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The microbiological analysis of composting

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THE MICROBIOLOGICAL ANALYSIS OF COMPOSTING

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ABSTRACT

Animal manures contain valuable nutrients which can be utilized for crop growth. Consequently, the wastes are often spread across field where produce is grown without treatment prior to land application. This practice is a potential threat to the environment and human health, as pathogens contained in the manures may have extended survival in soil, and could contaminate produce harvested for human consumption. Composting is a process that has been often implemented on-farm to inactivate pathogens resident in animal wastes. The objectives of this study were to: 1) perform a survey of South Carolina poultry farms to determine if the methods implemented resulted in the destruction of foodborne pathogens, and 2) determine the survival of *E. coli* O157:H7 in a dairy manure-based compost performed in uncontrolled environmental conditions.

In the survey of poultry farms, nine compost heaps at different stages in the composting process were analyzed on four poultry farms in upstate South Carolina. Both the materials used and composting methods differed among the farms surveyed. In the surveyed heaps, 71% of all internal samples contained moisture contents of less than 40%, which is considered as the minimum necessary for active composting. Ninety-one (91) of 141 compost samples analyzed were positive for coliform populations ranging from 1.00 to 6.00 $\log_{10}$ CFU/g. Approximately 94% of the surface samples analyzed were positive for coliforms, compared to less than 50% of the internal samples. Seventy-six percent of the surface samples were positive for presumptive *Salmonella* spp. Among all internal samples, ca. 26% and 19% were positive for presumptive *Salmonella* and

Listeria spp, respectively. Both *E. coli* O157:H7 and *L. monocytogenes* was not detectedin any of the samples. Among finished compost samples (n=21), ca. 62%, 33%, and 14% were positive for coliforms, presumptive *Salmonella*, and presumptive *Listeria*, respectively.

In the investigation of the survival of *E. coli* O157:H7, two trials were performed involving duplicate compost heaps constructed on an outdoor, fenced site. The compost heaps were comprised of dairy manure, old hay, feed waste, a sawdust-calf feces mixture, and fresh hay. Samples of the composting mixture were inoculated with *stx*-negative *E. coli* O157:H7 B6914 at initial cell numbers of ca. 10^7 and 10^5 CFU/g for Trial 1 and Trial 2, respectively. Individual sample bags were placed on the surface and at three locations (top, center, and bottom) within each heap. In Trial 1, *E. coli* O157:H7 was detected by enrichment through 14 days within the heaps. When inoculated with 10^5 CFU/g in Trial 2, *E. coli* O157:H7 was detected only through days 2, 2, and 5 at the top, center, and bottom locations, respectively. For both trials, the pathogen survived at the heap's surface for up to 4 months. The indicator microorganism, *E. coli,* was inactivated at a rate similar to that of *E. coli* O157:H7.

Our studies demonstrated that foodborne pathogens may persist for extended periods of time in the compost surface. This is important because it suggests that improperly compost manures may serve as vectors in the dissemination of foodborne pathogens on food products intended for human consumption.

DEDICATION

I would like to say thank you, to my Lord and Savior, Jesus Christ, for giving me the knowledge and strength to complete my thesis. I would like to dedicate this work to my mother, Vertrell B. Shepherd, my father, Marion W Shepherd, Sr., and my sister, Rashida J. B. Shepherd. Without their love, support, and encouragement this could not have been possible.

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CHAPTER ONE

LITERATURE REVIEW

Current agricultural practices

Food products, such as meats and vegetables, maybe contaminated with pathogens during the growing and processing stages of food production. Most foodborne pathogens are commonly shed in the feces of healthy animals, which serve as a major source of contamination. Ensuring the safety of the food supply is critically important in protecting the health of consumers. Many measures have been proposed in order to control the spread of foodborne pathogens from the farm to the table (Coleman, 1995; Cohen, 1998; Hafez, 1999; Curran, 2001, Crump et al., 2002; Scholthof, 2003). As a reaction to the outbreaks of *E. coli* O157:H7 in 2006 linked to lettuce and spinach, the legislature of the state of California approved three bills, in March 2007, designed to improve the safety of fresh produce. Included in the bills were provisions that would: regulate the use of water, fertilizer, and toilet facilities in the field; implement a system to track produce so that contaminated products would be easily identified in lieu of an outbreak; and mandate that leafy green vegetable growers be licensed by the state (Associated Press, 2007).

To limit the risks of contaminating fresh produce before harvesting occurs, many farms implement plans that ensure that "Good Agricultural Practices" (GAPs) are being followed. The GAPs include assisting workers in maintaining

good hygiene through the availability of toilet facilities, the use of manure that has been decontaminated of pathogens, the use of pathogen-free water for field irrigation, and the creation of barriers to limit the entrance of wildlife and insects into areas where produce is growing (Delazari et al., 2006). Adherence to GAPs is critically important, as the breakdown at any point could result in the contamination of produce. Introduction of untreated human or animal wastes, runoff associated with those materials, and insects may all serve as sources or vectors of pathogen contamination.

Procedures are also followed on the farms to control the spread of enteric pathogens among food animals. In an effort to decrease the exposure to limit infections the following has been suggested: slaughtering and/or quarantining animals, disinfecting areas where slaughtering has occurred, and screening animals for infection (Delazari et al., 2006). Undertaking these measures will serve to limit the exposure of animals to pathogens, which will in turn decrease pathogen concentrations in manures. Effective implementation should result in a decrease in the incidence of foodborne diseases caused by *Listeria monocytogenes*, non-typhoidal *Salmonella* spp., and *Escherichia coli* O157:H7.

Listeria monocytogenes

L. monocytogenes is a short, facultatively anaerobic, Gram-positive, non-sporeforming capable of growth over a wide range of temperatures. This organism causes approximately 2,500 illnesses and is responsible for 27.6% of all foodborne deaths each year in the United States (Mead et al., 1999). This pathogen is found across many

environmental sources, and has been detected in poultry, pig, and cattle manure (Pagotto et al., 2006).

Infection with *L. monocytogenes* may cause stillbirths, miscarriages, meningitis, or septicemia (Altekruse et al., 1997). The type of illness caused varies depending on the infected individual though healthy adults usually experience symptoms typical of gastrointestinal disorders. *L. monocytogenes* infection is caused due to the organism's ability to evade the host immune system and invade intestinal cells (Pagotto et al., 2006). Once the organism travels through the lymphatic system and enters the bloodstream, virulence factors such as hemolysin and internalin allow entrance and replication inside host cells (Pagotto et al., 2006).

***Salmonella* spp.**

Salmonella spp. are Gram-negative, rod shaped, facultatively anaerobic members of the family *Enterobacteriaceae*. Some *Salmonella* serotypes are highly adapted to causing human disease, but the focus of this study will be on serotypes that are not characterized by causing enteric fever (non-Typhi serotypes). There are over 2,500 different non-typhoidal *Salmonella* serotypes and they are commonly associated with mammals, insects, birds, and reptiles (Mølbak et al., 2006). Infections with salmonellae are second only to *Campylobacter* spp. as the most reported cause of bacterial foodborne illnesses with approximately 1.4 million illnesses and 580 deaths caused in the United States each year (Mead et al., 1999). Though there are numerous *Salmonella* serotypes, the four most common causes of human salmonellosis are, Typhimurium, Enteritidis, Heidelberg, and Newport (Finke et al., 2002).

Salmonella infections commonly occur through the fecal-oral route of transmission. As few as 10 salmonellae cells have been associated with causing disease; however, the actual number necessary may depend on the serotype and the function of the host's immune system (Møbak et al., 2006). Depending on the infectious dose, gastrointestinal symptoms may develop anywhere from 6 hours up to 10 days after infection. Most symptoms include diarrhea, vomiting, and abdominal and joint pain; though septicemia may occur without intestinal complications (Mølbak et al., 2006). The virulence factors associated with *Salmonella* induced diarrhea is thought to be caused by an enterotoxin, inflammation occurs due to activated genes on the *Salmonella* pathogenicity island 1, while *Salmonella* plasmid virulence genes are responsible for systemic infections (Mølbak et al., 2006).

E. coli O157:H7

E. coli O157:H7 is responsible for approximately 73,500 and 60 deaths each year in the United States (Mead et al., 1999). It is a Gram-negative, rod-shaped, facultative anaerobic organism that is a member of the family *Enterobacteriaceae*. The pathogen is usually associated with ruminant animals predominately, but has also been isolated from animals such as pigeons, raccoons, and flies (Shere et al., 1998). Cattle infected with the organism usually are asymptomatic carriers and shed the pathogen through their feces.

In a survey of fecal samples from cattle of different ages, *E. coli* O157:H7 was detected at populations less than 10^2 CFU/g (detected after enrichment culture) up to 10^5 CFU/g (Zhao et al., 1995). Shere et al. (1998) reported that dairy heifers shed *E. coli* O157:H7 at populations of ca. 2 – 5 $\log_{10}$ CFU/g for up to 16 weeks. It has been reported

that shedding of the pathogen is discontinuous. Grauke et al. (2002) reported that when inoculated orally with *E. coli* O157:H7, cattle shed the pathogen in three intervals: approximately 1 week, approximately 1 month, or 2 or more months. In this study, 50% of the cattle observed shed the pathogen for 29 to 33 days.

Though discontinuous, *E. coli* O157:H7 shedding is thought to vary based on cattle age, cattle diet, and seasonal variations. Zhao et al. (1995) report that weaned calves were more likely to shed *E. coli* O157:H7 in feces than pre-weaned calves. A study of fecal samples from 900 weaning calves across 15 different herds showed that 6.9% of calves tested shed *E. coli* O157:H7 in the feces (Laegreid et al., 1999). In that study, analysis of the serum of the calves revealed that 63.3 to 100% of all individuals in each heard contained antibodies to O157 antigens. These researchers conclude that calves are infected with *E. coli* O157:H7 before weaning. Additionally, Heuvelink et al. (1998) report that 21.7% of cattle between 4 and 12 months in age tested positive for *E. coli* O157, the highest rate among all ages of cattle sampled. Grauke et al. (2002) suggests that *E. coli* O157:H7 is more commonly isolated from the feces of younger cattle because the rumen microflora is still developing, as the environment in a mature rumen would limit the survival of the pathogen.

In regard to dietary impacts on the shedding of *E. coli* O157:H7, Dargatz et al. (1997) reported that cattle fed with soybean meal were less likely to shed the pathogen in their feces. Barley consumption and cattle on feed less than 20 days were more likely to shed *E. coli* O157:H7. Heuvelink et al. (1998) suggested that diets high in fiber and low in nutrients raise the pH in the rumen, creating conditions conducive for pathogen survival.

Chapman et al. (1997) reported that *E. coli* O157:H7 was isolated in cattle at a higher rate in the spring compared to the winter. Additionally, Heuvelink et al. (1998) indicated that cattle shed the pathogen more between the months of July through September than over other intervals studied. These researchers suggest that incidence of infection is greater during warmer months due to an increase in grazing resulting in manure-contaminated pastures, and environmental conditions favorable for pathogen survival.

Understanding *E. coli* O157:H7 shedding is important because this pathogen can result in severe illness. It is reported that the infectious dose of *E. coli* O157:H7 for humans is approximately 50 organisms (Fratamico and Smith, 2006). The incubation period of infection can range from 1 to 8 days, depending on the infectious dose and various host factors. In some cases, infection is asymptomatic, but is usually characterized by diarrhea. Expression of two Shiga-like toxins (stx_1 and stx_2), the predominate virulence factors of the pathogen, can result in bloody diarrhea, Hemolytic Uremic Syndrome – a condition which impairs kidney function and possibly death, especially in children under twelve years of age (Fratamico and Smith, 2006).

Developing strategies to limit *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 infections are important to food safety. Since three classes of pathogens combine to cause approximately 1.5 million illnesses and 60% of all deaths related to foodborne illnesses (Mead et al., 1999), procedures must be taken on the farm to treat animal wastes, materials harboring these pathogens, to limit the risks of contaminating commercially viable food products.

Animal mortalities, especially poultry carcasses, are often contaminated with *Listeria* and *Salmonella*, and, consequently, environmentally safe disposal methods are needed. It is estimated that a flock of 50,000 broiler chickens grown for 49 days will produce 2.2 metric tons (2,200 kg) of carcasses if the total mortality of the flock is 4.9% (Blake et al., 1990). Methods of poultry carcass disposal have been burial, incineration and rendering. Burial is considered as the most convenient method, but produces unpleasant odors due to anaerobic degradation and may affect ground water quality (Blake and Donald, 1992). Incineration is considered the safest disposal method for carcass management; however, it is slow, labor intensive, and expensive (Blake and Donald, 1992; CAST, 1996). In regard to rendering, while it is effective in carcass treatment, it is labor intensive and expensive, much like incineration (Sander et al., 2002). However, with rendering, a utilizable product is formed which may be sold for feeds or other uses to cover expenses and generate a profit. Implementation of practical, environmentally safe methods for the treatment of animal wastes, especially animal mortalities, is a great concern.

In agriculture, it is a common practice to spread raw animal manure onto land used for crop growth. The Council for Agricultural Science and Technology (1996) stated that "manure decreases soil loss by improving soil physical characteristics including structure, infiltration rate, permeability, bulk density and water holding capacity" (CAST, 1996). Manure is also an excellent source of nitrogen, phosphorus, and potassium – nutrients beneficial for crop growth. Dairy manure contains 5 – 16, 2 – 16, and 2 – 31 pounds per ton of nitrogen, phosphorus (in the form of P_2O_5), and potassium (in the form of K_2O) in non-liquid forms, respectively (Bates and Gagon,

1981). Poultry manure contains 4 – 111 pounds of nitrogen per ton, 1 – 96 pounds of phosphorus (in the form of P_2O_5) per ton, and 2 – 55 pounds of potassium (in the form of K_2O) per ton, in non-liquid forms. Importantly, many of the nutrients are present in forms that are readily available to growing plants.

Although manure contains resources that are valuable to the growth of crops, it also harbors pathogenic microorganisms that could cause sickness in humans. This is of great concern, as precipitation on fields covered with raw manure could create runoff, introducing pathogenic microorganisms and soluble minerals in area waters. Water supplies (rivers, lakes, and streams) are routinely monitored for fecal coliforms, as elevated levels indicate that fecal contamination, usually attributed to livestock, has occurred. As a consequence, bacterial, fungal, viral, and parasitic diseases may all be transmitted through food or water contaminated with fecal matter from animals (Diesch, 1969). It is suggested that runoff from fields that had been recently incorporated with manure contains nutrient concentrations and coliform counts comparable to fields that do not contain manure (CAST, 1996). While the immediate incorporation of manure is addressed, there still remains the possibility of runoff polluting water reservoirs used for food and recreation if raw manure is not incorporated.

Pathogen Survival in Soil

Most foodborne pathogens are enteric in nature, but may survive for extended periods in the environment. Lau and Ingham (2001) demonstrated that *E. coli* survived longer than enterococci in loamy sand and silty clay loam soils when incorporated with bovine manure. In this study, soils were exposed to environmental conditions similar to

what would be experienced in Wisconsin in late spring to early summer. Studies were conducted in the laboratory to investigate the survival of *E. coli* O157:H7 (Jiang et al., 2002) and *L. monocytogenes* (Jiang et al., 2004) in autoclaved and unautoclaved sandy loam soils mixed with contaminated bovine manure. In both studies, contaminated manures were mixed with soils at various ratios, and stored at 5, 15, and 21^{o}C. In the study of *E. coli* O157:H7, the pathogen survived for at least 35 days across all mixing ratios and storage temperatures in autoclaved soils. Pathogen detection was occurred for at least 138 days to greater than 226 days at 15^{o}C in manure mixed in autoclaved soils, depending on the manure to soil ratio. However, when manure was mixed with unautoclaved soils, detection occurred for 103 to 193 days over various manure to soil ratios at 21^{o}C. For *L. monocytogenes*, when held at of 5 and 15^{o}C, the pathogen was detected through 43 days and 21 days in manure amended autoclaved and unautoclaved soils, respectively. Additionally, at 21^{o}C the pathogen survived for 14 days in manure-amended autoclaved soil, compared to 21 days in manure-amended unautoclaved soil.

Researchers have also performed field-based studies to determine how pathogens will survive in soil. Avery et al. (2004) inoculated both bovine slurry and ovine stomach contents with *E. coli* O157:H7, and applied those materials to the surface and subsurface (ca. 25 mm in depth) into soil cores. At the completion of the 8 week study, analysis of soils samples revealed that *E. coli* O157:H7 was detectable in both surface and subsurface soils samples, even though there was an evident decrease in populations during the study. In an investigation of how bovine manure affects the bacteriological quality of organic lettuce, sandy loam soil contaminated with manure was analyzed to measure the persistence of several foodborne pathogens (Johannessen et al., 2004). In

this investigation, manures were not inoculated with any pathogenic microorganisms. In two trials, commensal *E. coli* and thermotolerant coliforms detected in soils treated with compost, firm manure, and manure slurry one week after fertilization. *E. coli* O157 was detected in soil 1 week after firm manure and manure slurry fertilization. Importantly, in trial 2, *E. coli* and thermotolerant coliforms were detected in soil 41 weeks after fertilization. You et al. (2006), in a study comparing the survival of multi-drug resistant (MDR) and drug susceptible (DS) *Salmonella* Newport in dairy manure, and soil contaminated with dairy manure, found that both strains had similar survival profiles. Detection of both pathogen strains occurred through direct plating for 107 and 158 days in manure mixed with non-sterilized soil and manure mixed with sterilized soil, respectively.

The studies described above illustrate the extended survival for both indicator and pathogenic bacteria in soil. Clearly, the soil type and resident microbial populations can affect pathogen survivability. Other environmental conditions such as pH and moisture content of the soil, along with the affect of ultraviolet light, all have some effect on pathogen survival. Knowing the affect of these factors could further the understanding of what contributes to the decline, or supports the persistence, in soils.

Treatment of the animal wastes before incorporation into the soil through proper composting will reduce and/or eliminate the likelihood of introducing pathogenic bacteria into the environment where survival can occur over extended time periods.

Microbiology of Composting

Composting is a method that can be used to effectively treat animal manures and carcasses. It is defined by Glanville and Trampel (1997) as a method where "biological degradation of organic residues under aerobic conditions…[result in] end products consist[ing] largely of water, carbon dioxide, ammonia, heat, and a humus-like material consisting of microorganisms, non-biodegradable inorganics, and organic compounds that are resistant to rapid biodegradation." Properly composted wastes are effective in the destruction of pathogens, viruses, weed seeds, and nematodes (Misra et al., 2003).

Though composting may be conducted using a variety of substrates, there are guidelines for ensuring successful composting. One major parameter of compost that should be monitored is the carbon to nitrogen (C:N) ratio. It is stated that the C:N ratio of 20:1 – 40:1 is acceptable for composting, while 25:1 – 30:1 is preferred (Sherman, 2005). If the right balance of these materials is not achieved, composting may be hindered, as microbes use carbon for energy and growth, while nitrogen stores are used for protein synthesis and reproduction.

Another important parameter of composting is the moisture content of composting materials. Moisture contents in the range of 40 – 65% are acceptable, while 50 – 60% is preferred (Sherman, 2005). Composts with too little moisture do not have enough available water for the microorganisms to effectively metabolize nutrients, a function essential for material decomposition. Conversely, material too saturated with moisture could operate under anaerobic conditions due to the lack of oxygen entrance into the heap.

Other factors that will influence the composting process are pH and heap size. The optimal range for pH in composting is 6.5 – 8.0. Numerous microorganisms involved in composting allow for continuation of the process without disruption due to pH fluctuations (Cochran and Carney, 2006). However, low pH may retard composts from entering into the thermophilic phase (Sundberg et al., 2004). Heap size is an important factor in composting, though it is dependent on the amount, and types, of material used and particle sizes of the composted materials. Heaps that are too small in size will not retain heat. As a consequence, the thermophilic phase cannot be reached resulting in pathogen persistence in compost materials.

While the substrates used in composting may vary, depending on the nitrogenous sources and bulking materials used, successful composting is usually characterized by the following three phases: mesophilic, thermophilic, and cooling/maturation. The composting phases are characterized by the changes of dominant microbial communities and soluble nutrients utilized during the composting process (Smith, 1992).

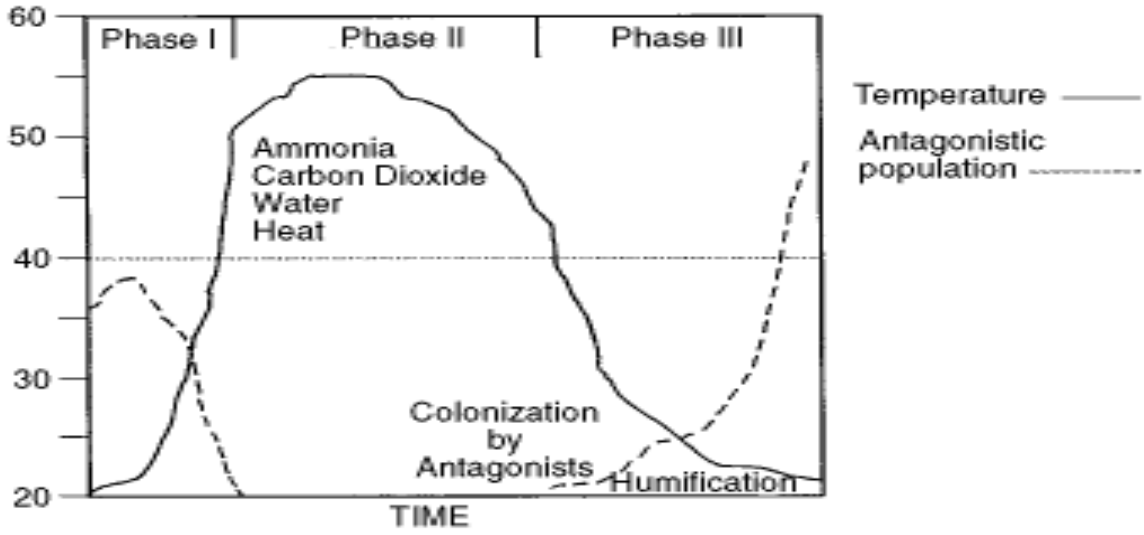

Figure 1. Phase changes during the composting process (Smith, 1992)

Many studies have been conducted to identify the organisms responsible for the breakdown of organic material that occurs in composting. Studying the changes in the microbial community when composting garbage, Ishii et al. (2000) analyzed the microbes present in the compost using Density Gradient Gel Electrophoresis (DGGE) of the 16s subunits of rRNA. Results indicated that 87% of the microorganisms in the thermophilic phase of composting were identified as belonging to the genus *Bacillus*. Further analysis showed that during the cooling phase, new organisms emerged that could metabolize complex nutrient substrates. Furthermore, it was reported that changes in temperature and available nutrients resulted in a final community much different than that from the onset of composting. Another study observed the changes in the 16s rDNA restriction fragment length polymorphism (RFLP) patterns in a compost held at 60^{o}C for 14 days (Nakasaki et al., 2005). In this study, dog food was used as a substitute for organic substrates in the compost. Incubation of the compost at 60^{o}C allowed the researchers to study microbial succession in compost over a shorter time period. In the study, RFLP patterns changed from day 0 through 4, days 5 through 7, and remained stable after day 9. Analysis of RFLP bands when compost temperatures were highest revealed that *Bacillus* species were dominant.

While analyses of 16s genetic subunits are very effective in the use of identifying microbes involved in composting, analysis of the phospholipids fatty acid content of microbes using Gas Chromatography – Fatty Acid Methyl Ester (GC-Fame) has also been a useful technique in organism determination. Ryckeboer et al. (2003) found that the genus *Bacillus* was most prevalent throughout the composting of vegetable, fruit, and garden wastes, especially in the thermophilic phase. Additionally, there was in inverse

relationship between temperature and microbial diversity. Steger et al. (2005) reported that when composting organic household waste and wheat straw under aerobic conditions (16% oxygen), the temperature increase in the compost could be attributed to a thermophilic bacterial community comprised of members of the genera *Bacillus* and *Thermus*. This determination was made as the two genera have similar fatty acid compositions. Bacterial communities of poultry manure, composted with either rice husk or rice bran, were also tracked using PFLA analysis (Kato et al., 2005). Though the temperature evolution of the composts varied, the establishment of a Gram-positive bacterial community during the thermophilic phase was evident using FAME analysis. The researchers, using data from past studies, inferred that *Bacillus* species, and possibly actinomycetes, were the organisms detected in the Gram-positive community that was detected from the thermophilic phase through the end of composting. Ishii et al. (2000) and Ryckeboer et al. (2003) reported that microbial diversity increases after the thermophilic phase to include members of the *Bacillus* taxon, various Gram-positive and Gram-negative organisms, and fungal species.

From the data in the presented studies, it can be concluded that Gram-positive communities, specifically the genus *Bacillus*, are the microorganisms that drive the thermophilic phase of composting. This is not surprising, as *Bacilli* are organisms that are found over a wide ranged of environmental niches, and can withstand elevated temperatures. The importance of this genus of bacteria cannot be understated, as the thermophilic composting phase is characterized the elevation of temperatures needed for pathogen inactivation.

Methods of Composting

The most common methods for composting include passive heaps, static heaps, windrows, or in-vessel composting systems. Passive and static heaps are similar in that they both involve the stacking of composting materials into mounds, referred to as heaps. Passive heaps, however, are managed very little, while static heaps are maintained through forced aeration or frequent mechanical turning (Sherman, 2005). In windrow composting, composted materials are mixed and formed into long and narrow heaps, which are subjected to regular agitation (Sherman, 2005). In-vessel composting is characterized as a group of composting methods that use a container or enclosed staging area to perform composting (Cochran and Carney, 2006). Using an in-vessel composting system can also include mechanical or forced aeration of compost material. Table 1 describes composting times typically experienced based on material and methods implemented (Rynk, 1992).

Table 1. Time necessary for composting depending on the implemented methods and substrates used (Rynk, 1992).

Method	Materials	Active composting time		Curing time
		Range	Typical	
Passive composting	Leaves	2–3 years	2 years	—
	Well-bedded manure	6 months to 2 years	1 year	—
Windrow—infrequent turning [a]	Leaves	6 months to 1 year	9 months	4 months
	Manure + amendments	4–8 months	6 months	1–2 months
Windrow—frequent turning [b]	Manure + amendments	1–4 months	2 months	1–2 months
Passively aerated windrow	Manure + bedding	10–12 weeks	—	1–2 months
	Fish wastes + peat moss	8–10 weeks	—	1–2 months
Aerated static pile	Sludge + wood chips	3–5 weeks	4 weeks	1–2 months
Rectangular agitated bed	Sludge + yard waste or Manure + sawdust	2–4 weeks	3 weeks	1–2 months
Rotating drums	Sludge and/or solid wastes	3–8 days	—	2 months [c]
Vertical silos	Sludge and/or solid wastes	1–2 weeks	—	2 months [c]

[a] For example, with bucket loader.
[b] For example, with special windrow turner.
[c] Often involves a second composting stage (for example, windrows or aerated piles).

While manures and other wastes have been composted using all varieties of techniques, chicken mortalities have been primarily treated using bin composting, which is considered an in-vessel system (Murphy et al., 1988). Gonzalez and Sanchez (2005) suggested that static heaps could successfully be used to compost straw, hen manure, and poultry mortalities.

To ensure that composts do not pose a risk to contaminate the environment and the food supplies with pathogens, governmental agencies of the United States have formulated the guidelines in which composting should be performed. When maintained under certain conditions, composting is known as a “Process to Further Reduce Pathogens”, or PFRP, by the United States Environmental Protection Agency (EPA)

(EPA, 1994). EPA regulations state that for Class A designation, which indicates that pathogens are below detectable levels, static aerated compost heaps containing biosolids should be maintained at temperatures of 55^{o}C higher for 3 days (EPA, 1994). Similarly, USDA recommends to organic growers that composting operations must maintain a temperature in the range of 55-77^{o}C for a minimum of 3 days for static aerated pile system and 15 days with 5 turns for windrow system (Misra et al., 2003). If guidelines are followed, the materials processed through composting may provide a pathogen-free soil amendment, which allows for the slow release of nitrogen and phosphorus into the soil.

Composting of Poultry Wastes

It is estimated that broiler chicken produce in between 12 and 23 billion kilograms of wastes each year in the United States (Nachman et al., 2005), which includes manure and carcasses. Poultry carcasses are commonly treated through rendering, burial, and incineration. Rendering is relatively expensive, and burial and incineration are detrimental to air and water quality (Gonzalez and Sanchez, 2005). Composting poultry wastes is an inexpensive process in comparison to rendering, and functions to improve soil conditions.

In a study by Chaudry et al. (1998) broiler poultry litter with moisture contents of 15, 25, and 35% were deepstacked (composted), and populations of indicator bacteria were monitored. Results of the study indicate that total and fecal coliforms can be eliminated after one week of deepstacking, regardless of the moisture content. It was found that the moisture content did influence temperature evolution in the heap; however,

as litter deepstacked with 35% moisture held higher temperatures at monitored locations than the other treatments. In a study investigating the survival of enteric bacteria with and without aeration, poultry litter was deepstacked (Kwak et al., 2005). This group revealed that enteric bacteria were inactivated between days 2 and 4 of litter deep stacking. However, this group also reported that in litter that was not deepstacked, *Salmonella*, *Shigella*, and *E. coli* were not detectable up to 4, 4, and 8 days post inoculation, respectively. This data indicates that the composting of poultry litter results in rapid elimination of pathogens.

Blake et al. (1994) conducted a field survey where 12 mini-composters were monitored for the presence of indicator and pathogenic microbes. Throughout the survey, temperatures in all heaps were above the 55^{o}C threshold and *Salmonella*, *Campylobacter jejuni*, and *L. monocytogenes* were not detected in any of the samples. Lawson and Keeling (1999) composted poultry litter and carcasses using the methods suggested by the USDA. Even at low ambient temperatures, the compost reached a peak temperature of 71^{o}C, inactivating *Salmonella*.

The composting of poultry carcasses is especially important, as "bacteria breakdown the carcass[es], leaving only feathers and bones" (Sander et al., 2002). This method of disposal provides a better option for poultry waste treatment over rendering and burial, techniques that are expensive and may attract scavenger animals, respectively.

Studies have demonstrated that composting poultry wastes (litter and carcasses) can result in the elimination of pathogenic bacteria. In addition to the increased temperatures that occur in stacked litter, Turnbull and Snoeyenbos (1973) suggested that ammonia production may result in *Salmonella* inactivation. However, this occurrence is

usually a result of composting poultry litter at below suggested C:N ratios (Elwell et al., 1998). Further studies composting litter under various initial pH ranges should be investigated for pathogen inactivation. Additionally, an investigation of microbial populations present in poultry litter may result in the discovery of microorganisms that release antimicrobial compounds into the surrounding environment, resulting in pathogen death.

Composting of Cattle Wastes

It is estimated that 43.3 million tons of dry cattle manure (including beef, feeder, and dairy cattle) are produced each year (Midwest Plan Service, 1985). Composting has been used as a practical way to treat bovine wastes. Lung et al. (2001) studied the inactivation of *Salmonella* and *E. coli* O157:H7 composting a fresh cow manure and sawdust mixture. In their study, bioreactors with forced aeration where held at external temperatures of 25 and 45^{o}C and samples were enumerated for pathogen detection. They reported that *E. coli* O157:H7 and *Salmonella* populations fell below the detection limits 3 and 2 days after the onset of composting incubated at 45^{o}C, respectively. When composted at 25^{o}C, bacterial populations remained somewhat constant. In an investigation of the fate of high levels (ca. 10^{7} CFU/g) of *E. coli* O157:H7, bovine manure was composted at 21 and 50^{o}C (Jiang et al., 2003). The researchers reported that external temperatures were maintained at 50^{o}C, *E. coli* O157:H7 was detected at all sampling locations through 7, but not 14, days of composting. In contrast, the pathogen was detected at all sampling locations through 36 days of composting, though inactivation occurred more rapidly at the bottom location, when the bioreactors were held

at 21°C. Hess et al. (2004) composted bovine manure inoculated with bovine-derived and laboratory-grown *E. coli* O157:H7 strains in an effort to compare the inactivation between the bacterial strains during composting. Results from the study indicate that laboratory-grown strains were more resistant to heat than those derived from the animal, as 300 degree-days were required for inactivation of the laboratory strain, while the bovine-derived strain could be eliminated in manures from infected cattle in 180 degree-days.

Laboratory-based studies, under well-controlled conditions, demonstrated that contaminated wastes can be effectively treated through composting. However, heating in the bioreactors must be induced through forced aeration or incubation at high external temperatures. However, the controlled conditions used in laboratory studies do not represent how composting may proceed in field conditions where the materials may be exposed to varying ambient temperatures and other environmental factors.

In a field study, Larney et al. (2003) composted beef cattle manure with two types of carbonaceous sources (barley straw and wood chips) and monitored total coliform and *E. coli* populations over two summers (1998 and 1999). The researchers reported that ca. 3 $\log_{10}$ CFU/g of the target bacteria were eliminated during the mesophilic phase of composting, where temperatures ranged between 33.5 to 41.5°C. The carbon source used for composting was reported not to have a significant effect on bacterial survival of total coliforms or *E. coli*. Total coliforms were detected through 94 composting days in 1998, whereas *E. coli* populations fell below the minimum detection limit after 45 and 7 days of composting in 1998 and 1999, respectively.

Nicholson et al. (2005) analyzed the survival of three foodborne pathogens inoculated into dairy farmyard manure. In the study, inoculated manure heaps were either maintained as static or static aerated heaps. Results of the study indicated that in unturned heaps, *E. coli* O157:H7 survived for 8 days, while *Salmonella* and *Listeria* both survived 4 days. These researchers also reported that there were no significant differences in survival of monitored foodborne pathogens in aerated and non-aerated heaps.

Field studies, presented above, demonstrated that composting may result in the inactivation of indicator and pathogenic bacteria. However, the constituents of and methods implemented in, composting appears to influence the rate of pathogen inactivation. Moreover, published studies of composting cattle waste under field conditions do not address the issue of pathogen inactivation rates at different locations within the compost. Similarly, though the compost surface is not subjected to the elevated temperatures that occur within the heaps, studies do not address how pathogens survive on undisturbed compost surfaces.

Inactivation of Viruses and Protozoans through Composting

In addition to bacterial pathogens, animal wastes are loaded with high numbers of viruses and parasites that may cause illnesses in humans. Composting, when conducted properly, can allow temperatures to reach thresholds high enough to inactivate viruses and parasites in addition to harmful bacteria. Pourcher et al. (2005) reported that infectious enteroviruses were inactivated after one month of composting a sewage sludge – straw mixture. Virus inactivation was determined after incubation of a virus loaded

solution in Buffalo Green Monkey cells. The researchers also stated that other genomes of enteroviruses were detected through 3 months of composting using PCR; however these were deemed as non-infectious as they were not detected after culturing in an active cell line. This study suggests that infectious enteroviruses can be effectively inactivated through composting.

Rimhanen-Finne et al. (2004) investigated the inactivation of *Cryptosporidium parvum* and *Giardia intestinalis*, two parasitic protozoans, and indicator bacteria remaining after sewage sludge disinfection at wastewater treatment facilities in Finland. Wastewater sludge, containing no animal manure inputs, were treated using the following techniques: windrow composting, mesophilic anaerobic digestion and windrow composting, drum and windrow composting, and tunnel and windrow composting. The researchers reported that after 30 weeks, the protozoan pathogens were not detected in sludge treated by any composting method.

The studies presented above have demonstrated that infectious viruses and parasites can be inactivated by composting wastewater sludge. However, further studies need to be performed to verify that viruses and parasites present in animals wastes may be inactivated through composting.

Pathogen Contamination of Vegetables

The inactivation of pathogens in manure and compost is important not only to decrease the transmission into the environment, but also to decrease the risk of contaminating fresh produce. This is especially important in organic farming, as manure

or manure based fertilizers are commonly used in lieu of synthetic fertilities, which are restricted by organic production guidelines.

Mukherjee et al. (2004) performed a study to determine the presence of indicator and pathogenic organisms on produce from organic and conventional farms. In the 40 farms from where vegetable samples were obtained, all organic farms (32) and four conventional farms reported using aged or composted animal manure as a crop fertilizer. The researchers found that 92% of all vegetables samples were positive for coliforms, while only 8% were positive for *E. coli*. Additionally, the study revealed that *E. coli* prevalence on organic produce was approximately three times higher than on conventional produce. Importantly, whereas *E. coli* O157:H7 was not detected on any of the produce (conventional or organic), *Salmonella* was detected on two organic produce samples: one head of lettuce, and one green pepper.

Islam et al. (2004, 2005) investigated the survival of *Salmonella* and *E. coli* O157:H7 on vegetables treated with contaminated compost and irrigation water. They report that *S*. Typhimurium was detected on carrots and radishes for 203 and 84 days, respectively, after seeds were sown for those crops. *E. coli* O157:H7 was detected on carrots 168 days, and onions 74 days after the introduction of contaminated compost or irrigation water into the soil. Avery et al. (2004) also reported the presence of *E. coli* O157 for six weeks (either through direct plating or enrichment culture) on vegetation when grown in soils where contaminated bovine and ovine wastes were applied to the soil surface. In examining the transfer of *E. coli* O157:H7 to fresh produce from manure, lettuce seedlings were grown in soil with contaminated bovine manure (Johannessen et

al., 2005). Though *E. coli* O157:H7 was not isolated from any parts of the analyzed lettuce samples, non-pathogenic *E. coli* was detected in lettuce during harvesting.

Guo et al. (2001) investigated the survival of salmonellae on and in tomatoes after inoculating stems and flowers before and after fruiting of the plant. They reported that 37% of the harvested tomatoes were positive for *Salmonella*. Also, salmonellae were detected in the scar tissue of the plant stem and the pulp of the fruit. This study suggests that *Salmonella* may be internalized in tomatoes, if present on an injured plant. Solomon et al. (2002) provided compelling evidence as to why untreated wastes should not be applied to agricultural crops. In their study, lettuce seeds were sown in sandy loam soil contaminated with manure, and lettuce plants were contaminated with green fluorescent protein-labeled *E. coli* O157:H7 at high pathogen concentrations (ca. $10^7 – 10^8$ CFU/g or ml). Using confocal microscopy, their results revealed that *E. coli* O157:H7 colonies were present in the edible parts of the plant, presumably taken up through the vascular system of the plant. In 2006, a massive *E. coli* O157:H7 outbreak occurred in the United States linked to contaminated spinach, which was due to fecal contamination in the field where the spinach was grown (FDA News, 2006). This outbreak resulted in two deaths and over 200 illnesses. In March 2007, it was reported that the spinach implicated in the outbreak was organically grown, though the producers of the spinach would not indicate whether raw or improperly treated manure was used during production (The American Conservative Union Foundation, 2007).

The incorporation of contaminated material necessary for the growth of crops, such as fertilizers and irrigation waters, can pose a great danger to the safety of the food

supply. Waste materials used when growing produce must undergo treatment before application to land to ensure that no pathogens are being introduced into the food chain.

Summary

It is evident that animal wastes, especially manures, are loaded with many microorganisms that may be pathogenic to humans. The use of untreated or improperly treated manures can be a biological hazard to the environment, animals, and food supplies. Studies and governmental regulations suggest that composting is an effective means of inactivating pathogens in manures. Treatment of manures on the farm could help limit transmission of foodborne pathogens to foods, especially fruits and vegetable which are commonly grown in field and consumed raw. Though it has been shown that composting results in pathogen inactivation, there are insufficient scientific studies concerning the mechanisms of pathogen inactivation under field conditions.

The objectives of our study were as follows:

- Surveying the different methods of composting poultry wastes implemented by poultry farmers.
- Determining the efficacy of composting methods implemented by poultry farmers by detection of indicator and pathogenic microorganisms over different compost locations.
- Investigating the inactivation of *E. coli* O157:H7 at different locations within the compost heap under field conditions.

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CHAPTER TWO

MICROBIOLOGICAL SURVEY OF COMPOSTING OPERATIONS ON SOUTH CAROLINA POULTRY FARMS

Abstract

It is well-known that poultry wastes are a reservoir for *Salmonella* spp. and *Listeria monocytogenes* which are microorganisms commonly responsible for foodborne illnesses. Active composting is a practical method used to treat poultry wastes on the farm. In this study, nine compost heaps at different stages in the composting process were analyzed on four poultry farms in upstate South Carolina. Both the materials used and composting methods differed among the farms surveyed. At Farms G and M, new materials were combined in heaps which had previously been composted In the surveyed heaps, 71% of all internal samples contained moisture contents of less than 40%, which is considered as the minimum necessary for active composting. Ninety-one (91) of 141 compost samples analyzed were positive for coliform populations ranging from 1.00 to 6.00 $\log_{10}$ CFU/g. Approximately 94% of the surface samples analyzed were positive for coliforms, compared to less than 50% of the internal samples. Seventy-six percent of the surface samples were positive for presumptive *Salmonella* spp. Neither *E. coli* O157:H7 nor *L. monocytogenes* was detected in any of the samples. Among finished compost samples (n=21), ca. 62%, 33%, and 14% were positive for coliforms, presumptive *Salmonella*, and presumptive

Listeria, respectively. Results indicate that the conditions in the compost surface were suitable for pathogen survival. The introduction of new materials into previously treated composting heaps could reintroduce indicator and pathogenic bacteria into the composting system, and allowed extended survival. Additionally, farmers should be educated in the composting process and adhere to composting guidelines, to ensure that composts produced at their facilities are free of bacteria which may be pathogenic to humans.

Introduction

In the United States, broiler chickens generate 12 to 23 billion kilograms of waste each year (Nachman et. al, 2005). Much of this waste is applied back to land for crop growth, since poultry litter contains valuable nutrients, such as nitrogen, phosphorus and potassium (CAST, 1996). However, this process may have adverse impacts on the environment as poultry litter contains microorganisms that could be pathogenic. In addition to litter treatment, safe disposal of poultry mortalities is also important, as analysis of broiler carcass rinses indicated that 20% and 15% of sampled carcasses were positive for *Salmonella* spp. and *Listeria monocytogenes*, respectively (USDA, 1995). While poultry mortalities can be treated through rendering, burial or incineration (Donald and Blake, 1992), those methods can be expensive, attract scavengers that could harm livestock, crops or humans, or aid in the transmission of disease, respectively. Poultry litter and carcasses need to be properly treated; studies report that pathogens are able to persist in feces, water and soils for extended periods of time (Islam et. al, 2005; You et.

al, 2006), and may have extended survival on produce (Islam et. al, 2004; Mukherjee et. al, 2004).

Composting is a microbe-driven process where organic material is digested into humus-like material (Richard et. al, 1998). Yard wastes, municipal solid waste, and agricultural wastes, such as animal mortalities and manures, are often used as substrates for composting.

While composting can be conducted using a variety of methods (Elwell et. al, 2001), if properly performed, all methods can safely result in the degradation of animal mortalities. Additionally, pathogens may be inactivated from manure and manure based materials, resulting in the creation of a biologically stable, nutrient rich product for crop utilization. The most widely accepted method for the composting of poultry wastes (litter and carcasses) in roofed bins was proposed by researchers at the University of Maryland and accepted by the United States Department of Agriculture (USDA) (Murphy and Carr, 1991). Though composting carcasses in roofed bins is the preferred method, open heaps and windrows may also be used depending on the carbonaceous material used in the compost (Gonzalez and Sanchez, 2005). Poultry litters are commonly treated through deepstacking, as it is a simple and cost-effective method of processing the material. Chaudry et al. (1998) and Kwak et al. (2005) demonstrate that deepstacking poultry litter can result in the complete inactivation of indicator and pathogenic bacteria.

Methods used in composting poultry wastes vary greatly among poultry farms, as the techniques employed depends on the facilities and equipment available. The objective of our study was to survey poultry farms in South Carolina to determine if the

methods used for composting on different sites and compost ingredients influenced the survival of indicator bacteria and pathogens in compost.

Materials and Methods

Farm recruitment: Four poultry farms (A, G, M, and S) in the upstate of South Carolina were recruited for participation in this study. Table 1 summarizes the composting information provided by the farm managers overseeing composting at the selected sites. The survey of compost heaps from Farm A, G, and M was conducted from November 2004 through August 2005, while Farm S heaps were monitored from November 2006 through January 2007. The compost heaps at Farms A, G, and M were sampled nearly monthly, whereas heaps at Farm S were sampled on days 0, 3, 7, 14, 21, 30, and 60.

Description of Surveyed Heaps: During the survey, Farm A had two heaps, known as initial and premix, that were analyzed. The initial heaps was constructed so that one part of chicken litter was combined with two parts of pine fines, and the premix heaps were composed of one part of the initially composted material (i.e, that had previously undergone a phase of heating) to two parts of pine fines. Three composting heaps were initially monitored at Farm G. The initial heap was composed of fresh wood chips, chicken mortalities and chicken litter. The cured heap was composed of initial composting materials that had been turned and composted for 8 weeks, and the intermediate heap was a mixture of fresh and cured compost material. Farm M had two heaps for analysis when the survey began. The first phase bin contained material that had not completed a heating phase of composting. The second phase bin contained material

that had completed one heating phase of compost, and been inverted after the addition of water so that another heating cycle may be reached. Farm S had two heaps for analysis during the survey; one contained pine fines and fresh poultry litter, while the other contained cured compost mixed with fresh poultry litter. Heaps surveyed were monitored for 185, 132 and 288 days into composting, on Farms A, G, and M, respectively. Farm S heaps were monitored from day 0 through 60 days of composting.

Temperature measurement in the compost heaps: Compost temperature and oxygen content data were collected using an OT-21 temperature and oxygen sensor (Demista Instruments, Arlington Heights, IL) during each sampling date prior to the opening of the compost heaps. Compost temperatures in duplicate measurements were taken at Farm M at the surface (0 – 5 cm), 40 cm, and 60 cm depths, while Farms A and G temperatures were monitored at the surface (0 – 5 cm), 60 cm and 90 cm depths. At Farm S, temperatures were monitored at the compost surface (0 – 5 cm), and depths 30 cm and 60 cm from the floor of the compost staging area. At Farms A, G, and M, measurements were recorded from the location in which the compost surface was breached.

Compost heap sampling: Compost heaps on Farms A and S were turned weekly, whereas heaps on Farms G and M were turned only when the materials were moved to another bin. Samples were taken from each available heap at different locations within the heap over sampling dates to ensure that prior openings of the heaps did not influence conditions present of the procured samples. During each sampling, the heaps were opened with a shovel that had been sanitized with 70% ethanol and wiped dry with paper towels.

Both the surface and internal samples were taken from the locations described above for temperature measurements. Five subsamples from different positions at each location were removed with a large sterile sampling spoon, and mixed well in a 2-gallon sampling bucket sanitized with 70% ethanol and wiped with dry paper towels. The composite samples (ca. 250 – 350 g), taken in duplicate, were placed into sterile sample bags. All samples were analyzed within 3 h upon return to the laboratory.

Bacterial enumeration and enrichment: Twenty-five (25) g of sample was combined with 225 ml of Universal Pre-enrichment Broth (UPB; Becton Dickinson, Sparks, MD) and homogenized using a stomacher (Brinkman Instruments, Inc., Westbury, NY) at medium speed for 1 min. Aliquots of the homogenized samples were used for the enumeration of total bacterial and *E. coli*/coliform populations. Sample homogenates were serial diluted (1:10) using 0.1% peptone water, plated on Tryptic Soy Agar (TSA; Becton Dickinson) using an Autoplate® 4000 spiral plater (Spiral Biotech Inc., Bethesda, MD), and incubated at 30°C and 55°C, for total mesophilic and thermophilic bacterial populations, respectively. *E. coli* and coliform populations were determined through plating 1-ml aliquots of the sample dilutions on *E. coli*/coliform Petrifilm™ (3M Microbiology Products, St. Paul, MN) and incubated at 37°C for 24 h.

The remaining homogenized sample-UPB mixtures were incubated at 37°C with shaking for 24 h, and 1-ml aliquots were transferred into 9-ml of Tetrathionate (TT) Broth (Becton Dickinson), Fraser Broth (Becton Dickinson), and modified tryptic soy broth (mTSB; Becton Dickinson) with novobiocin (Oxoid Ltd., Basingstoke, Hants, UK), for *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7, respectively. All three selective enrichment broths were incubated at 37°C for 24 h. One ml of each selective

enrichment culture was transferred to sterile Eppendorf tubes, and briefly treated with 10 µl of Anti-*Salmonella*, Anti-*Listeria*, or Anti-*E. coli* O157 DynalBeads® (Dynal Biotech, ASA, Oslo, Norway) following the manufacturer's procedure. The final DynalBead® suspension was then streaked on either XLT-4 agar (Becton Dickinson), Oxford agar (Becton Dickinson) with modified *Listeria* antibiotic supplements (Oxoid Ltd., Basingstoke, Hants, UK), or Sorbitol MacConkey agar (SMAC; Becton Dickinson) with Methylumbelliferyl Glucuronide (MUG) (Oxoid Ltd., Basingstoke, Hants, UK) for *Salmonella* spp., *L. monocytogenes*, or *E. coli* O157:H7 detection, respectively.

Presumptive positive *Salmonella* spp. colonies were confirmed using the *Salmonella* Latex Test Kit (Oxoid Ltd., Basingstoke, Hants, UK) and serotyping was performed at a Food and Drug Administration (FDA) laboratory. Presumptive positive *Listeria* colonies were confirmed by streaking on *Listeria* CHROMagar® for growth typical of *L. monocytogenes*.

Moisture content and pH determination: Approximately 1 g of each sample was weighed in a tared aluminum dish. The compost samples were then dried in an oven (Blue M Electric Company, Blue Island, IL) at 105^{o}C for 24 h for moisture content determination. Analysis of pH was performed by adding 1 g of sample into 50 ml of deionized water and stirring the solution for 5 min. A pH meter (Orion Research Inc., Boston, MA) was used to determine the pH of the samples.

Statistical Analysis: Bacterial count data were converted to $\log_{10}$ CFU/g for statistical analysis. An analysis of variance (ANOVA) for a completely randomized design with repeated measures across dates was conducted to determine if general differences existed between treatment means using the general linear model (GLM)

procedure. Specific comparisons among parameters determined at different locations in the compost heaps at each sampling date were accomplished with Fisher's least significant difference (LSD) test. All statistical analysis was performed using the Statistical Analysis System 9.1 (SAS; SAS Institute, Cary, NC).

Results

Parameters of surveyed compost heaps

Four poultry farms in the upstate area of South Carolina used windrow, static heap or bin composting systems as a means of treating poultry mortalities and chicken litter. The farm practices varied among the farms, especially in composting methods and composting components used (Table 1). Farm managers at Farms A and M were aware of and tried to adhere to composting guidelines, whereas managers at Farms G and S were experimenting with composting as a waste treatment method. Compost heaps at Farm A were considerably larger than those present at the other three farms, but the heaps were turned weekly, as were heaps at Farm S. New materials were periodically added into the heaps at both Farms G and M; additionally, there was no set schedule for heap turning at Farm G, whereas heaps were turned at Farm M after there had been an internal decline in temperature, indicating the completion of one phase of heating. Farm G was the only farm that did not monitor compost temperatures, while none of the farms monitored the moisture content and pH of the compost. The composts produced on Farms A and M sold their finished composts commercially, whereas the compost from the other two farms were applied directly to the fields on the respective farms.

Temperatures, pHs, moisture contents and oxygen levels of surveyed composting heaps

The temperature profile for the heaps at Farm S (Figure 1) indicates the complete heating cycle of composting. Maximum temperatures achieved in the initial and compost mix heaps were 52 and 38^{o}C, respectively. Some active composting heaps at Farms M and S did not achieve temperatures above 55^{o}C, the level recommended for pathogen inactivation, on sampling dates. In contrast, temperatures of up to 64 and 55^{o}C were detected during sampling days at Farm A and G, respectively (Table 2). In the cured compost heaps at Farms A and G, temperatures were detected ≥55^{o}C at internal locations; however, temperatures in excess of 26^{o}C were not detected on all sampling days at Farm M.

Higher temperatures (≥50^{o}C) were maintained in the heaps of Farm A, which employed windrow composting. In contrast, composting temperatures were generally lower than 45^{o}C in heaps at Farm M except for two sampling dates after new material was added into the heaps.

The pH values of active composting heaps at Farms A, M and S were mildly acidic to mildly alkaline, whereas pH values at Farm G were in the alkaline range during the survey (Table 2). The pH values for the cured heaps at Farms A, G and M followed the same trend that was observed for the heaps in active composting at those farms (Table 2). The average pH values from samples taken from all farms were in the following order: Farm G>Farm M>Farm S>Farm A.

Lower moisture contents were usually detected at the heap surface. However, in the Farm G heap undergoing active composting, the moisture content was 67.4% in the surface of the compost on one sampling date. This was due to the incorporation of

compost material into the sample that had been exposed to rain. Over all, it was found that approximately 71% of all internal samples contained less than 40% moisture.

Oxygen contents of the heaps were low among across all farms, however. Microaerophilic conditions existed within the heaps across Farms A, G and M, as oxygen contents ranged from 0 – 6% (Table 2). Heaps at Farm S had oxygen contents ranging from 2 – 17%, through the end of composting (Table 2). At Farm M, the heaps had low oxygen contents at the onset of composting; however, as composting progressed, the moisture content of the heaps decreased while the oxygen content increased.

Effect of adding new material into a composting heap

Figures 2a and 2b reveal the coliform populations present in the active composting heaps at Farms G and M, respectively. Active composting on Farm G resulted in the elimination of coliforms inside the heaps after 43 days of composting (Figure 2a). However, the addition of partially composted material between days 43 and 77 after the onset of composting resulted in an increase in coliforms greater than 1.5 $\log_{10}$ CFU/g in the surface of the heap (Figure 2a). Additionally, the introduction of fresh waste material between days 112 and 147 of composting caused increases of ca. 1.5, 3, and 1 $\log_{10}$ CFU/g in the surface, 60 cm, and 90 cm depths, respectively, of the heap undergoing active composting at Farm G (Figure 2a). In Farm M, application of a new layer of fresh waste material into the active composting bin resulted in increases of coliform populations of ca. 3 and 1 $\log_{10}$ CFU/g, respectively at the surface and 40 cm sampling locations between days 54 and 113 after the onset of composting. Coliforms

were not detected on the surface of the compost, but in the internal locations due to an inversion of the composted material one day prior to the final sampling date at Farm M.

Changes of bacterial populations during composting

Thermophilic bacteria: The mesophilic and thermophilic bacterial populations were in the range of 4.4 -9.6 and 5.1 – 10 $\log_{10}$ CFU/g, respectively, in the surveyed heaps. Thermophilic bacterial populations in the heaps in active composting at Farm S were ca. 2.5 $\log_{10}$ CFU/g less than those present in the heap at Farm A. During sampling dates, temperatures greater than 50^{o}C were commonly detected within the Farm A heap. This data illustrates that high populations of thermophilic bacteria are correlated with active composting.

Indicator and presumptive pathogenic bacteria: Detection of coliforms and presumptive pathogens in surveyed heaps during active and cured composting are presented in Tables 3a and 3b, respectively. Coliforms were detected in 100% (6 of 6), 83% (5 of 6), 86% (6 of 7), and 100% (14 of 14) of the surface samples from the active compost heaps of Farms A, G, M, and S, respectively (Table 3a). Inside the compost heaps on farms A, G, and M, coliforms were detected in ca. 22 – 71% of the internal samples. In contrast, at least 93% of all internal samples contained coliforms in the heaps of Farm S. In cured compost heaps on Farms A, G, and M 100% of all surface samples contained coliforms and up to 83% of all internal samples contained coliforms (Table 3b).

In all heaps of Farms A, G and M, presumptive *Salmonella* was not detected in any of the internal samples of the heaps except for one sample in the heap of Farm M

(Tables 3a and 3b). *E. coli*, coliforms and presumptive Salmonella spp. were detected in 100% of the surface samples in the heaps of Farm S, as compared with ca. 71% of the internal samples (Table 3a). Presumptive *Listeria* was detected in ca. 31% of all surface and interior samples of compost heaps in active composting, but were only detected on the surface of the cured compost heaps. Through further analysis on Chromagar®, no *Listeria* isolates collected exhibited growth typical for *L. monocytogenes*.

Abiotic and biotic analysis of finished compost

In the finished composts, pHs ranged from mildly acidic to mildly alkaline, depending on the farm and locations of the compost heaps (Table 4). Sixty-two (62) percent of all samples contained enumerable coliforms. *E. coli* was only detected in two samples, both from Farm S. Farm M was the only farm surveyed whose finished compost did not contain either presumptive *Salmonella* or *Listeria* at any sampled location.

Discussion

Poultry wastes, specifically litter, have many agricultural benefits. Poultry litter, along with other animal manures, contains nitrogen and phosphorus elements important to crop growth. As a result, application of poultry litter to land is an accepted "best management practice" routinely performed in agriculture (Chapman, 1996). Poultry litter is also used as a feed source for ruminant animals. In spite of the agricultural benefits of poultry litter, there are drawbacks to its use. Spreading untreated poultry litter could result in the introduction of pathogenic microorganisms *Escherichia*, *Pseudomonas*,

Salmonella, *Staphylococcus* (Srivastata et al., 1972), *Campylobacter* (Montrose et al., 1985), and *Clostridum* (Ogonowski et al., 1984) into soil and water where extended survival is possible. Additionally, feeding untreated poultry wastes to animals could result in the transfer of pathogens to other food animals. Due to these reasons, it is important that poultry wastes are treated before use. Guidelines have been proposed to suggest conditions that should be targeted to ensure that effective composting occurs (Richard et. al, 1998). Carbon-to-Nitrogen (C:N) ratios of 20:1 – 40:1, and moisture contents in the range of 40 – 60% are deemed as parameters that are either "acceptable" or "optimal" for composting. This survey revealed that the C:N ratios for Farm S heaps were in the range of 10:1 to 16:1, below acceptability standards for composting.

Studies have shown that co-composting poultry wastes with other substrates allow for increases in temperatures suitable for pathogen inactivation. Atkinson et al. (1996) composted a poultry litter – pine sawdust mixture (C:N ratio of 25:1) in compost reactors, and reported that the temperature increased to 55^{o}C after 20 h of composting and remained at that level for 16 days. When composting liquid poultry manure with barley wastes, Guerra-Rodríguez at al. (2003) reported that the compost reached and held temperatures in excess of 60^{o}C for more than 10 days.

Microbial inactivation in poultry litter during on-farm composting

Limiting pathogen introduction into the environment is one of the most important benefits in composting poultry litter, as it is widely known that these materials harbor bacteria associated with foodborne diseases. In poultry litter deep stacked in wooden bins (1.0 × 1.0 × 1.2 m), *E. coli*, *Salmonella*, and *Shigella* were not detected through 2, 2,

and 1 days when present in initial populations of 3.45 – 3.54, 2.18 – 2.32, and 1.40 – 1.70 $\log_{10}$ CFU/g, respectively (Kwak et al., 2005). It is generally accepted that elevated temperatures in composting is the major mechanism resulting in the elimination of indicator and pathogenic bacteria during composting. In this study, heaps at Farm A were large in size, and usually held elevated internal temperatures and moisture contents above 30%. Consequently, frequencies for detecting presumptive salmonellae and listeriae were low inside of the heaps (Tables3a and 3b). Our study also demonstrated that there was a positive correlation between heap moisture and elevated internal heap temperatures. The heap at Farm S which contained an initial moisture content of 37% (initial mix) reached an internal temperature in excess of 50^{o}C after one week of composting. Conversely, the compost mix heap at the farm had an initial moisture content of ca. 21%, and the maximum temperature reached was 38^{o}C. Chaudry et al. (1998) reported that deepstacking poultry litter at various moisture contents (15, 25 and 35%) resulted in 4 and 3 $\log_{10}$ CFU/g reductions of total and fecal coliforms, respectively, after one week. These researchers also report that there was a positive correlation between heap moisture and achieved temperatures.

Microbial inactivation in poultry litter and chicken carcasses during composting

Poultry litter and carcasses are routinely composted together in a farm environment. Studies have demonstrated that this practice can eliminate pathogenic bacteria. Blake et al. (1994) performed a field survey where 12 mini-composters were monitored for the presence of indicator and pathogenic microbes. Throughout the survey, temperatures in all heaps were above the 55^{o}C threshold and *Salmonella*, *Campylobacter*

jejuni, and *L. monocytogenes* were not detected in any of the samples. Lawson and Keeling (1999) composted hens according to the prescribed USDA method and reported heap temperatures in excess of 56^{o}C after 3 days of composting. Additionally, composting was completed in two months, with ambient temperatures ranging from -1 to 17.5^{o}C, resulting in the decomposition of carcass tissues and complete *Salmonella* inactivation. In a study of composting hatchery wastes with poultry litter, Das et al. (2002) reported that 99.9% of *E. coli* was eliminated and *Salmonella* was rendered non-detectable through composting. Those researchers suggest that elevated temperatures in excess of 60^{o}C were a major factor causing pathogen inactivation.

In our study, Farms G and M that participated in this survey composted poultry carcasses and poultry litter together. The presence of indicator bacteria and presumptive pathogens detected in the active compost heap of Farm M may be explained by the presence of sublethal temperatures in those heaps as detected on the sampling dates. In contrast, Farm G heaps usually held elevated temperatures inside the compost heaps.

Farm practices influencing pathogen survival in compost

A few studies surveyed the presence of either indicator bacteria or pathogens in poultry litter compost. Martin and McCann (1998) investigated the presence of indicator and pathogenic bacteria in poultry litter on Georgia farms. Out of the 86 samples tested, 64 samples were composted for various amounts of time (from ≤ 1 month through ≥ 4 months). Neither *E. coli* O157:H7 nor *Salmonella* spp. were detected in any sample analyzed. Additionally, only 5 out of 86 samples contained quantifiable coliforms, ranging in populations from 2.0×10^{1} – 8.0×10^{2} CFU/g. Hartel et al. (2000) compared the populations of coliforms present in interior and exterior locations of deepstacked poultry

litter heaps which were deepstacked from 1 to 104 weeks. Coliforms were not detected from the interior location of any of the deepstacked heaps, but 1 out of 13 heaps contained coliforms at the exterior location. Our results were in agreement with the above study, as we observed that the surface of the compost heaps allowed the extended survival of indicator and pathogenic microorganisms.

Surveys of poultry litter, mentioned above, suggest that the deepstacking of those materials effectively eliminates indicator and pathogenic bacteria. Recently, Lasardia et al. (2006) examined several types of composts available for sale on the Greek market for indicator and pathogenic bacteria. All composts analyzed, including those not derived from municipal solid wastes and animal manures, contained fecal coliforms in populations of at least 5×10^2 CFU/g. Although *Salmonella* spp. were not detected in any analyzed samples, 17 and 96% of the samples analyzed were found to contain *Staphylococcus aureus* and *Clostridium perfringens*, respectively. Though that study did not exclusively focus on the composting of waste materials, it serves as evidence that compost may not always completely render composts free of coliforms and pathogens from compost. In the analysis of finished compost produced at the farms in this survey, data revealed that coliforms, and often presumptive *Salmonella*, were detected in the samples, commonly on the surface. This is a potential hazard, as the presence of *Salmonella* may result in regrowth of the pathogen if the conditions are suitable. Furthermore, application of contaminated compost into agricultural land may allow for the contamination of soil, and more importantly, crops that may be grown in those fields.

During this survey, it was found that compost practices among farms were highly variable. On Farm G the temperature of the heaps were not monitored, while no moisture

was added or adjusted into the system or the ingredients during composting on Farm S. Though three of the farms indicated that they did monitor temperature, the monitoring was performed infrequently, and was only measured at the center of the heaps. This could be misleading, as the data from our survey shows that the temperatures in the compost heaps differ depending on location within the heap. Additionally, the farms which added moisture in the compost heaps had no procedures to determine the amount of moisture added, and if it was acceptable for proper composting to occur. Two farms, A and S, maintained set schedules for the turning of the composts. Overall, the oxygen content of the heaps were low. However, these heaps did not contain appropriate moisture, as it widely known that moisture decreases air spaces within compost heaps. A lack of oxygen in compost heaps could result in the uneven decomposition of composting substrates and reduce amount of heat produced, impacting pathogen abatement.

Importantly, both Farms G and M, which composted poultry carcasses, mixed fresh waste material into compost heaps that had already undergone a heating cycle. While this may be a common practice, it is unsafe microbiologically. The addition of new material into compost which has undergone heating allows for the possibility of pathogens surviving the compost process, as microbial activity will be reduced due to inadequate C:N ratios and moisture contents during composting Because these materials are not subjected to the elevated temperatures which occur during active composting, any pathogens associated with the fresh material may cause repopulation of the compost with pathogens. Also, there is a risk for any pathogens reintroduced into the heap to become heat resistant, as sublethal temperatures may cause the induction of heat-shock proteins. Staging areas and equipment used in housing and maintaining compost should also be

cleared of any untreated materials, as the use of contaminated equipment may result in repopulation of pathogens into compost. Russ and Yanko (1981) suggest that composts held in the mesophilic range with moisture contents of ca. 20% and C:N ratio greater than 15:1 may allow for *Salmonella* repopulation.

Other factors influencing pathogen survival in compost

Several environmental factors may contribute to the reduction of pathogens in compost. Exposure to ultraviolet light has been suggested as a factor (Nicholson et al., 2005); however, it is not applicable in this study as composting at each farm was conducted under a roofed area. Temperature on the compost surface can be "ruled out" as a factor, as Bush et al. (2007) state that temperatures near the outside edges of stacked poultry litter changes with ambient temperature. Sundberg et al. (2004) have suggested that low pH of compost may affect the transition into the thermophilic phase of composting, thereby influencing pathogen reduction; however, the pH values detected in this survey were not in the range that would delay entrance into the thermophilic phase. Although desiccation has also been suggested as a condition which would result in pathogen inactivation (Redlinger et al. 2001), in this study, presumptive pathogenic bacteria were often detected in the compost locations in which moisture contents were lowest. This data suggests that lower water activity (a_w) may result in prolonged pathogen survival. A study by Himathongkham (1999) reveals that salmonellae may survive for ca. 30 days at a_w of 0.5 and 0.38, and nearly 100 days at an a_w of 0.07.

Detection of presumptive pathogens (*Salmonella* and *Listeria*) also occurred during the survey in internal samples of the heaps; however, detection frequencies were

not comparable to that of the surface samples. An explanation for the lower detection rates found in internals samples could be the combination of the temperature along with the production of ammonia during composting. While ammonia release was not investigated in this study, production of this gas may have had a bactericidal effect on the pathogens investigated. Elwell et al. (1998) reported that due to low C:N ratios, the composting of unamended chicken litter results in the release of large amounts of ammonia. Additionally, Turnbull and Snoeyenbos (1973) indicated that elevated levels of ammonia have a "salmonellacidal" effect. Importantly, it has been demonstrated that decreased water activity and gassing with 1% ammonia resulted in reductions of 2.5, 3, and 4 $\log_{10}$ CFU/g for *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7, respectively, in chicken litter.

While it is likely that elevated temperatures and ammonia release could be the factors causing pathogen destruction inside of the compost heaps, confounding factors such as low oxygen content and microbial competition may have contributed to pathogen inactivation. The persistence of presumptive pathogens may be explained by the expression of heat shock proteins due to exposures to sublethal temperatures during the mesophilic composting phase (Brinton and Droffner, 1994).

Conclusions

Results from this study revealed that temperatures of compost heaps on poultry farms are commonly below levels recommended for pathogen destruction. Additionally, factors which may influence the progression of composting, such as moisture content, and C:N ratio, usually do not fall within the acceptable ranges for composting.

Importantly, indicator and pathogenic bacteria usually persist on the compost surface. The addition of new materials into treated compost may cause inadequate composting and permit the reintroduction of pathogens into composts.

The survey of composting on poultry farms revealed that the substrates and methods implemented in composting vary. When composting poultry wastes, carbonaceous materials and water should be added to increase the C:N ratio and moisture content, respectively, to ensure that conditions are acceptable for microbial activity. Composts should be regularly aerated in order to decrease the likelihood of both uneven decomposition of organic matter and pathogen destruction. Care should be taken that facilities used to house and maintain the compost are free of any materials that may re-introduce pathogens into the compost. Additionally, the addition of fresh, untreated materials into previously heated compost should be avoided.

Farmers should be educated so that they know under what conditions composting should be performed in order to produce safe compost. Also, practical measurements should be developed which make composting easier for farmers to correctly implement composting guidelines on their farms. Importantly, strategies should be implemented to reduce the survival of potentially pathogenic bacteria on the compost surface.

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Figure Legend

Figure 2.1: Temperature profiles of the composting heaps on Farm S. Values are the averages of results of two heaps at each sampling location. Results from the initial mix heap were obtained at the surface (×) and at two locations within the heaps (60 cm, ◊; 90 cm, □). Results obtained from the compost mix heap were obtained at the surface (○) and at two locations within the heaps (60 cm, ♦; 90 cm, ■).

Figure 2.2: Impact of adding new material on coliform populations in the initial compost heap (Farm G). The arrow (↓) indicates that new materials were added into the heaps between the sampling periods. The darkened circle (●) indicates an inversion of the heap one day before sampling.

Figure 2.3: Impact of adding of new material on coliform populations in Bin #1 compost heap (Farm M). The arrow (↓) indicates that new materials were added into the heaps between the sampling periods.

Table 2.1: Compost heap setup and composting practices on South Carolina poultry farms

Farm (# of heaps)	Composting method	Compost dimensions (L×W×H in meters)	Compost composition	Carbon:Nitrogen (C:N) ratio	Turning frequency	Temperature monitoring	Length of composting[d]	Use of compost
A (2)	Windrow	24.0 × 10.0 × 12.0	Chicken litter and "pine fines"	N/A[a]	Weekly	Yes	2 mon	Sold to consumers
G (3)	Static Heap	3.6 × 3.5 × 1.6	Chicken litter, chicken mortalities, and wood chips	N/A	Only when moved to new bin	No	6 – 8 wk	Land Application
M (2)	Bin	2.0 × 2.0 × 2.0	Chicken litter, chicken mortalities, and wood shavings	N/A	Only when moved to new bin	Yes	4 – 6 mon	Sold to consumers
S (2)	Static Heap	2.0 × 1.0 × 0.90	Chicken litter and "pine fines"	16:1[b]; 9.7:1[c]	Weekly	Yes	2 – 3 mon	Land Application

[a]: Initial compost sample was not examined for this parameter.
[b]: C:N ratio of the "Initial Mix" compost heap located at Farm S.
[c]: C:N ratio of the "Compost Mix" compost heap located at Farm S.
[d]: Denotes the length of time farm managers usually compost waste material on-farm.

Table 2.2: Summary of surveyed compost heaps during active composting

Composting type	Heap	Farm	Temp. range (°C) (inside heaps)	Oxy. content (%) (inside heaps)	pH range	Moisture content (%)	Total bacterial counts (Log CFU/g)		Coliform counts (log cfu/g)
							Mesophilic (30°C)[a]	Thermophilic (55°C)[b]	
Active	Initial	A	28 – 64	0 – 2[c]	5.4 – 7.7	10.6 – 44.6	4.5 – 9.6	6.3 – 10.0	ND[h] – 5.9
	Initial	G	36 – 55	0 – 8[d]	8.3 – 9.0	7.2 – 55.1	4.4 – 8.6	5.1 – 8.3	ND – 4.3
	Bin #1	M	14 – 51	6 – 18[e]	6.0 – 8.6	5.7 – 67.4	5.5 – 8.6	6.2 – 8.5	ND – 5.6
	Both[i]	S	13 – 52	1 – 18[f]	6.7 – 8.6	11.2 – 37.6	6.1 – 7.6	5.9 – 7.4	ND – 6.0
Cured	Premix	A	36 – 60	1 – 3[c]	4.8 – 8.4	8.2 – 52.6	5.3 – 9.3	6.0 – 9.5	ND – 6.0
	Cured	G	42 – 56	1 – 5[d]	8.0 – 8.6	8.1 – 37.8	5.5 – 8.3	5.5 – 7.5	ND – 3.9
	Bin #2	M	5 – 26	N/A[g]	6.4 – 8.9	10.9 – 52.9	7.1 – 8.6	6.7 – 7.9	ND – 3.5

[a]: Temperature value given represents the incubation temperature to enumerate mesophiles.
[b]: Temperature value given represents the incubation temperature to enumerate thermophiles.
[c]: Measurement began 130 days after the onset of composting.
[d]: Measurement began 43 days after the onset of composting.
[e]: Measurement began 203 days after the onset of composting.
[f]: Measurement began at day 0 of composting.
[g]: N/A: Not applicable, measurement was not taken
[h]: ND: Not detected, detection limit <1 log CFU/g.
i: Data from the Initial and Compost Mix heaps were combined.

Table 2.3: Prevalence of coliforms, *E. coli*, and presumptive pathogens in compost undergoing first heating phase

Farm	Heap	Location (in cm)[a]	Coliforms	*E. coli*	Presumptive *Salmonella* spp.	Presumptive *Listeria* spp.
A (n=18)[b]	Initial	<5 (n=6)[c]	6	2	6	1
		60 (n=6)	3	0	0	1
		90 (n=6)	4	0	0	1
G (n=24)	Initial	<5 (n=6)	5	1	4	1
		60 (n=9)	2	0	0	4
		90 (n=9)	2	1	0	1
M (n=21)	Bin #1	<5 (n=7)	6	2	3	1
		40 (n=7)	5	1	0	1
		60 (n=7)	4	0	1	0
S (n=42)	Both heaps[d]	<5 (n=14)	14	14	14	9
		60 (n=14)	13	10	13	7
		30 (n=14)	14	10	14	6

[a]: Sampling locations of compost heaps as described in Materials and Methods.
[b]: Total number of samples taken from a particular farm over the course of the survey.
[c]: Total number of samples taken from each location at a particular farm over the course of the survey.
[d]: Data from the Initial and Compost Mix heaps were combined, as both contained fresh poultry litter when composting began.

Table 2.4: Prevalence of coliforms, *E. coli*, and presumptive pathogens in compost undergoing second heating phase

Farm	Heap	Location (in cm)[a]	Coliforms	E. coli	Presumptive Salmonella spp.	Presumptive Listeria spp.
A (n=18)[b]	Premix	<5 (n=6)[c]	6	2	6	1
		60 (n=6)	5	0	0	0
		90 (n=6)	3	0	0	0
G (n=9)	Cured	<5 (n=3)	3	0	1	1
		60 (n=3)	1	0	0	0
		90 (n=3)	0	0	0	0
M (n=9)	Bin #2	<5 (n=3)	3	0	0	0
		40 (n=3)	2	0	0	0
		60 (n=3)	2	0	0	0

[a]: Sampling locations of compost heaps as described in Materials and Methods.
[b]: Total number of samples taken from a particular farm over the course of the survey.
[c]: Total number of samples taken from each location at a particular farm over the course of the survey.

Table 2.5: Abiotic and biotic analysis of finished compost samples

Farm	Heap	Days into composting	Location (cm)[a]	pH[b]	MC (%)[c]	Temp. (°C)	Thermo. (log CFU/g[d])	Meso. (log CFU/g[e])	Coliforms (log CFU/g)	*E. coli*	*Salmonella*[h]	*Listeria*[i]
A	Premix	193	<5	6.65±0.08	26.41±0.69	14±0.00	9.31±0.02	9.34±0.16	-	-	+	-
			60	5.60±0.03	42.57±0.45	48±0.00	7.56±0.03	6.82±0.67	2.48±0.67	-	-	-
			90	6.15±0.06	42.48±3.09	36±1.41	7.52±0.22	6.07±0.27	2.98±0.46	-	-	-
	Premix	193	<5	7.27±0.01	15.14±0.88	26±0.00	8.53±0.11	8.04±0.08	3.80±0.14	-	+	-
			60	7.18±0.00	22.86±0.91	53±1.41	6.60±0.15	5.39±0.55	-	-	-	-
			90	8.40±0.08	52.59±2.52	46±1.41	7.48±0.11	5.34±0.34	-	-	-	-
G	Cured	147	<5	7.70±0.30	8.17±1.30	19±0.00	7.36±0.15	7.76±0.03	2.72±0.45	-	+	-
			60	8.31±0.08	27.73±0.64	53±0.71	5.71±0.05	5.54±0.12	-	-	-	-
			90	8.02±0.15	25.51±0.72	39.5±0.71	5.52±0.08	5.56±0.04	-	-	-	-
M	Bin #1	342	<5	7.53±0.04	18.80±0.40	27±0.00	6.60±0.02	6.71±0.06	-	-	-	-
			40	7.90±0.02	34.58±2.52	29±0.00	6.52±0.13	6.52±0.14	2.78±0.25	-	-	-
			60	8.11±0.04	40.49±2.06	32±0.00	6.79±0.10	7.33±0.17	4.00±0.09	-	-	-
	Bin #2	342	<5	6.67±0.30	10.93±0.93	-1±0.00	7.47±0.01	7.28±0.08	3.05±0.21	-	-	-
			40	6.60±0.49	52.88±2.97	5±0.00	7.19±0.17	6.69±0.07	-	-	-	-
			60	6.67±0.16	48.79±2.33	6+0.00	7.18±0.05	6.65±0.01	-	-	-	-
S	Compost Mix	60	<5	8.27±0.06	14.77±2.03	3±0.00	6.23±0.01	6.84±0.09	2.86±0.04	1.40±0.00	+	-
			60	8.42±0.00	19.30±0.02	13±0.00	6.56±0.06	6.82±0.12	1.69±0.05	-	-	-
			30	8.55±0.01	20.48±0.27	13±1.41	6.49±0.01	6.61±0.06	1.95±0.01	-	-	-
	Initial Mix	60	<5	8.46±0.09	14.76±1.03	3±0.00	6.24±0.14	6.93±0.21	3.70±0.02	2.02±0.05	+	+
			60	8.38±0.01	20.34±0.90	13.5±0.71	6.35±0.04	6.60±0.03	1.62±0.03	-	+	+
			30	8.57±0.06	22.31±0.05	14.5±0.71	6.38±0.07	6.79±0.02	2.05±0.05	-	+	+

[a]: Locations of the samples of the composting heaps as described in the Material and Methods.
[b,c]: Values represent the mean of the data +/- the standard deviation.
[d,e]: Values represent the mean of the $\log_{10}$ counts of the thermophilic and mesophilic bacteria, respectively, +/- the standard deviation.
[f,g]: + and -, represents that the parameter has been detected or not detected in the sample, respectively.
h,i: Indicates the presence of presumptive *Salmonella* and *Listeria* isolates, respectively.

Figure 2.1.

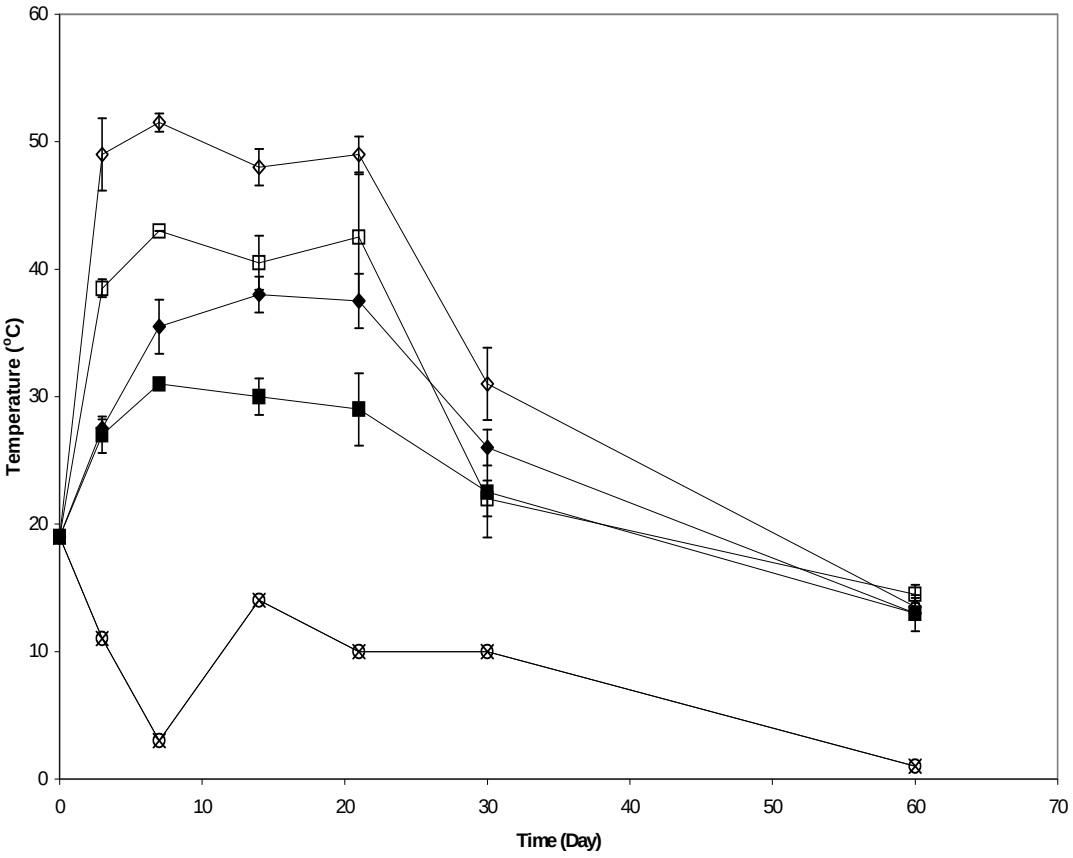
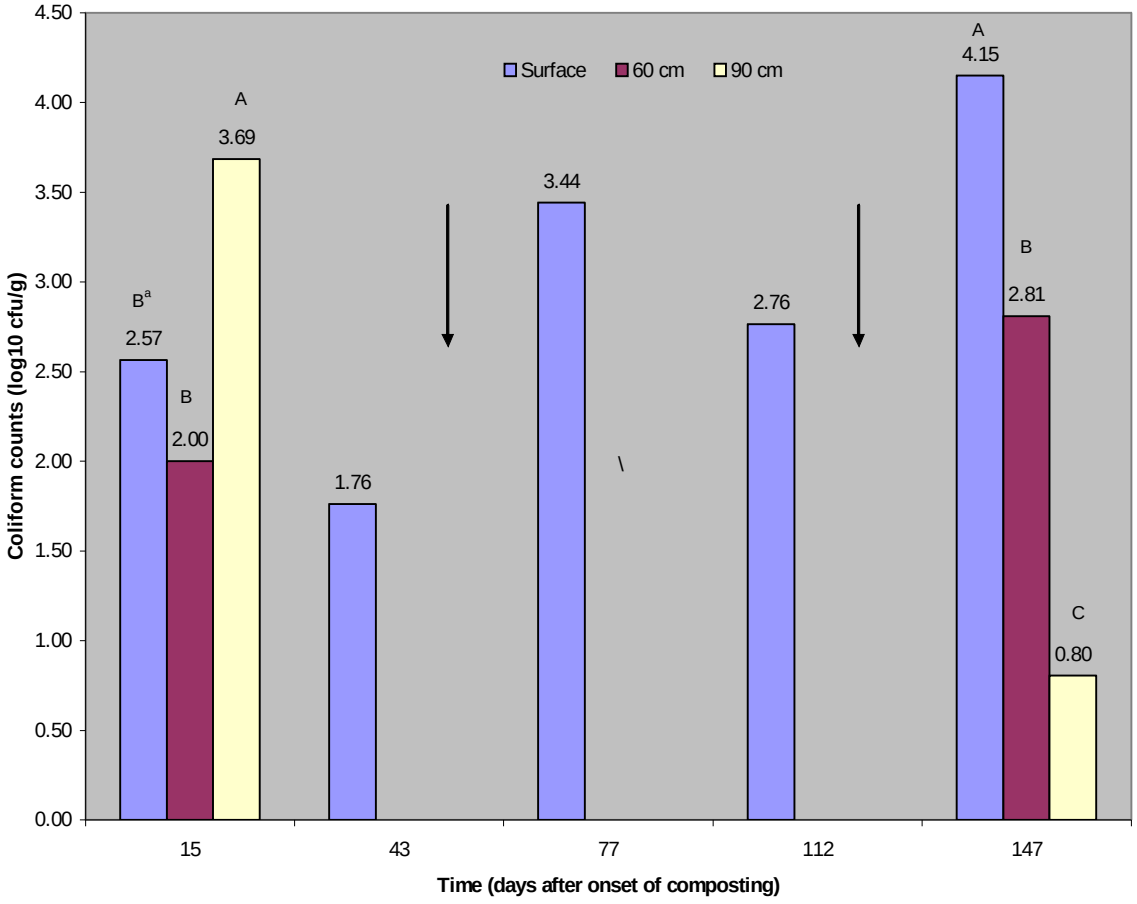
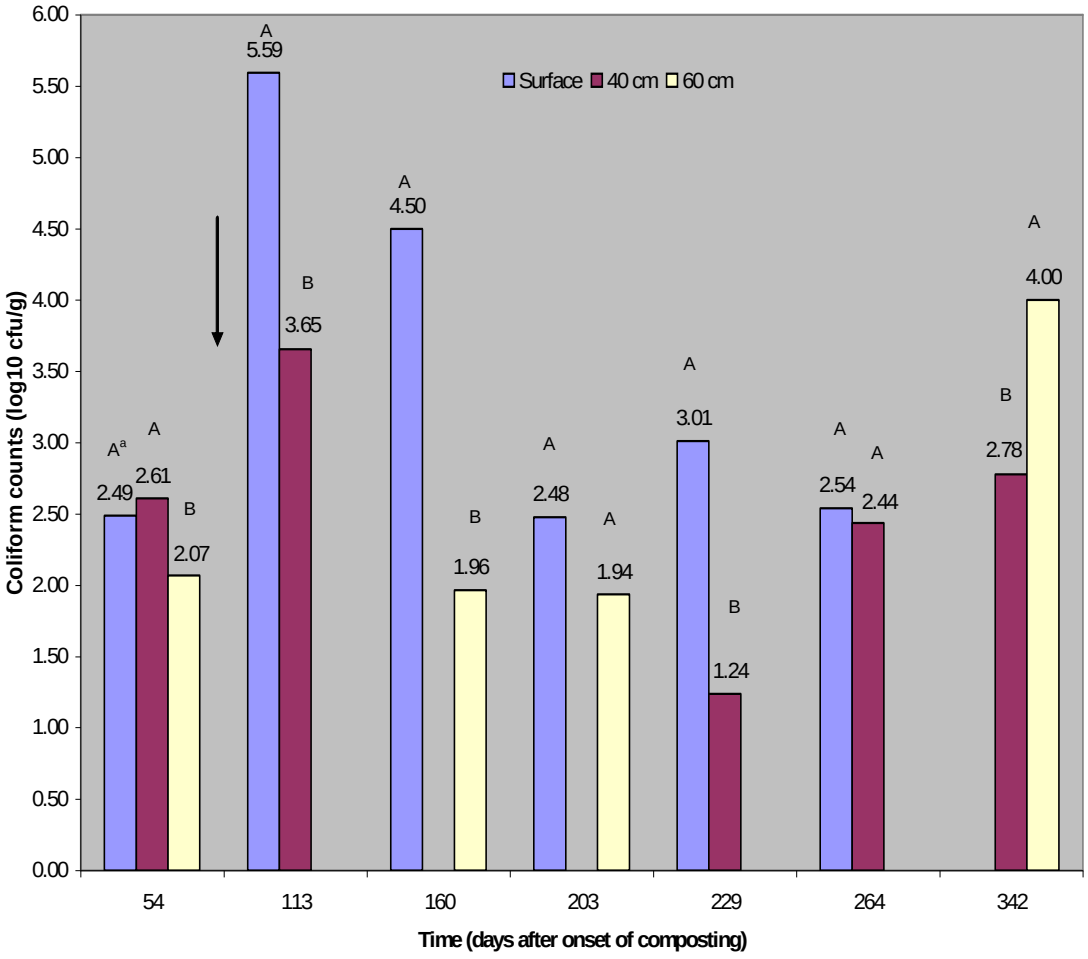


Figure 2.2.



[a]:Means with the same letter at each sampling date are not significantly different (P>0.05).

Figure 2.3



[a]:Means with the same letter at each sampling date are not significantly different (P>0.05).

CHAPTER THREE

FATE OF *Escherichia coli* O157:H7 DURING ON-FARM DAIRY MANURE-BASED COMPOSTING

Abstract

Studies were conducted to determine the fate of *Escherichia coli* O157:H7 in dairy manure-based compost in a field setting. Two trials were performed involving duplicate compost heaps constructed on an outdoor, fenced site. The compost heaps, ca. 1.3 m^3, were comprised of dairy manure, old hay, feed waste, a sawdust-calf feces mixture, and fresh hay. Samples of the composting mixture were inoculated with *stx*-negative *E. coli* O157:H7 B6914 at initial cell numbers of 10^7 and 10^5 CFU/g for Trial 1 and Trial 2, respectively. Individual sample bags were placed on the surface and at three locations (top, center, and bottom) within each heap. Though compost heaps achieved temperatures of 50^{o}C or above at all internal locations for at least 7 days, temperature stratification was observed. In Trial 1, *E. coli* O157:H7 was detected by enrichment through 14 days within the heaps. When inoculated with 10^5 CFU/g in Trial 2, *E. coli* O157:H7 was detected only through days 2, 2, and 5 at the top, center, and bottom locations, respectively. For both trials, the pathogen survived at the heap's surface for up to 4 months. The indicator microorganism, *E. coli,* was inactivated at a rate similar to that of *E. coli* O157:H7. Results indicate that composting, with periodic heap turning, can be a practical approach to inactivating *E. coli* O157:H7 in cattle wastes on the farm.

Our data also suggests that failure to maintain heaps through turning may allow *E. coli* O157:H7 survival for months at the compost surface.

Introduction

Cattle are a primary reservoir of *Escherichia coli* O157:H7 which shed the pathogen into the environment through their fecal matter. Application of animal waste to agricultural land as fertilizer or as soil conditioner is generally accepted as a practical waste management practice in the U.S. (CAST, 1996). However, application of manure to the land may introduce the pathogen into the soil that supports the growth of food crops such as vegetables. The application of manure and manure slurries to soil provides an opportunity for contamination of produce, drinking water, and irrigation water with the pathogen (Ogden et al., 2001; Islam et al., 2004; Islam et al., 2005; Mukherjee et al., 2005). Importantly, studies have revealed that *E. coli* O157:H7 can survive for extended periods of time in manure (Wang et al., 1996; Himathongkham et al., 1999), manure heaps (Kudva et al., 1998), and in manure-amended soil (Ogden et al., 2001; Jiang et al., 2003; Avery et al., 2004; Nicholson et al., 2005).

Some foodborne disease outbreaks have been associated with manure contamination of fresh produce with *E. coli* O157:H7 (Cieslak et al., 1993; Chapman et al., 1997). For example, an outbreak of *E. coli* O157:H7 infection among members of four families was associated with potatoes grown in soil fertilized with cattle manure on the family farm (Chapman et al., 1997). In another instance, a woman acquired *E. coli* O157:H7 infection from eating her garden-manured vegetables that were inadequately washed (Cieslak et al., 1993). Most recently, a large *E. coli* O157:H7 outbreak

associated with bagged baby spinach was linked to cattle feces present in a field of one of four California ranches implicated in the outbreak (CDC, 2006; FDA, 2006).

Composting is used often on farms to manage large amounts of animal wastes. When properly performed, composting is a practical method that can effectively kill pathogens in manure, as well as weed seeds, viruses, and nematodes (Misra et al., 2003). While some studies suggest that ammonia gas (Nicholson et al., 2005), desiccation (Redlinger et al., 2001; Nicholson et al., 2005), and microbial antagonism (Ichida et al., 2001) are factors that contribute to pathogen reduction during composting, the most influential mechanism is likely temperature elevation caused by metabolically active microorganisms during composting. Several studies investigating the survival of *E. coli* O157:H7 in compost have been performed in laboratory-scale bioreactors (Lung et al., 2001; Jiang et al., 2002; Hess et al., 2004). Results from these studies further confirmed that the increased temperatures were the predominant mechanism affecting the inactivation of *E. coli* O157:H7 and *E. coli*, and coliforms.

The objective of this study was to determine if the composting of dairy manure performed in a field setting under uncontrolled environmental conditions would effectively eliminate *E. coli* O157:H7 and indicator bacteria, *E. coli* and coliforms, throughout compost heaps.

Materials and Methods

Preparation of compost heaps: Two composting trials were conducted both in 2005; one during the summer and the other in the fall. For each trial, a sawdust-dairy manure mixture, fresh hay, waste cattle feed and old hay were mixed thoroughly with a

front-end loader at a ratio of 28:16:8:1, respectively, on a weight basis. All materials used in the composting trials were obtained from a single dairy farm and were accumulated no more than 2 weeks before the start of composting. The dairy manure used was collected daily from a herd of ca. 125 lactating cows and stored with minimal movement to prevent aeration, which could have potentially caused heating of the materials. None of the materials collected was subjected to any treatment prior to composting. The materials were thoroughly mixed, and split into two separate heaps with the aid of a front-end loader. Each heap was conical in shape and approximately 1.2 m in height by 2 m in width. During composting, the two heaps were on a 25-m by 16-m concrete slab surrounded by a gated fence and were not covered or protected from environmental conditions.

Preparation of bacterial cultures: An avirulent green fluorescent protein (GFP) - labeled *E. coli* O157:H7, strain B6914, provided by Dr. Pina Fratamico at the United States Department of Agriculture, Agricultural Research Service – Eastern Regional Research Center, was used for the study. A frozen stock culture of *E. coli* O157:H7 was thawed and streaked on tryptic soy agar (TSA; Becton Dickinson, Sparks, Md.) containing 100 µg ampicillin/ml (Sigma Chemical Co., St. Louis, Mo.) (TSA-A), and incubated at 37^{o}C for 24 h. After two transfers in tryptic soy broth (TSB; Becton Dickinson) containing 100 µg ampicillin/ml (TSB-A), one ml of the TSB-A culture was inoculated into 500 ml of TSB-A, and incubated with agitation (125 rpm) for 18 to 24 h. The bacterial culture was sedimented by centrifugation at 5,000 × g for 15 min at 4^{o}C, and twice washed with sterile 0.85% saline. The optical density of the bacterial

suspension was adjusted to ca. 0.5 at 600 nm to obtain cell numbers of ca. 5 x 10^8 CFU/ml. Cells were enumerated by plating serial dilutions of the inoculum on TSA-A.

Sample preparation and placement: For both composting trials, a small heap (ca. 30,000 g) of compost was set aside and underwent extensive mixing with a shovel. Ten kg of this compost mixture was weighed, and inoculated with 10 ml of a fresh culture of the GFP-labeled *E. coli* O157:H7 using a mister with a sterile spray nozzle, followed by rinsing with 6 ml of sterile deionized water. The compost was continually mixed by hand, wearing sterile gloves for approximately 10 minutes after inoculation to distribute the inoculum. Compost ingredients of Trial 1 were inoculated with ca. 10^7 CFU *E. coli* O157:H7/g, whereas those of Trial 2 were inoculated with ca. 10^5 CFU/g.

Approximately 30-g portions of the inoculated compost samples were inserted into Tyvek® self-seal pouches (8.89 cm x 13.33 cm, DuPont, Wilmington, Del.) with sterile spoons. Tyvek® is a chemically inert material made of spunbounded olefin and mylar that provides sterile packaging protection and is breathable to allow the exchange of oxygen and water. It does not rot or mildew. These pouches maintained environmental conditions that were similar to the environment inside the heaps and enabled control of their position inside the heaps. Compost samples were placed inside the composting heaps at three locations of 30, 50, and 70 cm depths measured from the base of the concrete slab (Fig. 1). Duplicate sample bags were placed at each location inside each heap. All sample bags were color-coded to enable identification of the samples, as settling of the compost could cause some movement of the samples. Surface samples (ca. 35 g) were positioned by placing the inoculated compost inside a sterile weighing dish

(14 cm x 14 cm x 5 cm), and sample dishes were secured to the top of the heaps with thin strings.

Temperature and oxygen measurements: An OT-21 oxygen and temperature sensor (Demista Instruments, Arlington Heights, Il.) was used to record the temperature and oxygen concentrations at three locations inside each heap. Both temperature and oxygen concentrations were measured daily for up to 14 days for Trial 1 and 21 days for Trial 2. Thereafter, temperature and oxygen concentrations were monitored at 30, 60, and 120 days for both trials.

Sample collection and heap maintenance: Samples were obtained at 0, 3, 7, 14, 21, 30, 60, and 120 days of composting for Trial 1 and at 0, 1, 2, 3, 5, 7, 14, 21, 30, 60, and 120 days for Trial 2. At each sampling, the temperature of the compost was determined at each specified location before any internal samples were removed from the compost heaps. Once the collection of data was completed, the compost heaps were opened with a shovel and the samples were collected aseptically. All tools and containers used for sampling were sanitized with Enviroquant sanitizer (Vestal Laboratories, St. Louis, Mo.), and wiped dry with sterile paper towels.

The compost heaps were turned and mixed well mechanically using a front-end loader, and the remaining sample bags were placed back to the previous locations as soon as the heap was turned and reconstructed manually to approximately the same height as achieved at day 0. For Trial 2, on sampling days 1, 2, and 5, the heaps were not turned but the sample bags attached to a long string were pulled out of the heaps manually. All samples were analyzed in the laboratory within 4 h of sampling.

Microbiological analysis of compost samples: Ten g of sample was added to 90 ml of Universal pre-enrichment broth (UPB; Becton Dickinson) in a stomacher bag and macerated using a Stomacher® 400 Circulator (Seward Ltd., West Sussex, UK) for 1 min. The mixture was then serially diluted (1:10) with sterile 0.85% NaCl solution, and plated on TSA in duplicate using an Autoplate® 4000 spiral plater (Spiral Biotech Inc., Bethesda, Md.). The plates were incubated at 30°C and 55°C for 24 h, for enumerating mesophilic and thermophilic bacteria, respectively. The remaining portions of the samples in UPB were incubated at 37°C for 24 h for pathogen detection.

TSA-A was used for enumerating the GFP-labeled *E. coli* O157:H7. The plates were incubated at 37°C for 24 h, and then examined under a UV light using a Gel Doc 2000 imager (Bio-Rad Laboratories, Inc. Hercules, Ca.) for typical green fluorescent colonies. Selected green fluorescent colonies were further confirmed using the *E. coli* O157 latex test kit (Oxoid Ltd., Basingstoke, Hants, UK). In addition to direct plating, the compost samples were enriched in UPB for 24 h, and then selectively enriched in 9 ml of TSB-A at 37°C for 24 h. The selective enrichment broth (500 µl) was mixed with 10 µl of anti-*E. coli* O157 Dynabeads® (Dynal Biotech, ASA, Oslo, Norway) according to the manufacturer's instructions. The anti-*E. coli* O157 Dynabeads® were washed twice with the buffer, and the final suspension of Dynabeads® was plated on TSA-A through quadrant streaking. Following incubation at 37°C for 24 h, the colonies were observed under UV light for green fluorescence, and confirmed using the *E. coli* O157 latex test kit as described above.

Both *E. coli* and coliforms were enumerated using 3M *E. coli*/coliform Petrifilm™ (3M Microbiology Products, St. Paul, Mn.), with 1-ml dilutions plated in duplicate and

incubated at 37°C for 24 h. Blue colonies surrounded with gas bubbles on Petrifilm™ were counted as *E. coli*, whereas all colonies producing gas were considered as coliforms. When *E. coli*/coliform counts were not detectable on Petrifilm™, the compost samples were analyzed using the 3-tube most probable number (MPN) method as specified in the Food and Drug Administration Bacteriological Analysis Manual (FDA-BAM) (FDA, 2002). MPN tubes exhibiting the presence of gas were streaked on Levine Eosin Methylene Blue (L-EMB; Becton Dickinson) agar to determine if the samples contained *E. coli*.

Moisture content, pH, and C:N ratio determinations. Approximately 1 g of each sample was weighed, and analyzed for both moisture content and pH determinations as described previously (Jiang et al., 2002). About 100 g of initial compost mixture in duplicate were sent to the Agricultural Service Lab at Clemson University for C:N ratio testing.

Statistical analysis: Bacterial count data were converted to $\log_{10}$ CFU/g for statistical analysis. Specific comparisons of plate counts among different locations in the compost heaps at any date, and in some cases over all dates, were accomplished with Fisher's least significant difference (LSD) test. Using the GLM procedure, an analysis of covariance was performed comparing the populations of commensal *E. coli* to those of *E. coli* O157:H7 at each sampling location over the duration of the study to determine if correlations between those two counts exist. For composting temperature data, an analysis of variance (ANOVA) for a completely randomized design with repeated measures across dates was conducted to determine if general differences existed among

different locations. All calculations were performed using either the GLM or the MIXED procedure of the Statistical Analysis System (SAS 2001, Cary, Nc.).

Results

Outdoor compost heap construction: Two trials were conducted in upstate South Carolina in 2005, with Trial 1 initiated in the summer and Trial 2 in the fall. In each trial, two compost heaps were constructed, and duplicate sample bags were placed in each heap at each location for selected sampling intervals. A summary of composting ingredients and results of analyses are shown in Table 1. Both the carbon to nitrogen ratio and the moisture contents of the compost heaps were in the acceptable ranges for composting.

Temperature profiles: Self-heating inside the compost heaps occurred rapidly soon after all compost ingredients were mixed. Figures 2a and 2b reveal the temperature stratification present in the compost heaps of two trials. Temperatures at all locations inside heaps were significantly ($p<0.05$) different for Trial 1, whereas the temperatures at only the top and center locations were significantly ($p= 0.2526$) different from the bottom location in Trial 2. Overall, the composting temperatures from the highest to lowest at those locations were in the following order: top>center>bottom>surface. While temperature stratification was present throughout the heaps in active composting, temperatures were elevated above 50^{o}C during thermophilic composting at all locations in the heaps for at least 7 days in Trial 1, and 14 days in Trial 2. The temperature at the top location for all heaps was above 50^{o}C for at least 30 days, and above 55^{o}C between 14 and 21 days. The maximum temperature achieved occurred at the top location of the

heaps in both trials, with 65^{o}C at day 6 and 62^{o}C at day 11, in Trials 1 and 2, respectively. At day 21, in Trial 1, temperatures gradually declined, whereas temperatures fell sharply in Trial 2. This was due to the lower ambient temperature in Trial 2 which concluded in the winter.

pH and moisture content determinations: The changes of pH values and moisture content in the compost through 120 days of composting for Trial 1 are shown in Table 2. The results for Trial 2 were very similar to those for Trial 1 (data not shown). In both trials, the compost mixture was mildly alkaline at day 0. Through day 7, however, the pH in the compost decreased slightly to neutral within the heaps. Thereafter, internal compost samples returned to mildly alkaline levels, reaching a maximum of 10.01 at day 21 in Trial 1 and 9.43 in Trial 2, both at the bottom location. Overall, pH values of samples inside compost heaps were not significantly ($p>0.05$) different during most sampling days for both trials. For the surface samples in both trials, the pH decreased gradually from mildly alkaline to near neutral by the end of the composting trials.

Throughout most of the sampling intervals in both trials, the moisture contents of the internal samples changed very little. In Trial 1, moisture contents were not significantly ($p>0.05$) different inside the heaps, except for days 14, 21 and 60 of composting. However, surface samples at most sampling days contained significantly ($p<0.05$) less moisture than the internal samples.

Soon after turning the compost heaps, the oxygen concentrations inside the heaps decreased rapidly, to 1 to 5 % depending on location (data not shown).

Survival of *E. coli*/coliform populations during composting: For Trial 1, both coliforms and *E. coli* cell numbers at the top and center locations of the compost heaps decreased ca. 4.9 and 4.6 $\log_{10}$ CFU/g within 3 days, respectively, and to non-detectable levels by direct plating at 7 days and beyond (Table 3). However, both *E. coli* and coliforms were positive after enrichment culture for up to 14 days of composting. Both *E. coli* and coliforms were inactivated slowly at the bottom of the compost heaps, and detectable by direct plating through 3 and 7 days, respectively. Both *E. coli* and coliforms were inactivated more rapidly in Trial 2 than in Trial 1 (Table 4). The populations of indicator bacteria decreased to undetectable levels by direct plating at 2 days of composting at top and center locations, and were eliminated after 5 days at the bottom location (Table 4). By day 7, all internal samples were negative for both *E. coli* and coliforms even after enrichment culture. In surface samples of Trial 1, both *E. coli* and coliforms were detected by direct plating for at least 14 days and 120 days of composting, respectively. *E. coli* were positive by enrichment culture in surface samples throughout 120 days of composting. In contrast, both indicator bacteria were inactivated more slowly in surface samples of Trial 2. Overall, the *E. coli*/coliform cell numbers were significantly ($p<0.05$) greater at surface and bottom locations than those at top or center locations up to 3 days and 5 days of composting for Trials 1 and 2, respectively.

Survival of *E. coli* O157:H7 during composting: Two different inoculation levels of *E. coli* O157:H7 were used. The lower inoculation level, ca. 10^5 CFU *E. coli* O157:H7/g, would likely be found in feces, whereas higher populations of ca. 10^7 CFU *E. coli* O157:H7/g allowed for the determination of pathogen inactivation rates through 5 log CFU/g during composting.

There was a ca. 6 $\log_{10}$ CFU/g reduction of *E. coli* O157:H7 at 3 days after composting began for samples obtained from both the top and center locations, as compared with a 4 $\log_{10}$ CFU/g reduction at the bottom location (Figure 3a). Samples from inside the heaps were *E. coli* O157-positive by enrichment culture through 14 days at all three locations. In contrast, *E. coli* O157:H7 was enumerated directly from compost on the surface of heaps for up to 2 weeks, and detected by enrichment culture for at least 4 months, when the study was terminated. Overall, the surface samples had significantly ($p<0.05$) greater *E. coli* O157:H7 cell numbers from day 3 until the completion of the study, whereas internal samples were not significantly ($p<0.05$) different over the same sampling interval.

Rapid inactivation of *E. coli* O157:H7 in compost heaps with a lower inoculum is shown in Fig. 3b. The pathogen was detected using enrichment culture through 2 days of composting at the top and center of heaps and through 5 days at the bottom. However, *E. coli* O157 was detected for at least 30 days by direct plating and for at least 4 months by enrichment culture in samples from the heap surface. Statistical analysis of pathogen survival data from Trial 2 revealed that there were no significant ($p>0.05$) differences in *E. coli* O157:H7 cell numbers among the different sampling locations on day 1 of composting. After 2 days, there were no significant ($p>0.05$) differences between the surface and bottom samples, or between the top and center samples. After day 3 of composting, *E. coli* O157:H7 cell numbers in the surface samples were significantly ($p<0.05$) greater than those from all internal samples.

In both trials, the correlations were positive between cell numbers of both *E. coli* O157:H7 and *E. coli* at each sampling location, indicating that inactivation of *E. coli* and

E. coli O157:H7 populations are correlated. The p-values of the correlation for Trial 1 were 0.5818, 0.7860, 0.7254, and 0.9518 for the surface, top, center and bottom locations, respectively. In Trial 2, p-values were 0.2565, 0.5880, 0.6410, and 0.6810 for the aforementioned locations, respectively.

Discussion

Only a few reported studies have addressed the inactivation of *E. coli* O157:H7 by composting in a field setting (Larney et al., 2003; Hutchinson et al., 2005; Pourcher et al., 2005). In those studies, entire compost heaps were inoculated with the pathogen and only composite samples were collected and analyzed for the presence of the target pathogen. Considering the highly heterogeneous nature of compost ingredients and the stratification of temperature that occurs throughout the heaps, the dynamics of *E. coli* O157:H7 inactivation at different locations of the heaps would be anticipated to be highly variable. To address this variability, we strategically placed small contained portions of thoroughly mixed, inoculated compost mixture at different locations of the compost heap. This method enabled us to easily control the position of compost samples and subsequently determined how the stratification of temperature, pH, and moisture content of compost at different locations in heaps affected *E. coli* O157:H7 inactivation.

USDA prescribes to organic growers that composting operations maintain a temperature in the range of 55-70^{o}C for a minimum of 3 days for static aerated pile systems and 15 days with 5 turns for windrow systems (NOSB, 2002). The optimal ratio of the carbon to nitrogen (C:N) for active composting is 25:1 to 30:1; however, 20:1 to 40:1 is considered an acceptable range for active composting (Richard et al., 1998). The

C:N ratio in both of our trials was within the acceptable range, and the temperature within each heap at the top location was above 55^{o}C for 14 to 21 days. However, the temperature profiles for both composting trials revealed there was temperature stratification throughout the heaps. The warmest location within the heap was slightly above the geometric center, and the coolest site was at the bottom near the concrete pad. Results of previous studies in bioreactors or compost heaps have revealed temperature stratification within the compost (Jiang et al., 2002; Hutchinson et al., 2005). Therefore, the temperature measurement for the compost heaps should be clearly defined in terms of location.

In addition to temperature, oxygen content, pH value, and moisture content were determined at each sampling location at each sampling interval, while C:N ratios of the Day 0 composting materials were determined. Results revealed that within the compost heaps a microaerophilic condition prevailed. Therefore, additional aeration should be applied to extend active composting, since only turning a heap, as was done in this study was not sufficient to maintain highly aerobic conditions. Our survey of several composting operations on poultry farms revealed generally low oxygen levels (0-6%) within composting heaps as well (data not shown). During active composting, microbial activity slightly reduced the pH (Table 2), which is consistent with previous studies (Sundberg et al., 2004). The moisture content of the internal samples changed very little in both trials during active composting (thermophilic phase), suggesting that moisture content had minimal influence on pathogen inactivation. However, the moisture content of the initial compost mixture may have affected the rapid onset of self-heating at the beginning of composting, which in turn also affected pathogen inactivation. For these two

trials, although the initial compost mixture and C:N ratio were very similar, Trial 1 heaps, which had a higher initial moisture content due to heavy rain prior to mixing compost ingredients, began heating more slowly than heaps in Trial 2. Moisture content fluctuations occurred in compost surface samples in both trials; however, this was not unexpected because all of the heaps were exposed to the elements of the environment. Periods of precipitation and dry conditions occurred throughout both trials, and were the cause of the variation in moisture content of surface samples. It is important that initial compost parameters fall with the acceptable to optimal ranges for composting to ensure that conditions for microbial activity are suitable; thereby allowing an the increase in temperature necessary for pathogen inactivation.

Several studies, using laboratory-scale bioreactors under well-controlled environmental conditions, have revealed that composting can effectively inactivate *E. coli* O157 in manure in times ranging from 72 h to less than 14 days (Lung et al., 2001; Jiang et al., 2002; Hess et al., 2004). Only a few studies have addressed the inactivation of *E. coli* O157:H7 in composted manure heaps under field conditions; however, it was not indicated whether or not those studies were performed using an optimal C:N ratio and moisture content of compost materials. Nicholson et al. (2005) determined composting dairy manure in unturned solid manure heaps reached temperatures greater than 55^{o}C, and *E. coli* O157 inoculated at ca. 2.7 – 5.2 $\log_{10}$ CFU/g could not be detected after one week In our study, the *E. coli* O157:H7 survived for up to 14 days when the initial cell numbers were ca.10^7 CFU/g and composting was performed during the summer, but became undetectable within 5 days when the inoculation cell numbers were ca. 10^5 CFU/g and composting was carried out during the fall. The *E. coli* O157:H7 inactivation

rates were also affected by the elevated temperatures and how rapidly the self-heating occurred within the heaps. At locations in the compost heap where temperatures were higher, i.e., the top and center, the reduction of *E. coli* O157:H7 and *E. coli*/coliform cell numbers was significantly ($p<0.05$) more rapid than that at the cooler locations such as the bottom and on surface (Fig. 3 & Table 3 & 4). Hutchinson et al. (2005) reported extended survival of *E. coli* O157 in heaped mixtures of dairy cattle manure and beef cattle manure for 32 and 93 days, respectively, despite heap temperatures in excess of 50^{o}C within 5 days of the onset of composting. The authors suggest that the pathogen may have survived by the induction of heat shock proteins during the longer mesophilic phase. However, it is also possible that detection of the pathogen was due to the sampling procedure used, as the samples for the study were composites of compost material throughout the heaps. An uneven inactivation of *E. coli* O157:H7 at different locations of the heaps would be expected. Variation in the composting length for *E. coli* O157:H7 inactivation from different studies can be explained by many variables that occur, including the types of raw materials, C:N ratios of compost mix, size of heaps, ambient temperature, frequency of turning, initial cell numbers of target pathogens, strain variation, seasons of the year and geographical locations.

Both *E. coli* and coliforms have been widely used as indicators of fecal contamination, although coliforms have minimal significance as fecal indicators (Doyle and Erickson, 2006). In this study, commensal *E. coli* cell numbers in the top locations of compost heaps were reduced by ca. 4 and 7 $\log_{10}$ CFU/g in 3 days at >55^{o}C for Trials 1 and 2, respectively, which are rates similar to those observed for *E. coli* O157:H7 in the same locations. Statistical analysis of bacterial survival at each sampling location

revealed that inactivation of *E. coli* and *E. coli* O157:H7 populations correlated. While complete pathogen and indicator organism inactivation occurred in internal samples within 2 weeks of composting for both composting trials, coliforms, commensal *E. coli*, and *E. coli* O157:H7 in surface samples survived for up to 4 months. This is in direct contrast with results of Hutchinson et al. (2005), who reported that the pathogens they inoculated could not be detected after 8 days in surface samples of the heaps they monitored, and Nicholson et al. (2005), who reported pathogen survival at the surface was comparable to survival in the main body of the heap. Although desiccation and UV exposure could contribute to pathogen reduction in surface samples, the lack of elevated temperatures was likely the primary factor influencing pathogen inactivation rates in this study. Greater survival of *E. coli* O157:H7, *E. coli* and coliforms in compost surface of Trial 2 most likely resulted because of the lower ambient temperatures to which the compost heaps were exposed. While it is unknown how the strain used in our study differs from other strains of *E. coli* O157:H7 in terms of desiccation resistance, there is evidence that the strain of *E. coli* O157:H7 can be a factor in its persistence in bovine feces at low moisture contents (Wang et al., 1996). The extended survival of *E. coli* O157:H7 on the compost surface is problematic because surviving pathogens can spread to the surrounding environment such as soil, water, and agricultural crops that are being harvested for human consumption. Results from a four-month study using the compost mixture described in this experiment, without any aeration of the heap, showed that the maximum internal heap temperature was 56^{o}C. Additionally, after the decline in temperature, regrowth of coliforms in the heaps occurred (data not shown). Hence, it is important to maintain active composting by frequent turning of heaps to ensure that all

composted materials are subjected to elevated temperatures generated during active composting.

Conclusions

Our results indicate that the compost composition and heap size utilized were adequate to achieve active composting. The stratification of temperature at different locations of the heaps affected the rates of *E. coli* O157:H7 inactivation; however, ca. 7 log CFU *E. coli* O157:H7/g inside compost heaps could be inactivated within 2 weeks if active composting occurred during this period of time. Importantly, our results revealed that both indicator bacteria and *E. coli* O157:H7 can survive up to 4 months on the surface of compost heaps and this could serve as a source of pathogen contamination of the surrounding environment. Finally, the methods used in this study can be used as a model to evaluate the fate of other pathogenic microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, viruses, and parasites in compost.

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Figure Legend

Figure 3.1: Temperature profiles of the composting heap for Trial 1. Values are the averages of results of two heaps at each sampling location. Results were obtained at the surface (◊) and at three locations within the heaps (Top, □; Center, Δ; and Bottom, ×) which were 70, 50, and 30 cm, respectively, from the surface of a concrete pad on which the compost heaps were built. Arrows indicate days of precipitation events.

Figure 3.2: Temperature profiles of the composting heap for Trial 2. Values are the averages of results of two heaps at each sampling location. Results were obtained at the surface (◊) and at three locations within the heaps (Top, □; Center, Δ; and Bottom, ×) which were 70, 50, and 30 cm, respectively, from the surface of a concrete pad on which the compost heaps were built. Arrows indicate days of precipitation events.

Figure 3.3: Fate of *E. coli* O157:H7 at different locations within the heaps during composting of Trial 1 on the Surface (◊) and at three locations within the heaps (Top, □; Center, Δ; and Bottom, ×).

Figure 3.4: Fate of *E. coli* O157:H7 at different locations within the heaps during composting of Trial 2 on the Surface (◊) and at three locations within the heaps (Top, □; Center, Δ; and Bottom, ×).

Table 3.1 Composition, and biotic and abiotic analyses of experimental compost heaps at the beginning of each trial.

Parameter	Description or results
Compost mixture	cow manure, calf barn sawdust bedding, feed waste, fresh hay[ab]
Compost dimensions	triangular shape, ca. 2 m in base width and ca. 1.1 - 1.2 m in height[ab]
C:N ratio	22:1[ab]
pH	7.9[a]; 8.0[b]
Moisture content	63.6%[a;] 56.5%[b]
Mesophilic bacterial counts	$1.3x10^8$ CFU/g[a]; $2.3x10^8$ CFU/g[b]
Thermophilic bacterial counts	$1.1x10^8$ CFU/g[a]; $1.4x10^9$ CFU/g[b]
E. coli O157:H7	absent in 25 g[ab]
Salmonella spp.	absent in 25 g[ab]

[a,b]Indicates parameters or results for Trial 1 or Trial 2 of composting, respectively.

Table 3.2 pH values and moisture content of Trial 1 compost samples.

		pH values or moisture content (%) on composting day[a]:							
Parameter	Location in heap	0	3	7	14	21	30	60	120
pH									
	Surface	7.90±0.06 A	8.58±0.21 B	9.17±0.13 B	8.34±0.27 A	8.45±0.09 A	7.79±0.09 A	7.39±0.04 A	7.14±0.04 A
	Top	7.90±0.06 A	7.37±.014 A	7.90±0.38 A	8.53±0.35 AB	9.88±0.45 B	9.94±0.31 B	8.62±0.46 B	8.53±0.69 B
	Center	7.90±0.06 A	7.20±0.39 A	8.42±0.17 AB	8.92±0.22 BC	9.98±.012 B	9.80±0.20 B	8.85±0.24 B	8.50±0.46 B
	Bottom	7.90±0.06 A	7.63±0.29 A	8.32±0.40 AB	9.25±.015 C	10.01±0.35 B	9.71±0.62 B	9.41±0.16 C	9.07±.039 B
Moisture content (%)									
	Surface	63.6±1.1A	68.9±1.2 B	64.2±6.4 A	4.25±0.52 A	5.30±0.08 A	8.50±0.01 A	3.27±0.45 A	5.77±0.78 A
	Top	63.6±1.1A	63.1±0.6 A	60.7±2.2 A	61.1±0.7 C	61.5±2.2 C	56.1±11.7 B	64.3±2.1 C	58.2±2.1 B
	Center	63.6±1.1A	63.7±1.5 A	60.7±1.7 A	59.2±2.6 BC	58.3±2.6 BC	57.9±5.9 B	58.9±3.4 C	51.4±15.2 B
	Bottom	63.6±1.1A	63.3±0.8 A	62.8±0.8 A	57.3±3.4 B	48.7±7.7 B	50.1±11.9 B	21.7±4.0 B	48.4±17.1 B

[a] Values with different capitalized letters are statistically different ($p<0.05$) on the sampling day.

Table 3.3 Fate of *E. coli* and coliforms at different locations of heaps during composting (Trial 1).

		Avg. log CFU/g at days of composting[a]:							
Indicator bacteria	Location in heap	0	3	7	14	21	30	60	120
Coliforms									
	Surface	7.20±0.11 A[a]	5.37±0.08 B	5.57±0.12 C	4.00±0.01 B	5.40±0.03	3.68±0.19	2.60±0.08	2.10±0.08
	Top	7.20±0.11 A	2.52±0.16 A	1.40±0.00A	1.40±0.00 A	ND[b]	ND	ND	ND
	Center	7.20±0.11 A	2.26±0.40 A	1.40±0.00 A	1.40±0.00A	ND	ND	ND	ND
	Bottom	7.20±0.11 A	4.86±0.04 B	2.52±0.16 B	1.40±0.00A	ND	ND	ND	ND
E. coli									
	Surface	6.40±0.26 A	5.12±0.07 B	5.29±0.01 B	4.00±0.01 B	1.40±0.00	1.40±0.00	1.40±0.00	1.40±0.00
	Top	6.40±0.26 A	1.96±0.00 A	1.40±0.00 A	1.40±0.00 A	ND	ND	ND	ND
	Center	6.40±0.26 A	1.79±0.27 A	1.40±0.00 A	1.40±0.00 A	ND	ND	ND	ND
	Bottom	6.40±0.26 A	4.72±0.08 B	1.40±0.16 A	1.40±0.00A	ND	ND	ND	ND

[a] Values with different capitalized letters are statistically different ($p<0.05$) on the sampling day.

[b] ND: not detected.

Table 3.4 Fate of *E. coli* and coliforms at different locations of heaps during composting (Trial 2).

Indicator bacteria	Location in heap	Avg. log CFU/g at days of composting[a]: 0	1	2	3	5	7	14	21	30	60	120
Coliforms												
	Surface	7.12±0.04 A[a]	7.10±0.02 C	7.05±0.01 C	7.04±0.01 B	6.75±0.04 B	6.04±0.04	5.52±0.43	5.93±0.06	4.97±0.07	4.61±0.03	3.35±0.41
	Top	7.12±0.04 A	5.69±0.04 A	1.40±0.00 A	ND[b]	ND	ND	ND	ND	ND	ND	N/Ac
	Center	7.12±0.04 A	5.72±0.07 A	1.40±0.00 A	ND	ND	ND	ND	ND	ND	ND	N/A
	Bottom	7.12±0.04 A	6.00±0.11 B	3.67±2.62 AB	1.40±0.00 A	1.40±0.00 A	ND	ND	ND	ND	ND	N/A
E. coli												
	Surface	7.05±0.03 A	7.02±0.03 C	7.00±0.01 C	6.94±0.03 B	6.65±0.02 B	5.70±0.06	4.85±0.43	5.59±0.11	4.54±0.02	4.06±0.08	2.71±0.23
	Top	7.05±0.03 A	5.15±0.06 A	1.40±0.00 A	ND	ND	ND	ND	ND	ND	ND	N/A
	Center	7.05±0.03 A	5.60±0.08 A	1.40±0.00 A	ND	ND	ND	ND	ND	ND	ND	N/A
	Bottom	7.05±0.03 A	5.91±0.11 B	3.62±2.56 AB	1.40±0.00 A	1.40±0.00 A	ND	ND	ND	ND	ND	N/A

[a] Values with different capitalized letters are statistically different ($p<0.05$) on the sampling day.
[b] ND: not detected.
[c] N/A: not applicable, sample not taken.

Figure 3.1

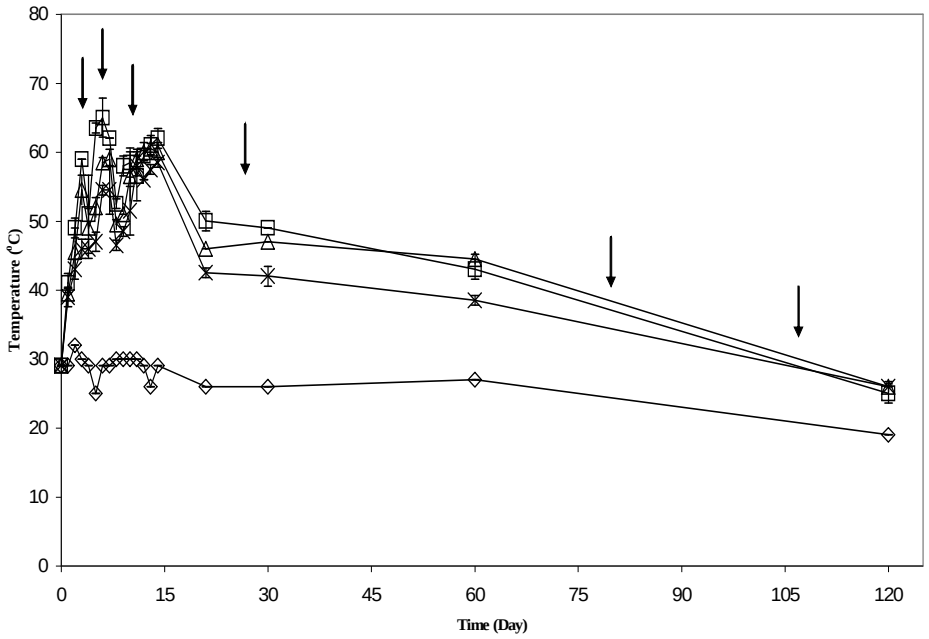


Figure 3.2

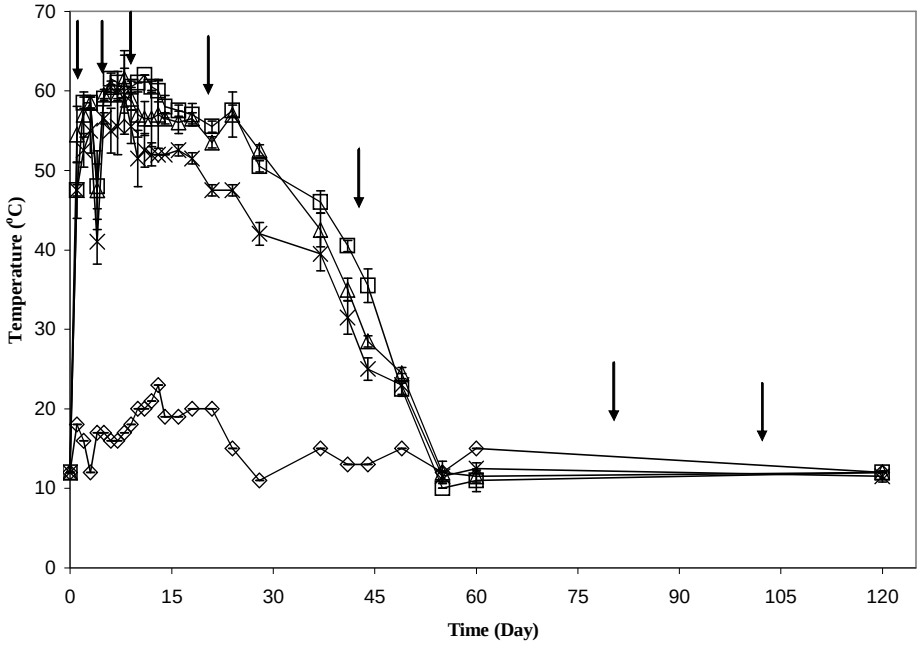


Figure 3.3

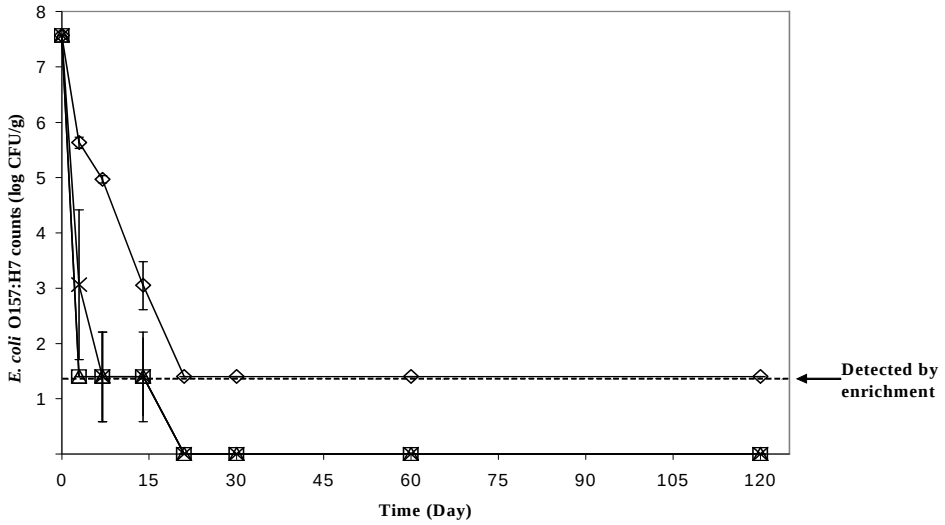
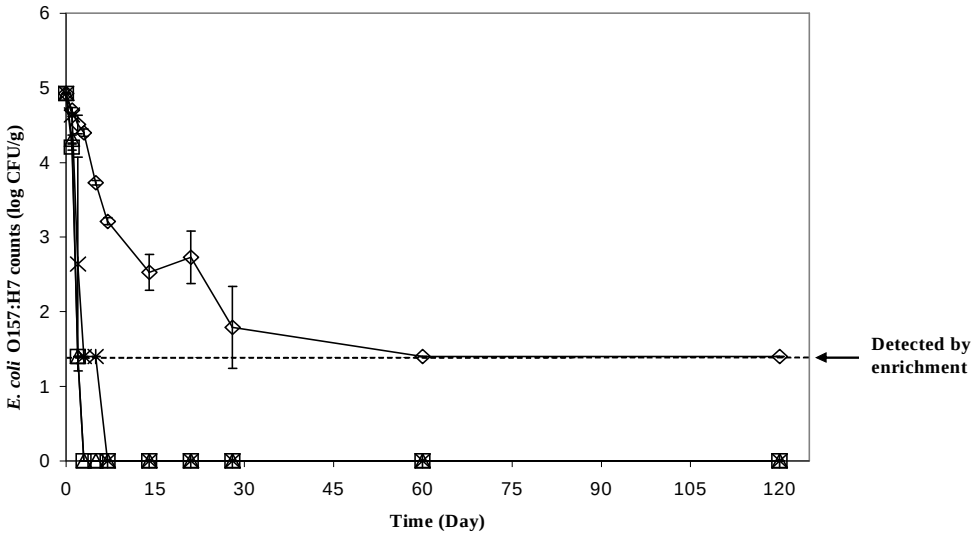


Figure 3.4



CHAPTER FOUR

CONCLUSION

In this study, a survey of South Carolina poultry farms and a challenge study determining the survival of *E. coli* O157:H7 were conducted. In both studies, extended survival of indicator and pathogenic microorganisms were detected in the compost surface for extended periods of time. However, within the body of the heaps, detection of pathogens was lower. In our survey of poultry farms, our results indicated that composting illustrated that composting was not performed under recommended conditions at the surveyed farms. Results from our challenge study revealed that *E. coli* O157:H7 may be inactivated within two weeks from the onset of composting within the body of the compost heap. In conclusion, our results demonstrated that composting under appropriate conditions results in the inactivation of pathogenic bacteria.