THE EFFECTS OF VERMICOMPOSTING AND PRE-COMPOSTING ON FECAL COLIFORM BACTERIA OCCURRENCE IN ANIMAL MANURES

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT HILO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL CONSERVATION BIOLOGY AND ENVIRONMENTAL SCIENCE

AUGUST 2020

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Acknowledgement

I would like to acknowledge all those who helped play a critical role in completing this research. First of all, I would like to thank my parents for all their love and support, without you I would not have attained such great success. I would like to thank my wife, whose encouragement throughout this process kept me focused through the difficult times. Additionally, I would like to thank my committee Dr. Arancon, Dr. Awaya, and Dr. Shintaku for all the guidance and direction throughout this exploration. Lastly, I would like to thank Hauʻoli Mau Loa foundation for seeing greatness in me and improving the future of Hawaiʻi one mind at a time. Thank you all for your unwavering support.

Abstract

The presence of *Escarichia coli* in animal manures and strict requirements for use in crop production emphasizes the need for viable treatment methods. The purpose of this study is to determine the efficacy of vermicomposting to suppress *E. coli* and total coliforms, from hog and horse manure that is either pre-composted or directly fed to *Eisenia fetida*, and to determine if there are significant differences among these methods. Using a general linear model this study analyzed the occurrence of *E. coli* and total coliforms in hog and horse manure that was subjected to thermophilic composting prior to vermicomposting and direct vermicomposting for 90 days. The occurrence of *E. coli* and total coliforms in hog and horse manure were non-detectable after vermicomposting for 90 days. This study definitively states that vermicomposting is an effective way to treat hog and horse manure for the use in crop production. Further research on the mechanisms behind suppression of *E. coli* would be beneficial to manure management.

Keywords: Vermicompost, Compost, *E. coli*, Total coliforms

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LIST OF ABBREVIATIONS

MPN:	**Most Probable Number**
BSAAO	**Biological Soil Amendments of Animal Origins**
CDC	**Center for Disease Control**
WHO	**World Health Organization**
MUG	**4-methyl-umbelliferly, β-D-glucuronide**
ONPG	**O-nitrophenyl, β-D-galactopyranoside**
CFU	**Colony Forming Units**
USGS	**United States Geological Survey**
FSMA	**Food Safety Modernization Act**

Chapter 1. Introduction

Use of Animal manures in agriculture

The use of animal manures in agriculture dates back 12,000 years to the Neolithic era of western Asia and Europe, where farmers manured fields prior to cultivation of cereal and pulse crops (Bogaard et al. 2013). Manure fertilization to increase growth and yield is still an integral part of crop production, but on much larger scales and intensity associated with confined animal feeding operations. In the United States alone there are 26,586 confined feedlots for cattle alone, that contribute to the roughly 31 million head of cattle in the United States (Drouillard 2018). The vast amount of manure from feedlot operations around the world is not specifically quantified but *Junior et al. (2013)* stated that 26% of the feedlots in Brazil produced 2 - 4 kg of manure per head of cow a day. This manure is collected, stored, used for fuel, or composted. Farmers apply manures directly to fields as solids or liquids, through spreaders or large irrigation lines. Application of manure on crops and pasture can increase production and reduce costs of fertilizer inputs (Wilkinson 1979). The use of animal manures classified as biological soil amendments of animal origin (BSAAO) in agriculture in the United States has strict guidelines for use in the commercial production of crops due to potential occurrence and transfer of harmful human pathogens. The Food Safety Modernization Act (Table 1) states that BSAAO must be treated by any chemical, physical and/or biological treatment process that is scientifically validated to meet microbiological standards (FDA. 2020). Manures which have undergone a treatment process that does not follow approved standards to remove harmful pathogens including incomplete

treatment, presence of any contaminant and other forms of hazards after treatment (FDA. 2020) are classified as untreated and are not approved for use in food production.

Biological Soil Amendments of Animal Origins (BSAAO)

BSAAO contain many enteric bacteria that can cause mild to severe harm to human health if not managed properly. Some of the most common and important pathogens are *Listeria monocytogenes* (*L.monocytogenes*), *Salmonella* species (*Salmonella spp.*), *Escherichia coli O157:H7* (*E. coli*), and other fecal coliforms. Although there are many other pathogens that can cause harm, salmonellosis, listeriosis, and infection with *E. coli* are of greater public health concern currently because these bacteria have multiple hosts, and the prevalence of infection has increased over the years (Pell AN. 1997). In particular *E. coli* has caused many outbreaks of human infection through the food supply from 2006 - 2020 in an assortment of products such as ground beef, spinach, salad mixes, clover sprouts, soy nut butter, lettuce, etc. (CDC. 2020.), *Fairbrother and Nadeau (2006)* mention the commonality of *E. coli* in a wide range of farm and wild animals. Beyond its infection of humans *E. coli* is regularly used as an indicator species for the detection of harmful human pathogens (Christensen et al. 2002).

Characteristics of E. coli

The family Enterobacteriaceae can be classified into 6 groups: 1) enteropathogenic, 2) enterotoxigenic, 3) enteroinvasive, 4) enteroaggregative, 5) diffusely adherent, and 6) shiga toxin- producing bacteria also referred to as enterohemorrhagic (Estrada-Garcia. et al. 2013). *E. coli* is a gram-negative, facultatively anaerobic (possessing both a fermentative and respiratory metabolism) rod-shaped bacteria. *E. coli* cells are typically 1.1 – 1.5 μm-wide, 2 – 6 μm-long and occur as single straight rods. They can be either motile or nonmotile, and when motile produce lateral, rather than polar, flagella. In addition to flagella, many strains produce other appendages such as fimbriae or pili, which are proteinaceous structures (or appendages or fibers) that extend outward from the bacterial surface and play a role in attachment to surfaces including other cells or host tissues (Desmarchelier & Fegan 2016).With many variations of *E. coli* among different classes the focus will be on serotype O157:H7 a common strain found among foodborne illness.

Etiology of E. coli

E. coli is a pathogen that is commonly found in the intestines of livestock and survives in their feces which contribute to the infection of humans. Many outbreaks of *E. coli* have been traced back to many cattle feedlot operations that led to secondary fecal contamination of agricultural fields. The ingestion of *E. coli* infected products attacks the alimentary tract and causes cramps with hemorrhagic diarrhea from the production of shiga toxins (Ameer et al. 2020). The incubation period of *E. coli* has not been definitively determined but the CDC & WHO have

reported 3 - 8 days and some outbreaks have reported a median of 9 days (Awofisayo-Okuyelu et al. 2019). The application of manure has therefore been recommended to be applied at a reasonable time usually 2 weeks or longer prior to harvesting of the crop or grazing to allow climatic control of *E. coli* from infecting humans and animals. The secretion of shiga toxins can cause life threatening hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura, and although ingestion is the main pathway for infection there have been accounts of person to person transmission (Padhye & Doyle 1992).

Epidemiology and Disease management

E. coli moves through the food system through the use of animal manures as direct field applications, contaminated surface water, and post harvest contamination in packing facilities (Fairbrother & Nadeau 2006). *E. coli* is fairly persistent in manures. *Nicholson et al. (2005)* recognized that *E. coli* survived for three months in manures of hog, beef, and chicken litter stored in feedlot yards in unturned piles and were detectable in field applications after one month although most field samples of *E. coli* could not be detected after four days. The removal of *E. coli* from soils that have been amended with manure have been linked to the antagonistic activity of soil bacteria (Jiang et al. 2002). Additionally, it has been reported that *E. coli* can also be removed by application of heat. *Hess et al. (2013)* reported that manures subjected to heat >55 ºC will kill *E. coli* effectively.

Composting

One of the processes that can eliminate *E. coli* due to heat is composting (Turner 2002), which is the process of biological decomposition of organic wastes. Compost is composed of carbon and nitrogen at an ideal ratio of 30:1 with adequate moisture creating a favorable environment for thermophilic microorganisms to thrive. Thermophilic microorganisms thrive at temperatures ranging from 39 – 77 ºC and feed off the carbon and nitrogen to produce a biologically rich humus that improves the structure of the soil, increases numbers of beneficial soil microorganisms (Fuchs 2010), promotes plant production, and pathogen suppression (Pace et al. 1995). *Ouédraogo et al. (2001)* found that the addition of 1 $g \cdot kg^{-1}$ of compost to soil in sorghum fields in West Africa increased the cation exchange rate by 4.3 $mol \cdot kg^{-1}$ and improved soil physical properties. *Logsdon et al. (2017)* found that an addition of two yard waste composts incorporated at a depth of 5 – 10 cm resulted in a 24 and 50% increase in infiltration rate on urban soils. The use of compost in the form of an actively aerated liquid extract or compost tea on the production of muskmelon, increased the fresh fruit weight (1.42 kg $plant^{-1}$) and ºBrix (13.62) content (Naidu et al. 2013). Compost not only improves yield, and soil chemical and physical properties, but it also suppresses harmful human pathogens that contribute to foodborne illness. Composting was shown to eliminate *E. coli* in infected cattle manure through the process of thermophilic decompositions of piles that were able to keep temperatures of 55 ºC for a minimum of 180 days (Hess et al. 2004). Additionally, *Turner (2002)* found that *E. coli* when heated to 55 ºC was rendered inactive after two hours. Although temperature is a key factor to quell pathogens, *Droffner & Brinton (1995)* found that *E. coli* and *Salmonella* both survived for

59 days at 60 ºC but were no longer detectable when temperatures dropped to 40 ºC for the curing process. *Piceno et al. (2017)* also reported the removal of *E. coli* and other opportunistic harmful pathogens in human waste was achieved with thermophilic composting with subsequent curing for one year. The presence of endemic microorganisms in compost have been shown to delay the reduction of *E. coli* in amended soils through competition and antibiosis (Cutler 2016). Other organic amendments that contain microorganisms and show potential to remove pathogens are vermicomposts. Unlike thermophilic composting, vermicomposting is a mesophilic process that may eliminate *E. coli* by factors other than temperature.

Vermicomposting

Vermicomposting is a process utilizing epigeic earthworm species and microorganisms in the biological decomposition and stabilization of organic wastes and residues at mesophilic temperatures to produce a fine humus-like structure with high porosity, aeration, drainage, and water holding capacity (Arancon et al. 2004). Vermicomposting occurs at temperatures of 15 – 27 ºC with an adequate moisture content of 80 - 90%. Vermicompost is a desirable soil amendment which increases plant yields, populations of beneficial microorganisms, and plant growth hormones while improving the structure of soil (Arancon et al. 2004) and accelerating rates of germination, flowering, and growth (Arancon et al. 2008). Further *Lazcano et al. (2008)* reported that vermicomposting degrades cattle manure at a higher rate than thermophilic composting. *Gutiérrez-Miceli et al. (2007)* noted a significant improvement on the growth of tomatoes, with plants amended with vermicompost being 11 cm taller and 0.40 cm thicker than

plants not amended. Vermicompost also substituted as potting substrate increased the rate of germination in tomato seeds, and subsequently improved overall productivity of mature plants even transplanted into fertilized field soil (Zaller 2007). Additionally, vermicompost has the ability to reduce the presence of harmful human pathogens.

Ravindran et al. (2016) stated a reduction of fecal coliforms in fermented tannery waste to non detectable levels after 21 day for vermicomposting, and 25 days for thermophilic compost. *Eastman (1999)* identified that pathogen reduction in biosolids through vermicompost without pre-composting significantly eliminated pathogens after 72 hours, with a 99.99% reduction of *Salmonella spp*. inoculated at 4.6 billion cells 25 ml^{-1}, a 98.7% reduction of fecal coliforms inoculated at 8.3 billion Most Probable Number g^{-1}(MPN), and a 98.9% reduction of enteric viruses inoculated at 197,000 Colony Forming Units 4 g^{-1}(CFU). Despite the occurrence of a number of reports that vermicomposting can significantly reduce or eliminate human pathogens, current studies seem to only refer to thermophilic composting followed by vermicomposting as an acceptable process that can safely remove human pathogens. *Nair et al. (2006)* stated that although composting was instrumental at reducing total mass of the organic waste, the process of additional vermicomposting of the pre-composted material was effective at inactivating harmful pathogens. The process *Nair et al. (2006)* used was pre-composting organic wastes for 9 days, followed by 2.5 months of vermicomposting rendering it a pathogen-free amendment. It is unclear, however, if the production of pathogen-free amendment was achieved by pre-composting alone, vermicomposting or the combination of both. Establishing which process could eliminate pathogens in manures would have tremendous implications on the current management and regulations on use of manures in crop production.

Research Objectives

The study aims to determine the efficacy of vermicomposting to remove *E. coli* and total coliforms from animal manures. It also aims to determine if pre-composting is a necessary step to remove *E. coli* and total coliforms prior to vermicomposting.

Chapter 2. Materials and Methods

Eisenia fetida was the species of epigeic earthworm used for this study. forty - 6¾" x 12¾" x 5" polypropylene plastic containers were used between two manures, (1) horse and (2) hog and wood chips. Each manure was divided into two classifications, vermicompost (non-heat treated) and pre-composted (heat treated) described below in section B.

A. Vermicomposting

1. Horse manure: was collected from the University of Hawaii at Hilo agricultural research farm equestrian program, manure was collected fresh 1 - 2 days prior to thermophilic compost initiation. Plastic containers measuring 6¾" x 12¾" x 5" with 8 cm of horse manure were inoculated with approximately 10g (∓ 0.01g) of epigeic earthworm species *E. fetida*, replicated 10 times. Water was added to meet 80 – 90% moisture requirement for vermicomposting. The containers were set aside for 90 days to meet the National

Organic Program (NOP) standards for vermicomposting, and moisture was monitored and maintained.

2. Hog manure: was collected from the University of Hawaii at Hilo agricultural research farm swine program, 1 - 2 days prior to thermophilic composting initiation. The hog manure was mixed with wood chips to balance carbon to nitrogen ratio. Eight centimeters of hog manure mix was added into plastic containers measuring 6¾" x 12¾" x 5" and were inoculated with approximately 10g (∓ 0.01g) of epigeic earthworm species *E. fetida*, replicated 10 times. Water was added to meet 80 – 90% moisture requirement for vermicomposting. The containers were set aside for 90 days to meet the NOP standards for vermicomposting, and moisture was monitored and maintained.

B. Pre-composting prior to vermicomposting

1. Horse manure: was collected from the University of Hawaii at Hilo agricultural research farm equestrian program, manure was collected in 114 L plastic containers for a period of 3 to 5 days until the desired amount was reached roughly 1 meter^3. Since horse manure has an ideal carbon to nitrogen ratio of 30:1, no other carbon was added. The horse manure was formed into a 1 meter^3 pile to have enough biomass to generate microbial activity and self insulate to retain heat. The horse manure compost was kept at a consistent temperature between 55 – 77 ºC for 15 days and turned at least 5 times, to meet NOP standards for compost. After 15 days at 55 – 77 ºC and at least 5 turns, eight centimeters of pre-composted horse manure was added into plastic containers measuring 6¾" x 12¾" x 5" and was inoculated with approximately 10g (∓ 0.01g) of epigeic

earthworm species *E. fetida*, replicated 10 times; water was added to meet 80 – 90% moisture requirement for vermicomposting. Once fed, the containers were set aside for 90 days to meet the NOP standards for vermicomposting, and moisture was monitored and maintained.

2. Hog manure: was collected from the University of Hawaii at Hilo agricultural research farm swine program, manure was collected in 114 L plastic containers for a period of 3 to 5 days till a desired amount was reached roughly 1 meter^3. Hog manure was mixed with wood chips, to obtain a 30:1 carbon to nitrogen ratio. The hog manure and wood chip mixture was formed into a 1 meter^3 pile to have enough biomass to generate microbial activity and self insulate to retain heat. The compost was kept at a consistent temperature between 55 – 77 ºC for 15 days and turned at least 5 times, to meet NOP standards for compost. After 15 days at 55 – 77 ºC and at least 5 turns, eight centimeters of pre-composted hog manure and wood chip mix was added into plastic containers measuring 6¾" x 12¾" x 5" and was inoculated with approximately 10g (∓ 0.01g) of epigeic earthworm species *E. fetida*, replicated 10 times.Water was added to meet 80 – 90% moisture requirement for vermicomposting. Once fed the containers were set aside for 90 days to meet the NOP standards for vermicomposting, and moisture was monitored and maintained. Additional note the room was temperature controlled to 21 - 23 ºC via A/C unit.

Sampling and Analyses

Baseline samples of both feedstocks (horse manure and hog manure wood chip mix) were collected prior to pre-composting, after 15 days of composting at 55 – 77 ºC and at least 5 turns, and finally after 90 days of vermicomposting, to test for MPN of *E. coli* and total coliforms using Colilert quanti tray. Colilert quanti tray uses specific nutrient indicators ONPG (O-nitrophenyl, β-D-galactopyranoside) for Coliforms, and MUG (4-methyl-umbelliferly, β-D-glucuronide) for *E. coli* to simplify detection. Each pathogen uses their respective enzyme to metabolize the indicators in the Colilert quanti tray. For coliforms they use β-galactosidase to metabolize ONPG to change it to yellow, and *E. coli* use β-glucuronidase to metabolize MUG to change it to become fluorescent (Brown 2019).

Samples for analysis were collected from 6 quadrants of each pile, and or container. Two large spoon fulls from 3 quadrants on the surface (end, end and middle), and 2 large spoon fulls from 3 quadrants of subsurface at least 1 inch deep (end, end and middle). For baseline and compost piles samples were also pulled from the center. Samples were placed in sterile bags mixed and shaken to have uniformity, then labeled accordingly. Prior to pathogen enumeration, samples were processed following *Economy & Garson-Shumway (2017)* standard operating protocol for processing soil sediment for enumeration. Samples were weighed to 10g then placed into 1L bottles for elution/shaking. 200 ml of 0.15M NaCl was added to each 1L sterile bottle then placed on a shaker table at 100 rpms for 45 minutes. After shaking, each elution bottle was

allowed to settle for 10 minutes. The specific aliquots were removed from the 1L bottle and serially diluted to 1:10 with 0.15M NaCl, to allow for proper enumeration with the Colilert quanti tray. For the baseline sample 4 serial dilutions were sent in for enumeration to determine best possible results from subsequent samples, thus utilizing the later dilution of 1:10. Total MPN g_{dw}^{-1} were calculated using USGS fecal indicator bacteria density calculations for MPN ($\frac{(\frac{MPN}{100ml})\times Sediment\ Dilution\ Factor}{Proportional\ Dry\ Weight}$)(Myers et al. 2014). All data was square root transformed for normal distribution, before running statistical analysis. SAS statistical package version 9.4 (SAS Institute, Carry, NC) was used to process data in proc GLM. Least significant difference (LSD) at 0.05 level of significance was used to separate differences between means.

Chapter 3. Results

Baseline

Baseline samples showed high occurrence of *E. coli* (310 MPN 100 ml^{-1} and 410 MPN 100 ml^{-1}) and total coliforms (141360 MPN 100 ml^{-1} and 840 MPN 100 ml^{-1}) in both horse and hog manure, respectively (Table.2). The reduction of *E. coli* and total coliforms in thermophilic composting were significant in horse manure with a 100% reduction from baseline samples to zero after 15 days and 5 turns at an average temperature of 63 ºC; whereas hog manure showed a

27% increase in the occurrence of *E. coli* and a 6,057% increase in total coliforms compared to the baseline sample after 15 days and 5 turns at an average temperature of 55.5 ºC (Table.2).

Vermicompost

After 90 day of vermicomposting both horse and hog manure had a significant ($P < 0.0001$) reduction in the occurrence of *E. coli* and total coliforms, with *E. coli* and total coliforms being a 100% reduction in both feedstocks compared to baseline samples (Figure 1 & 2). The reduction of fecal coliforms meets FSMA biological standards for BSAAO, with hog manure having 0.009 MPN g_{dw}^{-1} of *E.coli* and total coliforms and horse manure having 0.005 MPN g_{dw}^{-1} of *E. coli* and total coliforms (Figure 3 & 4).

Pre-Composting + Vermicomposting

The occurrence of *E.coli* and total coliforms in the composted horse and hog manure after 15 days and 5 turns followed by 90 days of vermicomposting was significant ($P<0.0001$), with a 100% reduction compared to the baseline samples of horse and hog manure (Figure 1 & 2). The reduction of fecal coliforms meets FSMA biological standards for BSAAO, with hog manure having 0.006 MPN g_{dw}^{-1} of *E. coli* and total coliforms and horse manure having 0.011 MPN g_{dw}^{-1} of *E. coli* and total coliforms (Figure 3 & 4).

Comparison of pre-composting and direct Vermicomposting

There was no significant difference between pre-composting and direct vermicomposting of horse and hog manure in the MPN of *E. coli* and total coliforms.

Chapter 4. Discussion

This study is unique for its use of baseline samples, which differed from other research by not inoculating with *E. coli*. Baseline samples of horse and hog manure both indicated a high occurrence of *E. coli* and total coliforms in this study. The use of heat through thermophilic composting was sufficient to suppress the occurrence of *E. coli* and total coliforms in horse manure following NOP standards with a consistent temperature of 63 ºC *Droffner & Brinton (1995)* found *E. coli* has been reported to survive temperatures in thermophilic compost piles at temperatures of 60 ºC but are undetectable during 40 ºC curing stage. Though no curing stage was done for this study *E. coli* occurrence was reduced in horse manure, but could explain the increase in hog manure. Similarly since both manures never underwent a curing time this increase in coliforms in just the hog manure is more likely linked to nutrient composition of the manures. *Cutler (2016)* reported similar results with an increase of *E. coli* in thermophilic composting where high levels of nitrogen composition in the compost sustained and increased the growth. There is potential that hog manure in this study contains higher nitrogen content compared to horse manure, as *Brown (2013)* compiled available nutrients from many livestock

manure sources, with solid hog manure having 3.8 kg tonne^{-1} average available nitrogen and horse having 1.2 kg tonne^{-1} available nitrogen on average.

Vermicompost reduced the occurrence of fecal coliforms to undetectable levels in 90 days, the reduction of *E. coli* and total coliforms could be attributed to microbial competition similar to *Hénault-Ethier et al. (2016)* which reported that high levels of microorganisms were shown to decrease the occurrence of inoculated *E. coli* in finished vermicompost with or without epigeic worms after 18 - 21 days. Though our study differed in the use of raw manure feedstocks to create finished vermicompost with non-detectable fecal coliforms with epigeic worms, the process of degradation supplied by worms physically alters the the feedstock as *Aira and Domínguez (2009)* reported that the process of fragmentation and conditioning of the substrate by earthworms, increasing surface area for growth of microorganisms and altering its biological activity. Though the reduction over time was not a part of this study, *Ravindran et al. (2016)* reported non-detectable levels of *E.coli* after 25 days, showing that there is some variation in pathogen suppression.

Pre-composting animal manures prior to vermicomposting reduced the occurrence of fecal coliforms to non-detectable levels which was similar to *Nair et al. (2006)* report stating that, the time difference in the combination of pre-composting to the length of vermicomposting was slightly different but resulted in no occurrence of *E. coli* and fecal coliforms. The shortened duration of time to achieve undetectable level in *Nair et al. (2006)* could be attributed to the feedstock material which contained less *E. coli* (>110 MPN g^{-1}) to the high amount of (410 &

310 MPN 100 ml^{-1}) of hog and horse manure in this study. Additionally *Ndegwa and Thompson (2001)* reported similar results with pre-composting prior to vermicomposting to meet a safe biological standard. The additional step of pre-composting feedstocks results in a reduction of harmful pathogens if temperatures are high enough but can also increase the occurrence of fecal coliforms if adequate temperatures are not met, thus the addition of vermicomposting ensures the reduction of harmful pathogens in these cases.

There was no difference in the reduction of fecal coliforms between either method of manure management between both feedstocks. Similar to *Nair et al. (2006)* the suppression of pathogens seems to have occurred in the vermicomposting process. *Monroy et al. (2009)* found that pig manure that passed through the gut of *E. fetida* in a vermicomposting operation significantly reduced the total number of coliform populations. The passing of manure through the gut of *E. fetida* is one mechanism in reducing the occurrence of fecal coliforms in the absence of thermophilic decomposition, additionally *Lazcano (2008)* reported that *E. andrei* appeared to modify the degrading activity of the manure to a much greater extent than the active phase of composting which was reflected by the lower EC, C to N ratio and pH, as well as by a more gradual release of P. Similar to findings by *Cutler (2016)* having a lower nutritional content drives microbial competition potentially decreasing the survivability of the fecal coliforms. This was achieved similarly in the manure feedstocks that were pre-composted prior to vermicomposting. With sufficient heat generation during the active composting stage, which reduced the total pathogen load, the subsequent effects of vermicomposting could have eliminated and rendered the manure inhospitable if any survival occurred. The presence of

endemic microbes from the vermicomposting process as described in *Hénault-Ethier et al. (2016)* reporting that antagonism in the form of antibiosis is a common defence in microbe rich and highly competitive environments with *Pseudomonas spp.* in their samples against *E. coli.*

Chapter 5. Conclusion

This research is important for the use of agricultural food production and waste management. With an increasing human population the need for a soil amendment that reduces impacts to the environment and that utilizes a harmful waste product is crucial. Direct vermicomposting of manures has potential to reduce the need for extra processing potentially increasing economic gain. Though the material has not undergone thermophilic processes to sterilize weed seeds the use in vermicompost tea would alleviate the economic pressure of weed competition and increase crop yields. There is a need for more research on the length of time finished vermicompost will stay *E. coli* and total coliform free. This is an important consideration for these regulations on the different applications pertaining to safe use of vermicompost and their teas in food production. This study focused on the efficacy of *E. fetida* to remove *E. coli* and total coliforms from animal manures and though positive results occurred, the use of many different earthworm species in vermicomposting operations poses the need for further research of their ability to remove fecal coliforms. Secondly, this study focused on the differences between management among vermicomposting animal manures which resulted in no significant difference in *E. coli* suppression, indicating there is no need to pre-compost prior to vermicomposting for meeting current microbial food safety standards. In spite of the fact that

direct vermicomposting can eliminate fecal coliforms in hog and horse manure , there are many other organic wastes that can be subjected to vermicomposting that need to be further investigated for their safe use in food production.

Clearly, vermicomposting is an effective way to process animal manures with high levels of fecal coliforms to be safely used in food production, from both pre-composting manures and direct feeding to *E. fetida* after 90 days. Future studies can be done using different feedstocks to determine if fecal coliform suppression could occur. Additionally the use of different epigeic earthworms and their ability to reduce fecal coliforms in manure and other substrates is important, since earthworm distribution and availability differ between climatic zones. Also future research to elucidate other mechanisms such as antibiosis, synergism, and induced system resistance on pathogen mediation would be beneficial to the management of harmful human pathogens from BSAAO.

Table 1: The minimum requirements for microbial standards of BSSAO for the Food Safety Modernization Act Produce Safety Rule.

21 CFR § 112.55	The Microbial Standard is-
L.Monocytogenes	Not detected using a method that can detect one colony forming unit (CFU) 5 g^{-1} (or milliliter, if liquid is being sampled) analytical portion.
Salmonella species	Not detected using a method that can detect three most probable numbers (MPN) 4 g^{-1} (or milliliter, if liquid is being sampled) of total solids.
E. coli O157:H7	Not detected using a method that can detect 0.3 MPN 1 g^{-1} (or milliliter, if liquid is being sampled) analytical proportion.
Fecal coliforms	Less than 1,000 most probable numbers (MPN) per gram (if liquid is being sampled) of total solids.

Table 2: MPN 100 ml^{-1} of *E. coli* and total coliforms in baseline feedstock samples and composted feedstock samples rendered from Colilert Quanti Tray.

Sample I.D.	*E.coli*		Total Coliforms	
	Baseline	Pre-composted	Baseline	Pre-composted
Horse	310	0	141360	0
Hog	410	520	840	51720

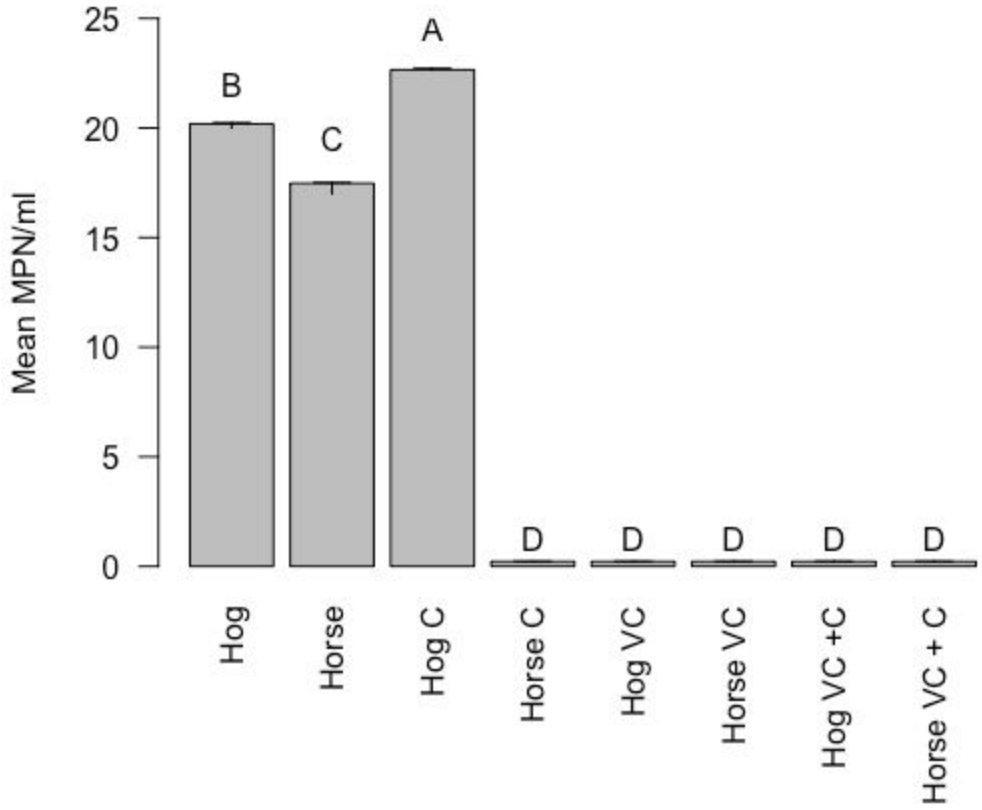

Figure 1: Means of MPN ml $^{-1}$ (square root transformed) of *E. coli* from samples of hog and horse manure under different decomposition management. Baseline samples (hog, horse), thermophilic composted manures (C) after 15 days with 5 turns at temperatures between 55 ºC - 70 ºC, vermicomposted manures (VC), and pre-composted followed by vermicomposting(VC+C) after 90 days of exposure to *E. fetida*. Means with the same letter(s) are not significantly different at 0.05 level of significance.

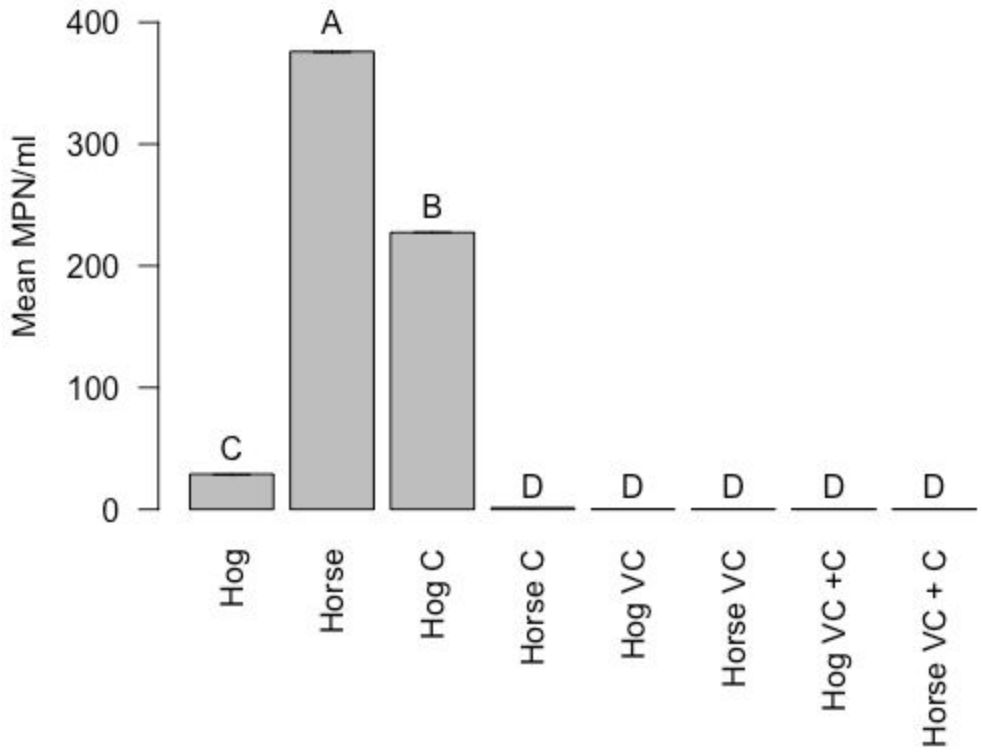

Figure 2: Means of MPN ml $^{-1}$(square root transformed) of total coliforms from samples of hog and horse manure under different decomposition management. Baseline samples (hog, horse), thermophilic composted manures (C) after 15 day with 5 turns at temperatures between 55 ºC - 70 ºC, vermicomposted manures (VC), and pre-composted followed by vermicomposting (VC+C) after 90 days of exposure to *E. fetida*. Means with the same letter(s) are not significantly different at 0.05 level of significance.

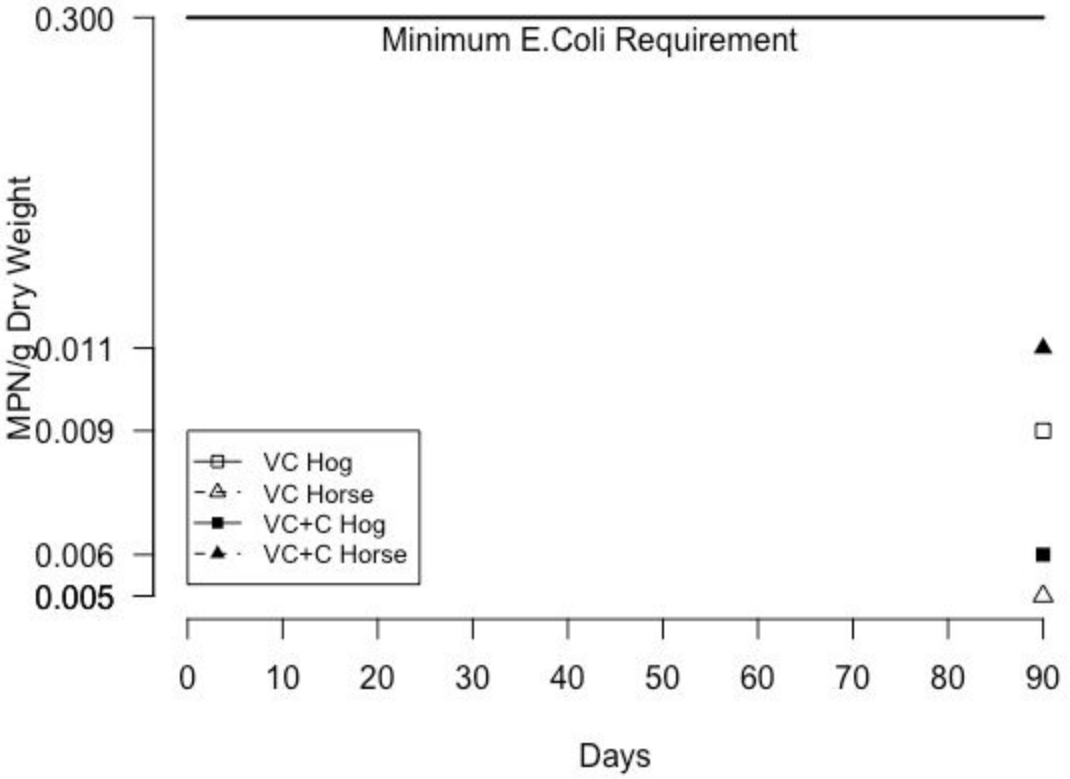

Figure 3: MPM g_{dw}^{-1} of *E. coli* of vermicomposted and pre-composted followed by vermicomposting manures after 90 days of *E.fetida* exposure compared with FSMA minimum biological requirements.

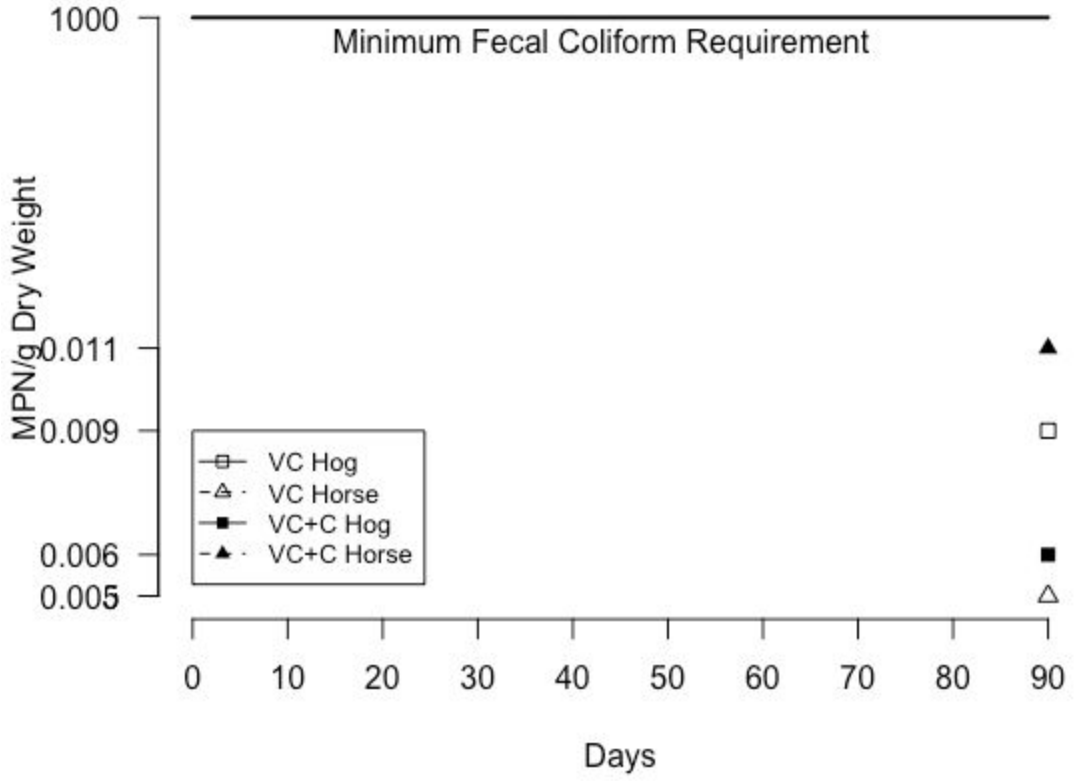

Figure 4: MPM g_{dw}^{-1} of fecal coliforms of vermicomposted and pre-composted followed by vermicomposting manures after 90 days of *E.fetida* exposure compared with FSMA minimum biological requirements.

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