. Poultry manure

On average, BSFL reduced the moisture content of poultry manure by 82.61% in the 18 g feed rate (Fig. 2a) and 45.63% in the 27 g feed rate (Fig. 2b). The non-digested manure moisture content was reduced an average 66.47% in the 18 g feed rate and 31.40% in the 27 g feed rate. The BSFL digested manure underwent a greater reduction in moisture at both feed rates compared to the non-digested manure.

3.1.2. Swine manure

On average, BSFL reduced the moisture content of swine manure by 81.69% in the 18 g feed rate (Fig. 2a) and 50.20% in the 27 g feed rate (Fig. 2b). The non-digested manure moisture content was reduced an average of 83.87% in the 18 g feed rate and 41.00% in the 27 g feed rate in manure. The BSFL digested manure, therefore, underwent a greater reduction in moisture than the non-digested manure at the 27 g feed rate; whereas the treatments had comparable reductions in moisture at the 18 g feed rate.

3.1.3. Dairy manure

On average, BSFL reduced the moisture content of dairy manure by 87.13% in the 18 g feed rate (Fig. 2a) and 54.25% in the 27 g feed rate (Fig. 2b). The non-digested manure moisture content was reduced an average of 87.64% in the 18 g feed rate and 55.62% in the 27 g feed rate. The BSFL digested manure therefore, reduced moisture content to comparable levels of that of non-digested manure regardless of the feed rate.

3.2. Number of VOCs

The number of VOCs in a given treatment differed significantly among the three manure types (Fig. 3). Both the initial and the BSFL digested manure emitted significantly more VOCs from poultry manure than dairy and swine. However, the number of VOCs from swine manure was not significantly different from that from...
dairy manure in either initial or BSFL digested manure. In non-digested manure, the number of VOCs emitted was not significantly different by manure type. Further analyses on the number of VOCs were conducted within a given manure type.

Across manure types, treatment and feed rate did not significantly interact as related to the number of VOCs present. Furthermore, neither trial nor feed rate were significant and were therefore excluded from analyses. For each manure type, the number of VOCs was significantly different between initial, BSFL digested and non-digested manure.

In poultry manure, BSFL digested manure underwent a 49.06% reduction in VOCs compared to a 13.00% reduction in non-digested manure. In swine manure, BSFL digested and non-digested manure had similar decreases in the number of VOCs, with reductions of 41.45 and 38.46%, respectively. In dairy manure, BSFL manure only underwent a 20.02% decrease in the number of VOCs compared to a 30.00% decrease in non-digested manure.

3.3. Select odorous VOCs in manure

A summary of the average relative amounts of nine select odorous VOCs across manure types is presented in Table 1. There was neither a significant interaction between trial and feed rate nor treatment and feed rate for any of the selected VOCs in any manure type.

With the exception of pentanoic acid, which was not detected in any swine manure samples, the amount of select VOCs present in BSFL digested and non-digested manure was significantly less than that of the initial manure in all manure types. The amount detected in BSFL digested and non-digested manure, however, did not differ significantly.

In several instances, BSFL digestion reduced the amount of a VOC below the detectable level. This was seen for 2-methylpropanoic acid in poultry manure, phenol, 4-methylphenol, and indole in swine manure, and 4-methylphenol, indole and 3-methyl indole in dairy manure. There were no fatty acids detected in either the BSFL digested or non-digested swine or dairy manure.

In poultry manure, BSFL digestion reduced relative amounts of the nine select odorous VOCs measured by 95–100%. A wider range of amount reductions were observed in the non-digested manure where relative quantities of select compounds were reduced 22–98%. In samples where the select VOCs were detected, BSFL diges-

Table 1

<table>
<thead>
<tr>
<th>Manure type</th>
<th>Volatile organic compounds</th>
<th>Treatment</th>
<th>Initial</th>
<th>BSFL digested</th>
<th>Non digested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry</td>
<td>Phenol</td>
<td></td>
<td>462.05 ± 57.5\textsuperscript{a}</td>
<td>3.00 ± 2.6\textsuperscript{b}</td>
<td>22.11 ± 6.4\textsuperscript{b}</td>
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<td></td>
<td>4-Methylphenol</td>
<td></td>
<td>512.06 ± 70.4\textsuperscript{a}</td>
<td>2.06 ± 1.5\textsuperscript{b}</td>
<td>16.03 ± 2.8\textsuperscript{b}</td>
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<td></td>
<td>Indole</td>
<td></td>
<td>82.90 ± 12.3\textsuperscript{a}</td>
<td>0.03 ± 0.3\textsuperscript{b}</td>
<td>15.32 ± 8.3\textsuperscript{b}</td>
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<td>3-Methylindole</td>
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<td>24.50 ± 3.8\textsuperscript{a}</td>
<td>0.04 ± 0.0\textsuperscript{b}</td>
<td>18.09 ± 7.2\textsuperscript{b}</td>
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<td>Propanoic acid</td>
<td></td>
<td>63.61 ± 27.3\textsuperscript{a}</td>
<td>7.64 ± 7.6\textsuperscript{b}</td>
<td>4.80 ± 3.5\textsuperscript{b}</td>
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<td>2-Methylpropanoic Acid</td>
<td></td>
<td>17.49 ± 9.7\textsuperscript{a}</td>
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<td>13.58 ± 9.4\textsuperscript{b}</td>
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<td>Butanoic acid</td>
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<td>1871.98 ± 1583.6\textsuperscript{a}</td>
<td>32.55 ± 32.6\textsuperscript{b}</td>
<td>39.34 ± 28.3\textsuperscript{b}</td>
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<td>3-Methylbutanoic acid</td>
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<td>347.34 ± 46.8\textsuperscript{a}</td>
<td>17.18 ± 16.9\textsuperscript{b}</td>
<td>49.96 ± 36.0\textsuperscript{b}</td>
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<td>Pentanoic acid</td>
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<td>832.40 ± 365.2\textsuperscript{a}</td>
<td>12.94 ± 12.9\textsuperscript{b}</td>
<td>49.52 ± 31.4\textsuperscript{b}</td>
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<td>Swine</td>
<td>Phenol</td>
<td></td>
<td>243.67 ± 64.2\textsuperscript{a}</td>
<td>0.00 ± 0.0\textsuperscript{b}</td>
<td>9.04 ± 9.0\textsuperscript{b}</td>
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<td></td>
<td>4-Methylphenol</td>
<td></td>
<td>860.95 ± 215.8\textsuperscript{a}</td>
<td>0.00 ± 0.0\textsuperscript{b}</td>
<td>33.49 ± 33.5\textsuperscript{b}</td>
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<td>Indole</td>
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<td>177.61 ± 37.8\textsuperscript{a}</td>
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<td>19.54 ± 19.5\textsuperscript{b}</td>
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<td>283.3 ± 224.2\textsuperscript{a}</td>
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<td>13.20 ± 14.3\textsuperscript{b}</td>
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<td>Propanoic acid</td>
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<td>2-Methylpropanoic Acid</td>
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<td>16.27 ± 5.2\textsuperscript{a}</td>
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<td>0.00 ± 0.0\textsuperscript{b}</td>
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<td>Butanoic Acid</td>
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<td>170.05 ± 43.7\textsuperscript{a}</td>
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<td>3-Methylbutanoic acid</td>
<td></td>
<td>177.95 ± 30.0\textsuperscript{a}</td>
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<td>Pentanoic acid</td>
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<td>N/A\textsuperscript{a}</td>
<td>N/A\textsuperscript{a}</td>
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<td>Dairy</td>
<td>Phenol</td>
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<td>93.7 ± 17.9\textsuperscript{a}</td>
<td>0.27 ± 0.9\textsuperscript{b}</td>
<td>0.77 ± 2.7\textsuperscript{b}</td>
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<td>4-Methylphenol</td>
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<td>414.00 ± 22.0\textsuperscript{a}</td>
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<td>0.85 ± 0.9\textsuperscript{b}</td>
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<td>Indole</td>
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<td>8.99 ± 4.5\textsuperscript{a}</td>
<td>0.00 ± 0.0\textsuperscript{b}</td>
<td>0.38 ± 0.4\textsuperscript{b}</td>
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<td>66.03 ± 17.3\textsuperscript{a}</td>
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<td>162.01 ± 13.9\textsuperscript{a}</td>
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<td>2-Methylpropanoic acid</td>
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<td>86.27 ± 4.2\textsuperscript{a}</td>
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<td>Butanoic acid</td>
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<td>146.71 ± 6.6\textsuperscript{a}</td>
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<td>90.28 ± 5.9\textsuperscript{a}</td>
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<td>Pentanoic acid</td>
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<td>74.46 ± 4.9\textsuperscript{a}</td>
<td>0.00 ± 0.0\textsuperscript{b}</td>
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</tr>
</tbody>
</table>

\textsuperscript{a} Different letters within a VOC and manure type indicate significant difference (P < .05).

\textsuperscript{ab} Relative areas were obtained by dividing the peak area of the VOC by the peak area of the internal standard, n-Octane, obtained from the chromatogram.
tion reduced the relative amounts by 99–100% compared to 89–96% in non-digested swine manure. Similarly, BSFL digestion reduced detectable VOCs by 99–100% compared to 95–99% in non-digested dairy manure.

4. Discussion

BSFL digestion of manure reduced the emission of select odorous VOCs across all manure types and feed rates tested when compared to amounts present in the initial manure. BSFL were adept at reducing select VOCs at rates of 87% or greater. In the case of 4-methylphenol, indole, skatole and the five fatty acids examined, no levels of VOCs were detected in either digested swine or dairy manure (i.e., 100% reduction). In addition to the benefits presented in previous studies, such as reductions in dry matter (Sheppard, 1994), nitrogen and phosphorus (Myers et al., 2008) and pathogenic Gram-negative bacteria (Erickson et al., 2004; Liu et al., 2008), the ability of BSFL to reduce VOC emissions, particularly those associated with the offensive smell of manure, is an additional benefit to their use as a manure-management tool.

The reduction of select odorous VOCs is believed to be in part due to a decrease of moisture (Brinton, 1998). In the current study, moisture content data (Fig. 2) indicate that manures both with and without BSFL were lower in moisture than freshly-thawed manure. At both feed rates (18 and 27 g), BSFL reduced the moisture content the most in dairy manure; particularly at the 18 g feed rate. Dairy manure also experienced the greatest decreases in moisture at the 18 g feed rate for non-digested manure. The differing levels of moisture reduction experienced by the manure types was expected due to the longer larval development times seen in swine and dairy manure compared to poultry. Furthermore, non-digested manure experienced greater moisture loss at the 18 g feed rates likely due to differing surface area to volume ratios compared to that at the 27 g feed rate. For example, Miller et al. (1974) found that poultry manure with a higher surface area was rendered into a more granular like texture when digested by Musca domestica (Miller et al., 1974). At both feed rates, larvae reached 90% prepupation significantly faster on poultry manure than on swine or dairy (data not presented).

The extended development time of BSFL on the certain manure types could be due to lower nutritional quality (i.e. calories, protein and fat content) (Zhou et al., 2013). For example, Ooincx et al. (2015) observed longer development times with larvae fed poultry, swine and cow manure compared to a control diet of poultry feed (144–215 vs 20 d). Similarly, Nguyen et al. (2013) found that larvae reared on pig manure experienced a significantly longer median time to adulthood (55.33 ± 2.14 d) compared to those reared on a standard diet of poultry feed (42.17 ± 1.30 d) (Nguyen et al., 2013). In fact, median time to adulthood was significantly longer for larvae fed manure compared to those reared on other substrates (liver, kitchen waste, fruits and vegetables) used in the study (Nguyen et al., 2013).

Possibly the increased BSFL development times observed with swine and dairy manure in our study gave the larvae more time to reduce the moisture content via digestion and aeration. Similarly, because VOCs were taken at 90% prepupation, BSFL digested and corresponding non-digested controls for these manure types incubated longer than poultry manure samples and continued to lose moisture in the environmental chamber. Thus, the digested and non-digested swine and dairy manure treatments had lower levels of moisture than poultry manure. The increased moisture reduction in dairy and swine manure might affect the presence of VOCs, such as volatile fatty acids in the samples. However, in some instances, the BSFL were more adept at reducing VOCs than manure without larvae. For example at the 27 g feed rate, the lower moisture content of BSFL digested manure could explain the complete reduction of 2-methylpropanoic acid in poultry manure, and phenol, 4-methylphenol, and indole in swine manure.

The ability of the BSFL to reduce the moisture content of the substrate on which it feeds likely impacts the microbial populations responsible for generating many odorous VOCs as many of these microbes are negatively affected by moisture content (Hansen, 1976). Tate (1978) determined survival of Escherichia coli to be greatest in organic soils that were under flooded conditions; Hagedorn et al. (1978) determined E. coli populations to be highest in a water table following a major rainfall. Similarly, Streptococcus faecalis degrades rapidly under low soil moisture conditions (Kibbey et al., 1978). Moisture content of manure and the effect on bacteria survival have been less thoroughly studied; however, Wang et al. (1996) suggested that dehydration of manure could contribute to the die off E. coli O157:H7 and observed this with fecal Streptococci spp. in a later study (Wang et al., 2004). Himathongkham et al. (1999) examined the survival of E. coli O157:H7 and Salmonella typhimurium in cow manure and slurry and reported similar results: these microbes persisted in wastes with higher moisture contents.

A reduction in microbial populations in manure digested by the BSFL could also contribute to reduction in targeted VOCs. Lalander et al. (2013) saw a 6 log reduction in Salmonella spp. in human feces with BSFL digestion compared to a less than 2 log reduction in the control feces, which had no larvae. Poultry manure inoculated with Salmonella enterica serovar Enteritidis that was digested by BSFL experienced pathogen populations 2.5 log lower than control samples without larvae after three days (Erickson et al., 2004). Lalander et al. (2015) observed a decrease from 10^7 CFU g^-1 to below 1 CFU g^-1 in Salmonella spp. in compost reactors that were supplied a mix of human and pig manure, and organic wastes and allowed to be digested by BSFL. Liu et al. (2008) inoculated dairy manure with E. coli and observed that larvae were successful at reducing the pathogen loads in the manure across all treatments; however, their ability to reduce the pathogen was affected by the amount of manure they were provided and the temperature at which the experiment was conducted. The greatest reduction of E. coli occurred with a manure amount of 50 g at 27 °C which decreased after 72 h from 7.0 log CFU g^-1 to 0.23 ± 3.39 log CFU g^-1.

Reduction in microbial populations reduces many odors associated with manure, as VOCs are the coproducts of different metabolic pathways, which serve as waste products or potential signals and cues for the microbes (Mayrhofer et al., 2006). Bacteria from several genera are responsible for many of the odorous VOCs found in manure (Mackie et al., 1998). Streptococcus spp. are capable of producing ammonia, five of which have been found in swine manure (Russell, 1979). Peptostreptococcus spp. metabolize peptone and amino acids into volatile fatty acids including acetate, formic, propionic, caproic, isobutyric, isovaleric, and isocaproic acids (Mackie et al., 1998). Other bacteria in genera such as Esuberteria, Lactobacillus, Escherichia, Clostridium Propionibacterium, Bacteroides and Megaphaera are responsible for the production of odorous VOCs such as volatile fatty acids indole and sulfur containing compounds (Mackie et al., 1998; Zhu, 2000; Le et al., 2005).

A study by Mayrhofer et al. (2006) found positive correlations between the production of VOCs and the enhancement of microbial growth and conversely negative correlations between microbial inhibition and VOC emissions during the breakdown of household biowastes over a course of 16 d. This relationship between microbes and specific VOC emissions has been demonstrated by several studies (Senecal et al., 2002; Lechner et al., 2005; Jungler et al., 2012) with the hopes of uniquely identifying human bacterial pathogens from specific volatile fingerprints to circumventing biases caused by DNA extraction procedures (Mayrhofer et al.,

![Image](image-url)
2006). Such studies support the concept that the microbial structure of manure affects VOC emissions.

Investigations into how different treatments of manure (e.g., freshly excreted versus thawed manure and rearing temperatures) affect digestion and subsequent VOC emissions are needed. Yasuhara (1983) saw differences in volatile profiles from samples of fresh poultry manure, manure that was immediately frozen and manure that was allowed to age before freezing. Husted (1994) found that methane production from dairy manure increased when stored at higher temperatures. Gibson et al. (1987) noted that different temperature regimes affected the growth of Clostridium botulinum spores in pork slurry. Therefore it is reasonable to hypothesize that different temperature regimes could affect the microbial communities in manure and alter VOC emissions.

5. Conclusions

This study demonstrated the ability of BSFL to reduce levels of odorous compounds emitted from different livestock manure types (e.g., 100% reduction of indole and fatty acids in swine and dairy manure, Table 1). This, along with aforementioned benefits, makes BSFL an attractive alternative means of manure management that is both environmentally and economically friendly. Not only would those using BSFL to process manure experience lower levels of odor emissions, but also an increased reduction in dry matter (Sheppard, 1983), pathogens (Erickson et al., 2004) and overabundant nutrients (Myers et al., 2008). All the while larvae are acquiring valuable biomass, and in turn it could be used as feedstock for poultry (Hale, 1973), swine (Newton et al., 1977), and several species used in aquaculture (Bondari & Sheppard, 1987; St-Hilaire et al., 2007; Kroekel et al., 2012). Furthermore, larval stock has also been successfully rendered into biodiesel (Li et al., 2011; Zheng et al., 2012). Those implementing a BSFL system on site could reduce the need for ancillary management equipment, lower feed costs, and generate profit by selling larvae or larval-based products.

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