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Evaluating the microbial safety status of products from sustainable organic agriculture

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Iowa State University

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Evaluating the microbial safety status of products from sustainable organic agriculture

by

Sai Deepikaa Elumalai

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Angela Shaw, Major Professor
Byron Brehm-Stecher
Kathleen Delate

Iowa State University
Ames, Iowa
2016

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DEDICATION

I want to dedicate this work to my family and friends for their help and support throughout my educational journey. I am grateful to my Father, Mother, Sister and Grandmother for always having faith in my abilities and giving me the freedom to make my own choices. Thank you for believing in me and staying by my side through thick and thin. I would not have been able to reach where I am without your support.

I would like to thank my Major Professor Dr. Angela Shaw and my POS Committee members for their help and encouragement.
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<tr>
<td>CFU/ml</td>
<td>Colony forming units/milliliter</td>
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<td>Cm</td>
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<td>Ft</td>
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<td>Mm</td>
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<td>PVC</td>
<td>Polyvinyl chloride</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>Watt</td>
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ABSTRACT

There has been a steady increase in the demand for organic and sustainable agricultural products. Consumers increasingly prefer non-GMO products grown without pesticides and antibiotics. In order to satisfy the increasing demand, several agricultural producers have opted for use of different types of modern agricultural practices which are more efficient and sustainable than conventional methods. My research focuses on two such modern agricultural practices. Because the products from these methods do not have many food safety interventions applied to them, it is important to make sure that they are free of pathogens and are safe for human consumption. My research 1) Identifies the food safety hazards associated with an aquaponic food production system and studies the efficacy of UV intervention, and 2) Evaluates the food safety status of the initial phase of an integrated crop-livestock organic agricultural system.

Aquaponics is a growing trend in food production as it is seen as a sustainable, space- and energy-efficient approach for production of fruits, vegetables and seafood. Within aquaponics, few microbial studies have been conducted to determine the food safety status of its units. The aim of this study was to determine the food safety status and the effectiveness of ultraviolet treatment (15 watt UV light, luminous flux of 900 lumens) as a food safety intervention in reducing the microbial loads of the water system, in a model aquaponic unit that is growing lettuce, basil and barramundi (Australian Sea Bass). Large Leaf basil, Buttercrunch Bibb lettuce, water and fish swab samples were collected throughout the 118-day production period and microbial analysis was conducted for the presence of *E. coli* O157:H7, *Aeromonas* and *Salmonella* spp. and the prevalence of aerobic plate counts (APC), coliforms, and fecal coliforms in the
systems in triplicates. Absence of foodborne pathogens was confirmed using ELISA technology (3M™ Tecra, Australia) and enumeration through petrifilms (coliform/E. coli Petrifilm™, 3M, St. Paul, MN) and agar (Aeromonas agar, OXOID, Hants, United Kingdom). A significant increase was observed in aerobic plate counts over the trial period (1 to 3 log CFU/mL), in the presence and absence of UV (p>0.05). Ultraviolet treatment did not significantly reduce the APC, Aeromonas or coliform counts when compared to the control systems samples. Though the UV intervention method was not effective in reducing microbial loads, future work should focus on improving the unit design, evaluation of bio-solid filtration and other food safety interventions that can be effective in the presence of living system while maintaining fish homeostatic environment.

Though several researchers have indicated the many benefits associated with the production and consumption of organic food such as restricted use of antibiotics and synthetic chemicals; it must be kept in mind that these benefits do not address the issue of microbial safety. With integrated crop-livestock production systems being relatively new practice in organic agriculture, the aim of this study was to develop and use a model agricultural system to check the food safety status of crops and beef and dairy calves (6-10 months old) produced in an integrated environment in Minnesota (MN), Iowa (IA), and Pennsylvania (PA). Pasture and fecal samples were collected 3 months apart and evaluated for presence of E. coli 0157:H7 and Salmonella using miniVIDAS and confirmation tests were performed according to FDA BAM and USDA standards. Results indicated very low probability for (0.0173-IA, 0.0032-MN, 0.0039-PA) E. coli 0157:H7 and (0.0077-IA, 0.0027-MN, 0.0022-PA) Salmonella occurrence (overall Pr<0.1). The
three states were studied individually for occurrence of *E. coli* O157:H7 or *Salmonella*. The probabilities of occurrence were again very low (0.0048-IA, 0.0003-MN, 0.0009-PA). Also, there was no significant difference between the three research sites (p>0.05) in terms of *E.coli* O157:H7 or *Salmonella* occurrence. At present, this model has low chance of *E.coli* O157:H7 and *Salmonella* being present in the feed and fecal matter, but long term studies including evaluation of meat products and rotational crops might help us better understand the stability of this system.
CHAPTER 1: INTRODUCTION

Over the years, the U.S. organic food industry has grown substantially in popularity. According to the Organic Trade Association's Organic Industry Survey (2011), total organic food sales accounted for $6.1 billion in 2000 and more than quadrupled in the past 10 years to $26.7 billion in 2010 (Van Loo et al., 2011). Several factors have led to this increased consumption of organic foods in the U.S., including consumer preference for lower pesticide residues (Baker et al., 2002), nutrition and health concerns (Williams, 2002, Magkos et al., 2003), negative environmental impacts associated with intensive conventional production (Venterea and Rolston, 2000), and the assurance of organic integrity through consistent federal organic standards (USDA-AMS, 2014).

With increasing demand, it is important to ensure that organic produce are safe for consumption. Some research conducted on organic-based foods has concluded that there is no evidence that organic food is safer, healthier, or more nutritious (Williams, 2002, Magkos et al., 2003), although others have found evidence of greater levels of antioxidants and vitamins (Callaway et al., 2009, Średnicka-Tober et al., 2016, Barański et al., 2014). Therefore, a food product produced organically is not necessarily indicative of it being safer. Consumers are often not aware that the organic standards are only based on production and processing practices and not on the final quality or safety of the product (Brennan et al., 2003). There are no stricter food safety standards for organic foods; organic foods are required to meet the same food safety standards as nonorganic foods (Van Loo et al., 2011).
Some of the common pathogens associated with produce are *E. coli* 0157, *Salmonella* and *Listeria monocytogenes*. *E. coli* outbreaks have been reported from conventional and organic lettuce and salad greens (Strain- number of cases or outbreaks; O145- 26 cases and 12 hospitalizations, O157- 60 cases and 30 hospitalizations, O157-33 cases and 13 hospitalizations), sprouts (O26- 29 cases and seven hospitalizations, O121- 19 cases and seven hospitalizations), berries (O157- 15 cases and two deaths, O26- five cases and one hospitalization), and melons (O157- nine and six cases in two outbreaks) (CDC, 2012a, CDC, 2014a, Danyluk et al., 2014).

Related to produce, in 2006, a multistate outbreak of *Salmonella Typhimurium* in tomatoes led to 183 cases of illness including 22 hospitalizations. A large outbreak in 2008 linked to pepper and tomatoes resulted in 1,500 illnesses, 308 hospitalizations, and two deaths (CDC, 2011). Additional produce outbreaks have been linked to avocado (2007; 46 illnesses), blueberries (2009; 14 illnesses, 2010; six illnesses and one hospitalization), salads (multiple outbreaks and hospitalizations), cantaloupe (18 outbreaks resulting in multiple deaths), sprouts (1999; 112 illnesses, two hospitalizations), cucumber (2012; 49 illnesses, 14 hospitalizations), tomatoes (2004; 429 illnesses and 129 hospitalizations, 1998; 86 illnesses and three deaths), and watermelon (2008; 594 illnesses, 31 hospitalizations, and 2010; 17 illnesses, 11 hospitalizations) (CDC, 2012a).

Produce has also been associated with listeriosis on several occasions in U. S. history. Consumption of *Listeria*-contaminated celery resulted in 10 illnesses and five deaths in Texas, 2010 (CDC, 2012a). Sprouts have also been a vehicle of *Listeria* transmission in several cases, one in 2008 in which 20 individuals became ill, and another
in 2012 that resulted in six illnesses and one death (CDC, 2012a). *Listeria* was identified as the only microorganism (compared to *Salmonella* and *E. coli*) to be capable of growth on the surface of cantaloupe (Behrising et al., 2002), which was the vehicle of one large outbreak in 2011. This outbreak spread across 28 U. S. states, resulting in 147 illnesses and 33 deaths, several of which were associated with pregnancy and/or newborns (CDC, 2012b).

Produce related outbreaks have also been linked to other bacterial pathogens; *Shigella* (carrots, lettuce, parsley, tomatoes, lemons, strawberries, and melons), *Bacillus* spp. (onion, sprouts, and potatoes), *Campylobacter* (pepper, lettuce, peas, watermelon, tomato, and spinach), *Clostridium* spp. (cabbage, mushrooms, onions, lettuce, and peppers), and *Staphylococcus aureus* (peppers, potato, salad greens). Additionally, outbreaks have occurred as a result of parasites; *Cryptosporidium* (apple cider and salad greens), *Cyclospora* (berries, green beans, arugula, and peas), and *Giardia* (unspecified vegetables) as well as viruses; Hepatitis A (strawberries, green onions, tomato, and salad greens), Norovirus (melons, strawberries, salad greens, tomato, green beans, grapes, broccoli, cucumber, asparagus, and onion), and Norwalk (melons, salad greens, and celery) (Beuchat, 2002, Olaimat and Holley, 2012, CDC, 2012a, CDC, 2013a).

Others have also reported that extended exposure to outdoor conditions may cause organically raised farm animals to be more likely infected by *Salmonella* and *Campylobacter* (Lund, 2006, Hansen et al., 2002). Organic meat production has the potential to have higher microbiological safety risks because of the strict restrictions in the use of pharmaceutical agents for therapeutic use (such as antimicrobials or
parasiticides), raising the animals outdoors, use of slow-growing breeds and the smaller slaughtering facilities (Engvall, 2002, Doyle et al., 2006, Thamsborg, 2002).

My research focused on evaluating the presence of some of the above mentioned pathogens. The work in this article can be divided into two research goals. 1. Develop and study the food safety status of a model aquaponic system, and 2. Study the food safety status of a pasture-livestock agricultural system. Both the systems are modeled to be sustainable and do not employ pesticides, antibiotics or any other genetically modified organisms.

References


Consumers' willingness to pay for organic meat: Experimental evidence from

following fertilizer application to agricultural soil: Biotic and abiotic mechanisms

CHAPTER 2: LITERATURE REVIEW ON “INFLUENCE OF UV TREATMENT ON THE FOOD SAFETY STATUS OF A MODEL AQUAPONIC SYSTEM”

Introduction to Aquaponics

The market for locally produced is growing; cost and space-efficient means for producing them are needed to help meet demand. Aquaponics, a method integrating both aquaculture and hydroponics for the production of both fish and produce, is garnering attention among organic producers. Aquaponics easily fits into a local and regional food system model in part because it can be practiced in or near large population centers (Love et al., 2014). This approach can be replicated anywhere, irrespective of geographic location and weather to overcome the environmental pollution caused by several other agricultural systems (Salam et al., 2014).

What is aquaponics?

Aquaponics is an environmentally friendly agricultural practice that involves the cultivation of crops in a non-soil medium (known as hydroponics) by feeding the plants with nutrient-rich water from intensively cultured aquatic organisms such as fish (i.e., aquaculture). Aquaponic systems are recirculating aquaculture systems that incorporate the production of plants without soil. Recirculating systems are designed to raise large quantities of fish in relatively small volumes of water by treating the water to remove toxic waste products and then reusing it. In the process of reusing the water many times, non-toxic nutrients and organic matter accumulate. These metabolic by-products need not be wasted if they are channeled into secondary crops that have economic value or in some way benefit the primary fish production system. Fish excrete ammonia from their gills as part of their waste-elimination metabolism. This ammonia is then converted into
nitrite and then to nitrate by beneficial bacteria (such as *Nitrosomonas* and *Nitrobacter* species). When plant roots find nitrate in the solution around their roots, it is taken up and converted into amino acids, the building blocks of proteins. These systems that grow additional crops by utilizing by-products from the production of the primary species are referred to as integrated systems. If the secondary crops are aquatic or terrestrial plants grown in conjunction with fish, this integrated system is referred to as an aquaponic system (Rakocy et al., 2006).

There are many benefits to aquaponic crop production such as:

- Plants exhibit twice the growth rate of those observed in soil culture
- Production footprint is up to 75% smaller than normal soil culture
- Water consumption is reduced by 90% as compared to conventional methods
- Extended season to year-round production possible in high tunnels or greenhouses
- Soil-borne plant pathogens are eliminated
- Multiple crops are produced simultaneously

**Features of an Aquaponic System**

Most aquaponic systems are constructed similar to hydroponic systems. The main difference between the two systems would be dissolving nutrients in water in case of hydroponics and nutrients being available to crops from fish in aquaponics. The major types of aquaponic systems are similar to hydroponic systems as their differences can be attributed to differences in nature of plant bed. Liquid hydroponic systems employ the nutrient film technique (NFT), floating rafts, and non-circulating water culture. Aggregate hydroponic systems employ inert, organic, and mixed media contained in bag, trough, trench, pipe, or bench setups. Aggregate media used in these systems include
perlite, vermiculite, gravel, sand, expanded clay, peat, and sawdust. In aquaponics, nutrients are delivered via aquacultural effluent. Fish effluent contains sufficient levels of ammonia, nitrate, nitrite, phosphorus, potassium, and other secondary and micronutrients to produce hydroponic plants (Rinehart, 2010).

Naturally, some plant species are better adapted to this system than others. The selection of plant species adapted to hydroponic culture in aquaponic greenhouses is related to stocking density of fish tanks and subsequent nutrient concentration of aquacultural effluent. Lettuce, herbs, and specialty greens (spinach, chives, basil, and watercress) have low to medium nutritional requirements and are well adapted to aquaponic systems. Plants yielding fruit (tomatoes, bell peppers, and cucumbers) have a higher nutritional demand and perform better in a heavily stocked, well established aquaponic system. Greenhouse varieties of tomatoes are better adapted to low light, high humidity conditions in greenhouses than field varieties (Rinehart, 2010).

Also, picking the right kind of fish is critical to the success of an aquaponic system. Several warm-water and cold-water fish species are adapted to recirculating aquaculture systems, including tilapia, trout, perch, Arctic char, and bass. However, most commercial aquaponic systems in North America are based on tilapia. Tilapia is a warm-water species that grows well in a recirculating tank culture. Furthermore, tilapia is tolerant of fluctuating water conditions such as pH, temperature, oxygen, and dissolved solids. Tilapia produces a white fleshed meat suitable to local and wholesale markets. Barramundi and Murray cod fish species are raised in recirculating aquaponic systems in Australia (Rinehart, 2010).
In the 1980s, Mark McMurtry and Doug Sanders at North Carolina State University developed an aqua-vegeculture system based on tilapia fish tanks sunk below the greenhouse floor. Effluent from the fish tanks was trickle-irrigated onto sand-cultured hydroponic vegetable beds located at ground level. The nutrients in the irrigation water fed tomato and cucumber crops, and the sand beds and plant roots functioned as a biofilter. After draining from the beds, the water recirculated back into the fish tanks. The only fertility input to the system was fish feed (32 % protein). He identified several benefits of an integrated aquaponic system. He pointed out that the water consumption in an integrated aqua-vegeculture system amounts to 1 percent of that required in pond culture to produce equivalent tilapia yields. Such low-water-use symbiotic systems are applicable to the needs of arid or semi-arid regions where fish and fresh vegetables are in high demand (Rinehart, 2010).

In the early 1990s, Tom and Paula Speraneo – owners of S & S Aqua Farm near West Plains, Missouri – modified the North Carolina State method by raising tilapia in a 500-gallon tank, with fish effluent linked to gravel-cultured hydroponic vegetable beds inside an attached solar greenhouse. Later, they expanded to a full-size commercial greenhouse. The Speraneo system was practical, productive, and wildly successful (Rinehart, 2010). James Rakocy and associates at the University of the Virgin Islands (UVI) developed a commercial-scale aquaponic system that ran continuously for more than five years. Nile and red tilapia were raised in fish rearing tanks, and the aquacultural effluent was linked to floating raft hydroponics. Basil, lettuce, okra, and other crops were raised successfully, with outstanding quality and yields. Yields of aquaponic basil were
three times greater than field-grown, while yields of aquaponic okra were 18 times
greater than field-grown (Rakocy et al., 2004).

Lennard et al. (2006) used Murray cod, Maccullochella peelii (Mitchell), and
Green Oak lettuce, Lactuca sativa, to test for differences between three hydroponic
subsystems, Gravel Bed (GB), Floating Raft (FR) and Nutrient Film Technique (NFT), in
a freshwater aquaponic test system, where plant nutrients were supplied from fish wastes
while plants utilized nutrients from the waste water before it was returned to the fish.
Their results suggest that NFT hydroponic sub-systems are less efficient at both removing
nutrients from fish culture water and producing plant biomass or yield than GB or FR
hydroponic sub-systems. Aquaponic system designers need to take these differences into
account when designing hydroponic components within aquaponic systems.

**Economic Impacts**

Aquaponics can be implemented using low-cost materials, which keeps capital
overhead low and thus feasible for small-farm applications. Additionally, the multiple
crops produced in an aquaponics system (plants and fish) allow small, family-scale
farmers to diversify their incomes, which both reduces risk of crop failure and increases
revenue by providing products for multiple market outlets. Economic sustainability of
aquaponics, the combination of aquaculture and hydroponics, depends on a variety of
factors, including system and feed design, animal welfare or parasite and pathogen
control (Palm et al., 2014a). Benefits of aquaponics are conservation of water resources
and plant nutrients, intensive production of fish protein and reduced operating costs.
Water consumption in integrated systems is less than 1% of that required in pond culture
to produce equivalent yields (Khater, 2015). The Aquaculture Research and Education
Laboratory in Cheyney University, Pennsylvania, under Dr. Steven Hughes produces 600 dozen basil plants from its aquaponic system which incorporates tilapia as its aquaculture. Not only has it lead to monetary profit, it has also created employment for people at each of its site (Hughes, 2015).

In a study conducted in Manchester, Jenkins et al. (2015) utilized empirical research on crops grown in an elevated aquaponic system on a building top and extrapolated the findings across a whole city. It was stated that with 87 percent of people in developed regions estimated to be living in cities by 2050, it can be assumed that the majority of localized production will be occurring in and around cities. Hence, it is important to explore the possibilities and difficulties associated with integrating food production systems like aquaponics with existing buildings as aquaponic systems can re-purpose space.

In 2011, there were 70 aquaculture operations in Hawaii producing a total sales value of $39.97 million. That number increased to a record high of $55.74 million in 2012. In 2015, a study investigating economic feasibility of small-scale commercial aquaponics was carried out through comprehensive study of three aquaponics farms in Hawaii. It was found that small-scale commercial aquaponics is economically feasible, but their findings were not as optimistic as those previously published. Output prices and operational cost parameters affect the overall economic outcome. It was shown that investment in commercial aquaponics cannot be supported if annual sales revenue falls by 11%. Though there are several challenges and risks faced by commercial aquaponic farms, there might be potential economic gain from organic certification and renewable energy implementation (Tokunaga et al., 2015).
There is tremendous potential to increase economic, social, and environmental sustainability of Iowa agriculture through aquaponics. However, there is currently minimal research for aquaponics in Iowa, thus leaving the question of food safety unanswered.

**An Overview of Food Safety Risks in Aquaponics**

**Produce Pathogens**

*Escherichia coli*

A large number of fatal multi-state outbreaks have been reported in fresh produce over many years. Produce is a prominent vehicle for *E. coli* transmission, accounting for approximately 20% of all produce outbreaks (Rangel et al., 2005). *E. coli* outbreaks have been reported from lettuce and salad greens (Strain- number of cases or outbreaks; O145-26 cases and 12 hospitalizations, O157- 60 cases and 30 hospitalizations, O157- 33 cases and 13 hospitalizations), sprouts (O26- 29 cases and seven hospitalizations, O121- 19 cases and seven hospitalizations), berries (O157- 15 cases and two deaths, O26- five cases and one hospitalization), and melons (O157- nine and six cases in two outbreaks) (CDC, 2012a, CDC, 2014, Danyluk et al., 2014). In case of sprouts, the source of outbreak was traced backed to the seeds. However, the source of contamination in case of other breakouts could not be identified.

As many as 400 Shiga toxin-producing *Escherichia coli* (STEC) are known to exist but not all have been identified as causing human illness and not all cause human disease in the same severity (Johnson et al., 1996, Gyles, 2006, Liu, 2010). The STEC strain most commonly associated with severe forms of disease is *E. coli* O157:H7, but it is not the only STEC known to cause disease. In fact, at least 60 strains of STEC have
been linked to human illness worldwide (Bettelheim, 2003), and a U. S. study completed by the CDC between 1983 and 2003 demonstrated as many as 14 different serogroups were implicated in human disease resulting from \textit{E. coli} infection, in addition to illnesses that resulted in undetermined serotypes (Brooks et al., 2005). However, the same study demonstrated that approximately 70\% of the infections caused by non-O157 STEC infections, that could be serotyped, were attributed to only 6 serotypes: O145, O121, O111, O103, O45, and O26, which have been identified by the CDC and USDA-FSIS as the “Big 6” non-O157 STEC (USDA-FSIS, 2011). Non-O157 STEC is of major concern in many areas of the world. Some European countries report that over one half of confirmed STEC infections are caused by non-O157 STEC (Arthur et al., 2002, Brooks et al., 2005, Monaghan et al., 2011). It has been estimated that \textit{E. coli} O157:H7 strains cause two-thirds of all \textit{E. coli} human infection cases in the U. S., while non-O157 strains are responsible for the remaining cases (Mead et al., 1999).

When compared to \textit{E. coli} O157:H7 and other enteric pathogens, non-O157 STEC are infrequently isolated and implicated in foodborne illness outbreaks. It is believed that this group of pathogens is largely under-accounted for, presumably due to ineffective laboratory screening and culturing methods (Possé et al., 2008). As indicated, non-O157 STEC are not newly emerging pathogens. They have been implicated in clinical cases of human disease and have been of increasing public health concern since the early 1990’s (USDA-FSIS, 2010).

\textit{Salmonella}

\textit{Salmonella} spp. have been implicated in outbreaks linked to produce; sprouts, tomatoes, cantaloupe, spinach, peppers, papaya, beets, cabbage, cauliflower, onion, and
letuce (Beuchat, 2002, Olaimat and Holley, 2012, CDC, 2013b). In addition to animal products, nuts and produce have also been often implicated as vehicles for transmission of *Salmonella*. Outbreaks with peanuts include one in 2006 when 715 became ill and 129 were hospitalized, in 2008 were 714 illnesses, 166 hospitalizations, and nine deaths, and also in 2012 when 42 became ill and ten were hospitalized (CDC, 2012a).

Related to produce, in 2006, a multistate outbreak of *Salmonella* Typhimurium in tomatoes led to 183 cases of illness including 22 hospitalizations. A large outbreak in 2008 linked to pepper and tomatoes resulted in 1,500 illnesses, 308 hospitalizations, and two deaths (CDC, 2011). Additional produce outbreaks have been linked to avocado (2007; 46 illnesses), blueberries (2009; 14 illnesses, 2010; six illnesses and one hospitalization), salads (multiple outbreaks and hospitalizations), cantaloupe (18 outbreaks resulting in multiple deaths), sprouts (1999; 112 illnesses, two hospitalizations), cucumber (2012; 49 illnesses, 14 hospitalizations), tomatoes (2004; 429 illnesses and 129 hospitalizations, 1998; 86 illnesses and three deaths), and watermelon (2008; 594 illnesses, 31 hospitalizations, and 2010; 17 illnesses, 11 hospitalizations) (CDC, 2012a). Though the outbreaks could be traced back to the firm/company producing the product, the actual source of outbreaks has not been identified.

*Salmonella* spp. is a common inhabitant of the intestinal tract of birds, reptiles, mammals (livestock such as pigs and cattle, and humans), and insects (Jay et al., 2005). It is therefore naturally secreted in feces, and transmitted to water, plants, and soil. Once contracting *Salmonella*, a person or animal can become a carrier; shedding the organism in its feces without showing symptoms of the disease to infect others. Cattle have been
shown to shed the bacteria at rates of 14.0% in feedlots (Tabe et al., 2008), and 21.1% in milking facilities (Wells et al., 2001).

Much research has indicated that *Salmonella* is capable of survival in the environment for extended periods of time. A prevalence study by Jensen et al. (2006) demonstrated the ability of *Salmonella* to persist in a hog production environment for up to five (soil) or seven (shelters) weeks, and to be present in water associated with production. It has also been shown to survive in manure and manure-amended soils for up to 184 and 332 days, respectively (You et al., 2006). Transfer of *Salmonella enterica* serovar Typhimurium onto the surface of produce (carrot and radish) was shown when contaminated manure and irrigation water was applied throughout the growing season (Islam et al., 2004). Yang and group (2001) demonstrated the ability of *Salmonella Typhimurium* to persist in chilled chicken processing water after treatment with chlorine, and Mezrioui et al. (1995) found *Salmonella* to have better survival rates in treated sewage water than that of *E. coli*.

*Listeria monocytogenes*

Produce has also been associated with listeriosis on several occasions in U. S. history. Consumption of *Listeria*-contaminated celery resulted in 10 illnesses and five deaths in Texas, 2010 (CDC, 2012a). Sprouts have also been a vehicle of *Listeria* transmission in several cases, one in 2008 in which 20 individuals became ill, and another in 2012 that resulted in six illnesses and one death (CDC, 2012a). *Listeria* was identified as the only microorganism (compared to *Salmonella* and *E. coli*) to be capable of growth on the surface of cantaloupe (Behrising et al., 2002), which was the vehicle of one large outbreak in 2011. This outbreak spread across 28 U. S. states, resulting in 147 illnesses.
and 33 deaths, several of which were associated with pregnancy and/or newborns (CDC, 2012b). Being present in natural environments, unsanitary conditions and poor equipment maintenance are major causes of *Listeria* contamination.

*Listeria monocytogenes* is naturally found in the environment (such as soil), making it an easy transfer onto growing produce, especially root crops. This human pathogen is unique in that it can grow at refrigeration temperatures which make it environmentally persistent in the production fields and processing environments (Kathariou, 2002). *Listeria monocytogenes* has been linked to outbreaks associated with the consumption of celery, sprouts, cabbage, cucumber, lettuce, tomato, and cantaloupe (Beuchat, 2002, Olaimat and Holley, 2012, CDC, 2012b).

*Listeria monocytogenes* is widely found in the environment; present in soil, vegetative material, fecal and water samples. Diverse sampling has found its presence in stream water, mud, sewage, slaughterhouse waste, human and animal feces, and animal feed sources such as silage world-wide (Farber and Peterkink, 1991, Sauders et al., 2012, Haase et al., 2014). Ability to survive in moist soils more than 295 days as well as thrive in low-salinity coastal waters has been demonstrated (Colburn et al., 1990, Welshimer, 1960). Prevalence studies have found *L. monocytogenes* in raw milk, soft cheeses, meat, poultry, seafood, and fruit and vegetables (Jay et al., 2005). A large number of mammals host the bacteria, including cattle, sheep, goats, and humans, as well as poultry and other fowl, ticks, flies, fish, and crustaceans (Gray and Killinger, 1966).

The high prevalence of *Listeria* in the environment, combined with its ability to grow at low temperatures and readily form biofilms (Valderrama and Cutter, 2013) leads to high concern over this pathogen in food processing environments. Persistence of some
strains within these processing environments (Holch et al., 2013) has led to zero tolerance for this pathogen in ready to eat (RTE) products (Jay et al., 2005).

**Other Pathogens**

Produce related outbreaks have also been linked to other bacterial pathogens; *Shigella* (carrots, lettuce, parsley, tomatoes, lemons, strawberries, and melons), *Bacillus* spp. (onion, sprouts, and potatoes), *Campylobacter* (pepper, lettuce, peas, watermelon, tomato, and spinach), *Clostridium* spp. (cabbage, mushrooms, onions, lettuce, and peppers), and *Staphylococcus aureus* (peppers, potato, salad greens). Additionally, outbreaks have occurred as a result of parasites; *Cryptosporidium* (apple cider and salad greens), *Cyclospora* (berries, green beans, arugula, and peas), and *Giardia* (unspecified vegetables) as well as viruses; Hepatitis A (strawberries, green onions, tomato, and salad greens), Norovirus (melons, strawberries, salad greens, tomato, green beans, grapes, broccoli, cucumber, asparagus, and onion), and Norwalk (melons, salad greens, and celery) (Beuchat, 2002, Olaimat and Holley, 2012, CDC, 2012a, CDC, 2013a).

**Human Pathogens Associated with Aquaculture**

Another area of microbial interest in aquaponics would be aquaculture pathogenicity. A multistate outbreak of *Salmonella bareilly* and *Salmonella nchanga* infections associated with raw scraped ground tuna products were reported in 2012. A total of 425 persons infected with the outbreak strains of *Salmonella bareilly* (410 persons) or *Salmonella nchanga* (15 persons) were reported from 28 states and the District of Columbia of which 55 ill persons were hospitalized. No deaths were reported. It was later identified that the company exporting tuna did not have adequate HACCP policies and tracing back to the source of outbreak was difficult (CDC, 2012). CDC, state
and federal partners in 13 states are monitoring an increase in vibriosis since May 2013. As of September 30, 2013, 104 cases of illness caused by a specific strain *Vibrio parahaemolyticus*, in 13 states with 6 hospitalizations and no deaths were reported to CDC (CDC, 2013). In 2015, a multistate outbreak of *Salmonella Paratyphi* B variant L (+) tartrate (+) infection linked to frozen raw tuna has been reported. As of July 20, 2015, 62 people infected with the outbreak strain of *Salmonella Paratyphi* B variant L (+) tartrate (+) have been reported from 11 states. Eleven ill people have been hospitalized. No deaths have been reported. Of the three strains isolated in relation to this outbreak, one of the strains was resistant to certain antibiotics, however, the source of contamination remains unknown (CDC, 2015).

Topically (skin) acquired zoonotic diseases (diseases transmitted from animals to humans) include those caused by bacterial species such as *Aeromonas, Edwardsiella, Erysipelothrix, Mycobacterium, Streptococcus (iniae)*, and *Vibrio spp.* as discussed by (Harper, 2002b). These topical infections usually occur as the result of injuries from the spines of fish or through contamination of open wounds. Although most humans have a strong natural immunity to wounds infected by marine bacteria, more serious infections are often associated with immune-compromised individuals, deep puncture wounds, and highly virulent strains of bacteria (Harper, 2002b). Ignorance of the microbial profile of aquacultural products can also affect human health and has led to the transmission of streptococcal infections from tilapia to humans (Weinstein et al., 1996).

According to Noga (1996), motile aeromonad infection (MAI) is likely the most common bacterial disease of freshwater fish, all of which are probably susceptible. By far the most important bacterial pathogen of fish is *Aeromonas hydrophila* (synonyms: A.
liquefaciens, A. formicans) (Chalmers, 2004). In a study conducted by Su Shiung Lam et al. (2015), an attempt to culture fish in earthen ponds and cages failed because of the disease problem caused by Aeromonas hydrophila. As it has become common to cultivate shrimp in aquaponic systems, it is important to keep in mind the common pathogens associated with shrimp such as Vibrio spp. and Aeromonas spp. Vibrio spp. form part of the indigenous microflora of aquatic habitats of various salinities (Sung et al. 2001). Testing water for its microbial status and using appropriate safety interventions can help eliminate this problem. Previous research indicated that the prevalence of Aeromonas in fresh water systems is well expected; however, methods to eliminate this problem are not yet well identified.

Food Safety in Aquaponics

Though there are several beneficial bacteria that help turn fish waste products into plant food, there are several bacteria and other organisms which cause disease in animals called zoonosis, which can be transmitted to humans. Zoonotic pathogens represent a health risk to people contacting the water used in an aquaponic system or to people consuming food that has zoonotic pathogens on them (Hollyer et al., 2009).

In 2013, 818 foodborne disease outbreaks were reported in the United States, resulting in 13,360 illnesses, 1,062 hospitalizations, 16 deaths, and 14 food recalls (CDC Annual Report, 2015). Over the years, foodborne diseases have become more severe, making it a huge global concern. Some of the common pathogens associated with fresh produce outbreaks are E. coli, Salmonella spp. and Listeria monocytogenes. There are also several foodborne pathogens (parasites, bacteria, viruses, dinoflagellates) and toxins
associated with aquatic species (Harper, 2002). However, the food safety status of all produce from an aquaponic system is not well known.

There are several means for the above mentioned pathogens to enter into an aquaponic system. It must be noted that aquatic culture are natural carriers of a variety of microbes and introducing them into an aquaponic system would lead to contamination of the whole system. Fish are in intimate contact with their environment, which can contain very high concentrations of bacteria and viruses. Many of these are saprophytic, some are pathogenic and both are very capable of digesting and degrading the fish's tissues. However, under normal conditions the fish maintains a healthy state by defending itself against these potential invaders by a complex system of innate defense mechanisms. These mechanisms are both constitutive and responsive (i.e. pre-existing or inducible) and provide protection by preventing the attachment, invasion or multiplication of microbes on or in the tissues (Ellis, 2001). Though the aquatic species are capable of establishing a good defense against most microbes, the microbes attached to the surface of aquaculture can enter the water system and attack the hydroponic unit. Culture water with nutrients, being a good system for the microbes to multiply, enhances the survival probability of these pathogens in the aquaponic system.

Under such conditions, when crops are being harvested from the systems on a rotational basis, the probability of cross contamination between the systems, by the harvester, becomes high. Most often during harvest, there might be spillage of water on the floor or other harvesting tools. Proper sanitation would reduce the probability of these pathogens’ survival and subsequent transfer to other systems.
There have been several other food safety concerns arising with aquaponics. This can be attributed to the cultivation of crops in water containing fish feces and other organic matter including fish and plant part residuals. The specific food safety concern with aquaponics is with the proximity of the fish culture water, containing fish excrement, to the edible plant culture component. Although fish, generally are not regarded as a food safety threat because the temperature of the culture water are low enough to not promote the establishment of pathogens such as *E. coli* and *Salmonella* (Fox et al., 2012), the potential for survival and growth still remains. Additionally, the potential for cross contaminations from animal and insect vectors does raise concerns for food safety. Several disease outbreaks in food have been traced to *E. coli* and *Salmonella* species (CDC, 2015). Very few studies have been conducted which identify the pathogenicity of these bacteria that result from aquaponic systems.

The fecal waste which fish generate, uneaten feed and organisms (e.g., bacteria, fungi and algae) that grow in the system can also become toxic to the system if allowed to accumulate. If this organic matter accumulates in the system, it will depress dissolved oxygen (DO) levels as it decays and produce carbon dioxide and ammonia. If deep deposits of sludge form, they will decompose anaerobically (without oxygen) and produce methane and hydrogen sulfide, which are very toxic to fish. Suspended solids have special significance in aquaponic systems. Suspended solids entering the hydroponic component may accumulate on plant roots and create anaerobic zones that prevent nutrient uptake by active transport, a process that requires oxygen. This may suffocate the plant roots leading to death. Use of suitable filters to eliminate the waste
accumulation will be critical in preventing the accumulation of solids in plant roots (Rakocy et al., 2006).

**Greenhouse Environment Sanitation**

Sanitation in greenhouse can also be of prime importance in determining the microbial safety of produce. In June of 2014, a multi-state foodborne outbreak of *Salmonella* Saintpaul was linked to greenhouse grown cucumbers in Culiacán, Mexico, that caused 84 infections and 17 hospitalizations in the U.S. Like the majority of foodborne outbreaks, poor sanitation and not following GAPs/GMPs caused this outbreak (Shaw et al., 2015). The Federal Food, Drug and Cosmetic Act and Food Safety Modernization Act provide mandatory guide lines specific to fruit and vegetable growers. According to Shaw et al. (2015), greenhouse food crop safety programs should focus on four main areas: substrate, water, facilities and people.

One of the biggest risks to fresh produce safety is our hands, which are in continual contact with the environment. When we harvest produce, it is important to be mindful of what you have touched before you touch the food product that you will sell or serve to others. Before harvesting your plant crops, wash your hands using liquid soap, rinse them for at least 20 seconds with potable water, and dry them with single-use paper towels. Wash hands every time after using the bathroom, eating, smoking, petting animals, shaking hands with someone, changing diapers, handling fish, putting your hands into the system’s water, touching your head (mouth, nose, ears, hair), etc (Hollyer et al., 2009).

Cranston (1987) indicated that the larva of the non-biting mosquito, *Chironomus* sp., feeds on roots of horticultural plants and can damage indoor horticultural crops,
particularly lettuce, *Lactuca sativa* L., and young tomatoes, *Solanum lycopersicum* L. *Chironomus* sp. and *Bradysia* sp. can annoy operators because the insects fly around the face making activities in the enclosed aquaponics system difficult. Sticky traps can be used to indicate abundance of the insects in the greenhouse, but larger sticky traps can be used as a method of control by mass trapping (Campos-Figueroa et al., 2015).

**Safety Interventions in Aquaponics**

**Chlorine**

Water sanitation would be of utmost importance among aquaponic safety interventions. The water treatment process should be monitored and controlled. Control of the sanitary quality of water is technologically feasible but requires strict management of operating practices (Lopez-Galvez et al., 2010, Luo et al., 2011, Suslow, 1997). Generally, chlorine or other disinfection agents are added, to control microbial load in the process wash water (Gil et al., 2009). Chlorine in the form of sodium hypochlorite granules, tablets or liquid is the most commonly used disinfection agent (Suslow, 2001). The use of other disinfection techniques such as electrolyzed water, UV-C light, ozone, hydrogen peroxide and peroxycetic acid have also been recommended (CAC, 2003, FAO, 2003, FDA, 2009, Suslow, 2004). The levels of disinfection agents should be monitored and controlled to ensure that they are maintained at effective minimum concentrations (Lopez-Galvez et al., 2009).

In aquaponic systems, microbial and physio-chemical quality of process water decreases rapidly due to the continuous addition of organic matter to the washing tanks. To maintain the quality of the process water the use of a residual concentration of a disinfection agent in the water is critical for preventing pathogen survival and transfer
Maintaining a relatively consistent level of a disinfection agent during the process is a technical challenge in practice because of the rapid reaction of the disinfectants with organic materials released into water (Luo et al., 2011). Recent studies highlight that maintaining a residual concentration of 1 mg/l free chlorine in the process wash water, kept bacterial contamination below 2.7, 2.5, and 2.5 log CFU/100 ml for tap water and artificial process water with COD values of 500 and 1,000 mg O₂/l, respectively (Van Haute et al., 2013). However, residual concentrations between 3 and 5 mg/l completely inhibited microbial contamination in artificial process water with COD values of 500 mg O₂/l (Gil et al., 2013a). Being a system with live organisms, this method however does not work well with aquaponics. High levels of chlorine can be harmful to the fish and plants in the system. Hence chlorine treatment is not compatible with aquaponic sanitation despite its ability to kill microbes. The sensitive nature of the live system should be kept in mind while designing safety intervention strategies for aquaponic systems.

**Ozone**

Another potential contestant for aquaponic system disinfection is ozone. Ozone has a strong microbiocidal action against bacteria, fungi, parasites and viruses when these microorganisms are present in low ozone-demand media. The use of ozone as an antimicrobial treatment in foods has been established for some time (Sapers, 2001), but its potential for use on fresh fruits and vegetables is limited. This is largely due to strong oxidizing abilities which cause physiological damage to produce tissues (Parish et al., 2003). Bananas treated with ozone were reported to develop black spots, and carrots were reported to dull in color (Liew and Prange, 1994, Parish et al., 2003) In addition to tissue
damage, nutritional components including vitamins and enzymes have been reported altered by ozone oxidation (Kim et al., 2003).

Ozone is also reactive with organic matter, causes corrosion of metals, is costly to implement in a processing system due to cost of generation and ventilation necessary to remove toxic gases, and relatively instable when compared to other antimicrobial agents. However, ozone does not leave a residue and is able to penetrate below tissue surfaces, making it a useful tool in processing water and equipment for whole produce products.

Ozone is shown to be a strong oxidizing agent, acting on lipids found in the cellular membrane and lipopolysaccharide layer, enzymes and genetic material within the cell (Kim et al., 2003). Research has indicated ozone to effectively inactivate bacteria and fungi in water (Restaino et al., 1995), enteric viruses (Finch and Firbaim, 1991), and to a lesser and more variable extent, parasitic pathogens (Peeters et al., 1989, Korich et al., 1990). An extended shelf life of fruits has also been reported, attributed to oxidation of ethylene to slow ripening (Parish et al., 2006), as well as reduction of fungal contaminants on berries and grapes treated with ozone gas (Barth et al., 1995, Sarig et al., 1996). Rodgers and group (2004) reported ozone treatment for 15 seconds resulted in *E. coli* and *L. monocytogenes* reductions by 5 logs when in solution, which was the fastest acting sanitizer of all compared (including chlorine dioxide, chlorine, and peroxacetic acid). This group also observed pathogen reductions of 5.6 log when the treatment was applied to apples, lettuce, strawberries, and cantaloupe at 5 ppm with no sensory changes; however, no reductions in yeast and mold populations were observed on the produce. In contrast, Bermúdez-Aguirre and Barbosa-Cánovas (2013) reported much lower reductions of 2.2 log for *E. coli* on the surface of tomatoes, and minimal (less than 1 log)
reductions on carrots and lettuce even after 15 minutes. This study also reported color effects from treatment of lettuce. Singh et al. (2002) reported similar results of 1.6 and 2.5 log reductions of *E. coli* on lettuce and carrots, respectively, and Neal and group (2012) reported only 1.0 and 0.6 log reductions of *Salmonella* and *E. coli*, respectively on spinach when treated with 1 mg/L ozone water for 30 minutes. Readily available organic constituents in food, however, compete with microorganisms for applied ozone and thus efficacy of the treatment is minimized. The antimicrobial efficacy of ozone can be enhanced considerably when ozonation is combined with physical (e.g., ultraviolet radiation) or chemical treatments (Kim et al., 2013).

**Organic Acids**

Sirsat et al. (2013) showed that aquaponically grown lettuce in 2.5% acetic acid had significantly lower concentration of spoilage and fecal microorganisms compared to in-soil grown lettuce. The intervention study showed that diluted vinegar (2.5% acetic acid) significantly reduced *Salmonella, E. coli, coliforms*, and spoilage microorganisms on fresh lettuce by 2 to 3 log CFU/g. However, adding acid to the system would lower its pH which might not be suitable for the survival of several crops and aquatic species. High acid environment might be toxic to the aquaponic cultures and put them under stress. This will considerable reduce the survival rate of aquaculture and crops.

**Ultraviolet Light**

Of the available solutions, UV disinfection has gained most interest. UV irradiation is a non-thermal technology that has been traditionally used to disinfect air, water, and surfaces (Bintsis et al., 2000). It has gained considerable interest in the liquid food pasteurization field because it is easy to use and lethal to most types of
microorganisms. It does not generate chemical residue, and it is a dry cold process that can be effective at a low cost (Guerrero-Beltrán et al., 2004, Tran et al., 2004). UV treatments are potentially capable of inactivating bacterial spores (Hijnen et al., 2006). UV irradiation was used in 1985 (Chang et al., 1985) to inactivate bacteria *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis* spores, the enteric viruses poliovirus type 1 and simian rotavirus SA11, the cysts of the protozoan *Acanthamoeba castellanii*, as well as for total coliforms and standard plate count microorganisms in effluent waste water at different intensities. Aquaponic water being almost similar in nature to effluent waste water can potentially be treated likewise.

However, UV light has poor penetrating power so efficacy against pathogens is restricted by proximity of generated radiation to the intended treatment as well as product translucency and surface variability, with greatest results seen when bacteria are dispersed in clear to semi-clear liquids, in the air or on the surface of smooth products (Wong et al., 1998, Worobo and Hartman, 2013). In addition to this, bacterial spores, stationary phase cells, and norovirus have been shown to have resistance to UV light treatments (Fino and Kniel, 2008, Warriner et al., 2009). These factors can be disadvantageous and treatments should be decided based on the nature of the system and pathogens to be treated.

A more recent application of combined UV light and hydrogen peroxide to generate free radicals has been utilized to control human pathogens on the surface as well as internalized in vegetable tissues. This treatment (1.5% H₂O₂ at 50°C and UV-C of 37.8 mJ/cm²) has been reported to reduce viable *Salmonella* cells by 4.1 log on the surface of
lettuce, and 2.8 logs when internalized in lettuce structure, without significant
discoloration (Hadjok et al., 2008). Combinatorial sanitation methods can sometimes be 
more advantageous than the individual methods. Identifying the optimum treatment 
methods for the system will be critical in determining its success.

**How does UV work?**

When bacterial spores are treated with UV-C radiation (200–280 nm), which is 
considered the most germicidal wavelength range, their DNA absorbs photons from the 
UV and induces the formation of bipyrimidine dimers (Moeller et al., 2007). These 
transiently block DNA transcription and replication, leading to cell death (Friedberg et 
al., 2006). Variables such as flow rate, exposure time, type of crops grown, water 
composition, among other variables, need to be studied to obtain products with reduced 
microbial load, increased shelf life and adequate sensory and nutritional characteristics 
(Guerrero-Beltran et al., 2004).

**Industrial Uses of UV Disinfection**

In the past, several researchers have shown successful application of UV 
treatment to sterilize several food items. Research in 2008 indicated that the UV-C 
wavelength of 254 nm was used for the disinfection of several fruit juices and had a 
germicidal effect against microorganisms (Keyser et al., 2008). Research in 1987 
indicated that UV light had been effective in destroying bacteria on the surface of fresh 
meat without causing any deleterious effects (Stermer et al., 1987). Later on, UV 
treatment became very popular and is now used for sterilizing water, pasteurization of 
several fruit juices and is also used as a surface disinfectant. The success of UV treatment 
in food industry can be extended to aquaponics, as it an integrated food system itself.
Part 1: Scope of UV in Aquaponics

Researchers indicate that UV treatments are expected to reduce the level of several harmful disease-causing bacteria in both aquaculture and crops (Timmons and Ebeling, 2007). There has been research which studied water and plant samples from aquaponic systems over a two year period to determine the presence of total and fecal coliforms, and *Salmonella*, and whether UV treatment makes a significant difference. Tests for *Salmonella* and *E. coli* were negative proving that UV treatment did significantly reduce levels compared with non-treated fecal and total coliforms (Pablo González-Alanis et al., 2011).

In a recent study involving lettuce, it was shown that there were no *E. coli* found in both sterilized and non-sterilized aquaponic systems. However, total coliforms under UV disinfection showed counts well below 1 CFU ml\(^{-1}\) and a reduction in microbial loads higher than 99% with no significant difference in the productive traits of lettuce (Pantanella et al., 2010). This suggests that use of UV treatment in aquaponics is a valid method to produce vegetables with high hygienic standards. Using the knowledge gained from previous research, we examined the efficacy of UV treatment in reducing the pathogen levels in our greenhouse cultivation environment.

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CHAPTER 3: LITERATURE REVIEW ON “FOOD SAFETY STATUS OF
ORGANIC LIVESTOCK AND FEED”

Introduction to the Organic Food Industry

What is Organic Agriculture?

A modern definition of organic farming provided by Lampkin (1994) states that
the aim is: “to create integrated, humane, environmentally and economically sustainable
production systems, which maximize reliance on farm-derived renewable resources and
the management of ecological and biological processes and interactions, so as to provide
acceptable levels of crop, livestock and human nutrition, protection from pests and
disease, and an appropriate return to the human and other resources”.

According to Hill and MacRae (1992), organic farming comprises a range of
approaches within the broader sustainable agriculture spectrum. In its most developed
form, ecologically sustainable agriculture (including organic farming) is both a
philosophy and a system of farming. It is based on a set of values that reflect an
awareness of both ecological and social realities, and on a level of empowerment that is
sufficient to generate responsible action. Efforts to ensure short-term viability are tested
against long-term environmental sustainability, and attention to the uniqueness of every
operation is considered in relation to ecological and humanistic imperatives, with an
awareness of both local and global implications. It emphasizes benign designs and
management procedures that work with natural processes and cycles to conserve all
resources (including beneficial soil organisms and natural pest controls), and minimize
waste and environmental damage, prevent problems, and promote agroecosystem
resilience, self-regulation, evolution and sustained production for the nourishment and
optimal development of all (including rural communities both here and abroad). Special attention is paid to the relationships between soil conditions, food quality and livestock health, and livestock is cared for in the most humane way possible. In addition, organic farmers tend to avoid the use of synthetically compounded fertilizers, pesticides, growth regulators and livestock feed additives. Instead, they rely on crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, mechanical cultivation and mineral-bearing rocks to maintain soil fertility and productivity. Insects, weeds and other pests are managed by means of natural, cultural and biological controls.

**Current Demand in Organic Food Industry**

Driven by consumer demand, the U.S. organic food industry has grown substantially in popularity. According to the Organic Trade Association's Organic Industry Survey (2011), total organic food sales accounted for $6.1 billion in 2000 and more than quadrupled in the past 10 years to $26.7 billion in 2010 (Van Loo et al., 2011). Several factors have led to this increased consumption of organic foods in the U.S., including consumer preference for lower pesticide residues (Baker et al., 2002), nutrition and health concerns (Williams 2002, Magkos et al., 2003), negative environmental impacts associated with intensive conventional production (Venterea and Rolston, 2000), and the assurance of organic integrity through consistent federal organic standards (USDA-AMS, 2014). Farmers also are interested in producing organic crops that meet the “triple bottom line” of environmental sustainability, economic viability, and social equity. In recent years, organic farmers have become increasingly concerned about farm product/food safety, particularly important for farmers practicing integrated crop/livestock production (Pereira et al., 2013). Assuring food safety is critical in any
farming operation, and, in the case of produce, has been the subject of the recent federal law, "FDA Food Safety Modernization Act” (21 USC 2201, FDA, 2011), which will have far-reaching effects on the entire food supply chain.

**Standards for Organic Food Production**

The USDA is committed to help organic agriculture grow and thrive. It defines organic agriculture as a process that produces products using methods that preserve the environment and avoid most synthetic materials, such as pesticides and antibiotics (USDA, 2015). Organic farmers, ranchers, and food processors follow a defined set of standards to produce organic food and fiber. Congress described general organic principles in the Organic Foods Production Act, and the USDA defines specific organic standards though it’s National Organic Program (USDA-AMS-NOP, 2016a). These standards cover the product from farm to store, including soil and water quality, pest control, livestock practices, and rules for food additives. Organic farms and processors preserve natural resources and biodiversity, support animal health and welfare, provide access to the outdoors so that animals can exercise their natural behaviors, only use approved materials, do not use genetically modified seeds or ingredients, and receive annual onsite inspections and separate organic food from non-organic food on store shelves.

The Organic Foods Production Act (OFPA) was enacted under the 1990 Farm Bill. The Act authorized creation of USDA’s NOP for the production, handling, and processing of organically grown agricultural products. Organic regulations are set forth under Title 7, Part 205 of the Code of Federal Regulations. These regulations describe the specific requirements that must be verified by a USDA-accredited certifying agent before
products can be labeled USDA organic. For organic crops, the USDA organic seal verifies that irradiation, sewage sludge, synthetic fertilizers, prohibited pesticides, and genetically modified organisms were not used. With organic livestock, the USDA organic seal verifies that producers met animal health and welfare standards, did not use antibiotics or growth hormones, and used 100% organic feed and provided animals with access to the outdoors. The NOP oversees mandatory certification of production and handling of all products to be marketed or represented as organic within the United States. Producers who meet USDA Organic Regulations may only label their products as “USDA Certified Organic.”

**Organic versus Conventional Food Production**

Food safety practices to reduce toxins and microbial contamination are on the minds of all farmers, but particularly for farmers who integrate animals and crops in the same system. Studies comparing organic and conventionally raised livestock and pasture crops have found, in general, no significant food safety differences between conventional and organic systems (Bourn and Prescott, 2002, Maffei et al., 2013, Oliveira et al., 2010, Blanco-Penedo et al., 2012). In one study, it was found that conventional wheat had greater mycotoxins in a pilot project, but no significant differences were found in a more extensive experiment (Edwards, 2009). In a livestock comparison in Spain, there were no food safety differences in organic or conventional beef cattle, but organic beef was reported to have higher quality (Blanco-Penedo et al., 2009). In a comparison survey of organic versus conventional broiler chickens, no significant differences were found in *Salmonella* presence but *Campylobacter* was higher in the organically raised broilers (Tuyttenssa et al., 2008). Grazing systems that reduce the larval or shed load of internal
parasites will enhance cattle productivity on organic pastures (Larsen, 2006). Continuously grazed pastures fail to break the life cycle of these parasites, whereas rotational grazed pastures frequently reduce the parasite larval load (Stromberg and Averbeck, 1999). This project has been designed taking into account all the above mentioned factors. It will explore a relatively new research area of integrating livestock within rotational cropping systems and examine its effect on plant and animal health and product safety.

**Food Safety Risks Associated with Manure and Livestock Feed**

The interactions between cropping and grazing systems on organic and conventional farms are not fully understood (Franzluebbers, 2007), particularly in organic systems, where the collection and distribution of manure is critical to the crop’s nutrient balance. The significance of fresh vegetable consumption on human nutrition and health is well recognized. Human infections with *Escherichia coli* O157:H7 and *Salmonella enterica* linked to fresh vegetable consumption have become a serious public health problem inflicting a heavy economic burden. In the United States, surface water is commonly used to irrigate a variety of produce crops and can harbor pathogens responsible for foodborne illnesses and plant diseases. Understanding when pathogens infest water sources is valuable information for produce growers to improve the food safety and production of these crops (Jones et al., 2014). The use of manure and manure slurry in crop production is believed to be one of the principal routes of fresh vegetable contamination with *E. coli* O157:H7 and *S. enterica* at preharvest stage because both ruminant and non-ruminant livestock are known carriers of *E. coli* O157:H7 and *S. enterica* in the environment (Ongeng et al., 2014). USDA-AMS-NOP has mandated that
unless composted, raw manure should be incorporated into the soil not less than 120 days prior to the harvest of a product whose edible portion has direct contact with the soil surface or soil particles; or not less than 90 days prior to the harvest of a product whose edible portion does not have direct contact with the soil surface or soil particles (USDA-AMS-NOP, 2016b).

A number of challenge-testing studies have examined the fate of *E. coli O157:H7* and *S. enterica* in the agricultural environment with the view of designing strategies for controlling vegetable contamination preharvest. Mathematical modeling approaches were used to study the behavior of *E. coli O157:H7* and *S. enterica* in the manure, manure-amended soil, and manure-amended soil–plant ecosystem during cultivation of fresh vegetable crops. However, these models had significant limitations and could not predict pathogen survival associated with the risk of preharvest vegetable contamination (Ongeng et al., 2014).

Feed has been reported as a vehicle for transmission of *Salmonella enterica* in cattle (Glickman et al., 1981, Jones et al., 1982, Anderson et al., 1997, Krytenburg et al., 1998, Lindqvist et al., 1999, Hinton, 2000 and Kidd et al., 2002), and several lines of evidence suggest that feed can be a vehicle for transmitting *Escherichia coli O157:H7* (O157) as well (Hancock et al., 1997, Hancock et al., 2001 and Lynn et al., 1998). Because food-producing animals are the primary source of O157 and pathogenic *Salmonella* infections in humans, it follows that bacterial contamination of animal feed contributes to the burden of foodborne illness (Crump et al., 2002).

Among the factors considered hazardous in forage crops and silages are pathogenic enterobacteria, such as *Salmonella* and toxin-producing *Escherichia coli*
Pathogenic strains of *E. coli* can cause severe illness in humans and animals, and the toxin-producing organism *E. coli* O157:H7 is of special concern; if conditions in silage are favorable for growth of this bacterium, it may cause intestinal disorders and mastitis in animals that consume the silage (Lindgren, 1991). Cattle are a primary source of pathogenic *E. coli* O157:H7 (Bach et al., 2002), but this organism may be transmitted to crops and their products via shedding or through fertilization of fields with manure (Russell et al., 2000).

Lynn et al. (1998) showed that sixty-three of 209 (30.1%) samples of cattle feed collected from multiple commercial sources and farms were found to contain *Escherichia coli*. However, none of the feed samples examined were culture-positive for *E. coli* O157. Replication of fecal *E. coli*, including *E. coli* O157, was demonstrated in a variety of feeds at temperatures that were similar to those found on farms in summer months. Fresh mixed rations containing corn silage were sampled from 16 dairies. Rations from 12 of these dairies were found to contain *E. coli*, and the rations from 5 dairies had concentrations of *E. coli* that were greater than 1000 cfu/g. The ability of experimental mixed rations to support the replication of *E. coli* was positively correlated with the concentration of organic acids in the corn silage that was used in the ration. Widespread contamination of cattle feeds with *E. coli* and the ability of *E. coli* to replicate in feeds suggest that feeds are a potentially important factor in the ecology of organisms that can be transmitted from feces to mouth, such as *E. coli* O157.

In a research conducted by Franz et al. (2005), the type of feed fed to cattle determined the level of *E. coli* O157:H7 in the feces. Roughage type significantly influenced the rate of decline of *E. coli* O157:H7 in feces, with the fastest decline being
from pure straw diet and the slowest from the diet of grass silage plus maize silage. However, this did not have much effect on *Salmonella enterica* serovar Typhimurium when tested. The success of this research with *E. coli* O157:H7 can be used as a good basis for identifying other feed formulae which might potentially have a decreasing effect on other pathogens.

In several studies, the presence of low levels of *S. enterica*, *Bacillus cereus*, *E. coli*, and various other food spoilage micro-organisms have been reported in wheat and flour in different parts of world (Eyles et al., 1989, Richter et al. 1993, Berghofer et al., 2003, Sperber, 2007). The plant phyllosphere, in particular cereal crops with longer growing times, present a hostile environment for bacterial pathogens to survive (Brandl, 2006). The cereal phyllosphere is subjected to rapid and large fluctuations in temperature, humidity and osmotic pressures (Wilson et al., 1999) which may adversely affect the survival of enteric pathogens (Cox, 1993, Casanova et al., 2010). Environmental factors, such as ultraviolet (UV) radiation and desiccation, have been identified as important factors that influence pathogen survival on the phyllosphere (Heaton and Jones, 2008). Competition for limited nutrients and moisture also makes enteric pathogen survival more difficult (Mercier and Lindow, 2000). Plant-specific factors, such as waxes, may also restrict bacterial attachment to leaf surfaces (Aruscavage et al., 2006). However, pathogens may survive better under certain conditions such as in the shade or under increased moisture content, usually between the leaves and stems of the plants (Brown et al., 1980, Lindow and Brandl, 2003, Ibekwe et al., 2004). Once deposited in the phyllosphere through rain splash, pathogens could also migrate into the biofilms
established by autochthonous microflora which is reported to shield them from desiccation and UV (Elasri and Miller, 1999, Fett, 2000, Monier and Lindow, 2005).

Recently, corn byproducts such as distiller’s grains (DG) are commonly utilized as livestock feed. The inclusion of DG has been shown to appreciably affect rumen microbiology and fermentation (Fron et al., 1996). Others have speculated that the relative lack of starch (compared to corn) and the high concentration of fiber and protein escaping ruminal digestion in DG are more likely to influence the hindgut (Jacob et al., 2009). Taken together, these reports suggest that feeding DG, compared with a traditional corn or sorghum grain-based diets, alters the gastrointestinal environment and that it likely influences bacterial populations within the gut (Edrington et al., 2010). Recent research attempted to determine whether feeding DG influenced fecal prevalence of *Escherichia coli O157:H7* and *Salmonella* in feedlot cattle with mixed results (Jacob et al., 2008b, 2008c, 2009). Researchers report that feeding grain processed by dry-rolling compared with steam-flaking decreased fecal shedding of *E. coli* O157:H7 (Fox et al., 2007), whereas others (Jacob et al., 2009) reported no effect of dry-rolled corn (DRC) on fecal prevalence in cattle.

Kutter et al. (2006) investigated the colonization behavior of different food-borne pathogenic bacteria of roots and shoots of barley plants in a monoxenic model system. They selected two strains of *Salmonella enterica* serovar Typhimurium (LT2 and S1) and pathogenic (*L. monocytogenes*) as well as apathogenic (*L. ivanovii* and *L. innocua*) strains of the genus *Listeria* for these investigations. Both *S. enterica* strains were found as endophytic colonizers of barley roots and reached up to $2.3 \times 10^6$ CFU per g root fresh weight after surface sterilization. The three *Listeria* strains had 10-fold fewer cell
numbers after surface sterilization on the roots and therefore were similar to the results of nonendophytic colonizers, such as *E. coli* HB101. They demonstrated not only high-density colonization of the root hairs and the root surface by *S. enterica* but also a spreading to subjacent rhizodermis layers and the inner root cortex. By contrast, the inoculated *Listeria* spp. colonized the root hair zone but did not colonize other parts of the root surface. Endophytic colonization of *Listeria* spp. was not observed. Finally, a systemic spreading of *S. enterica* to the plant shoot (stems and leaves) was demonstrated using a specific PCR analysis and plate count technique.

Irrigation and surface run-off waters can be sources of pathogenic microorganisms that contaminate crops in the field. Irrigation water containing raw sewage or improperly treated effluents from sewage treatment plants may contain hepatitis A, Norwalk viruses, or enteroviruses (poliomyelitis, echoviruses, and Coxsackie viruses) (Bagdasargan, 1964). Rotaviruses are known to retain viability on the surface of vegetables held at 4°C for up to 30 days (Badaway et al., 1985).

*Listeria* and other potentially pathogenic bacteria have been reported in sewage. Watkins and Sleath (1981) analyzed 52 sewage, river water, and industrial effluents for pathogens. Effluents were from abattoirs, cattle markets, and poultry packing plants. *L. monocytogenes* was isolated from all samples. In many instances, populations of *L. monocytogenes* were higher than those for *Salmonellae* and, in some instances, *L. monocytogenes* was isolated when no *Salmonellae* were detected. Application of sludge containing *L. monocytogenes* and *Salmonellae* to soil showed that *L. monocytogenes* could survive longer. Populations of *L. monocytogenes* in soil remained essentially unchanged during 7 weeks after application.
Treatment of sewage does not always yield a sewage sludge cake or a final discharge free of *Listeria* (Al-Ghazali et al., 1986). The use of sewage as a fertilizer could contaminate vegetation destined for consumption. MacGowan et al. (1994) examined sewage at 2-month intervals in 1991 to 1992 and found 84% to 100% contained *L. monocytogenes* or *L. innocua*.

Application of sewage sludge or irrigation water to soil is one avenue through which parasites can contaminate crops. *Ascaris ova* sprayed onto tomatoes and lettuce remain viable for up to 1 month, while *Endamoeba histolytica* could not be recovered 1 week after spraying (Rudolfs et al., 1951). If sewage irrigation or night soil application is stopped 1 month before harvest, the produce would not likely be vectors for transmission of diseases caused by these parasites.

In the poultry industry, feed is the major component of the total cost of production for meat and egg production (Yegani and Korver, 2008). Corn and soybean meal remain the main ingredients of choice for poultry diets worldwide. It seems that there are currently no globally applicable alternatives to corn and soybean meal, although inclusion of high levels of wheat in poultry diets is common in some parts of the world (Tucker and Taylor-Pickard, 2004). Feed is probably the most important entity in the poultry industry that can expose the birds to a wide variety of factors through the gastrointestinal (GI) tract (Yegani and Korver, 2008).

The latest concern in the food industry is the prevalence of antibiotic-resistant bacteria in the food chain. When the impact of resistant bacteria on the food chain is considered, an important area for investigation is people coming into contact with livestock and farms who are at risk of infection by antibiotic-resistant bacteria that are
present in that environment (Friedman, 2015). For example, a study in Thailand (Boonyasiri et al., 2014) found that, among 544 healthy adult food factory workers, 75% were positive for a particular resistant bacterium of interest, extended-spectrum β-lactamase (ESBL)-producing \textit{E. coli}. The value for 30 healthy animal farm workers was 77.3%. Among the farm animals, the value was 76.7% in pigs and 40% in poultry broilers. The ESBL-producing \textit{E. coli} was more prevalent in fresh meat samples than in fresh vegetables, in fresh than in cooked foods, and in water samples collected from animal farms than in those from canals and fish and shrimp ponds.

\textbf{Microbial Status of Livestock}

The ability for animals to be raised free range is important in organic farming, but by the very nature of this type of environment, this production system has the potential for greater food safety risks. With free-range practices, there is a greater risk of transfer of zoonosis from wildlife to farm animals via greater exposure to pathogens carried by parasites, rats, mice, birds, and pathogens in soil. Because of extended exposure to outdoor conditions, organically raised farm animals may more likely be infected by \textit{Salmonella} and \textit{Campylobacter} (Lund, 2006, Hansen et al., 2002).

Consumers often buy organic meats because they believe they are healthier or safer (O'Donovan and McCarthy, 2002). However, this may not be true. The microbial safety of conventional animal production has been widely studied, but organic animal production practices have not been examined to this same degree (Young et al., 2009).

There are only limited data on the prevalence of pathogens in cattle raised under organic or natural conditions for meat. Reinstein et al. (2009) reported \textit{E. coli} contamination at slaughter in USDA-certified organically (14.8%) and naturally raised
(14.2%) cattle similar to those previously reported for conventionally raised cattle (11.2%). Likewise, Miranda et al. (2009) did not detect any significant differences of *E. coli* prevalence in conventionally (43%) and organically (48%) raised beef at retail outlets. Miranda et al. (2009) also found no significant differences in *S. aureus* (55% versus 51%), *L. monocytogenes* (29% versus 36%) and *Salmonella spp.* (0% for both) contamination on organically and conventionally raised beef from cattle.

Cattle are a major reservoir of *Escherichia coli* O157:H7 and can shed this bacterium asymptomatically after it colonizes the lower digestive tract (Gyles, 2007). The prevalence and shedding of *E. coli* O157:H7 may be influenced by multiple factors, including host stress, seasonal variation, and feeding practices (Rhoades et al., 2009). For the latter, the impact of a number of dietary components on the shedding of *E. coli* O157:H7 have been assessed. Berg et al. (2004) showed that feeding barley increased the shedding of *E. coli* O157:H7 by cattle compared with feeding corn. It was proposed that this difference was due to alterations in pH and carbohydrate flow to the lower tract. An epidemiological study revealed a positive correlation between cattle receiving barley grain in their diet and the prevalence of *E. coli* O157:H7 in feedlot cattle (Dargatz et al., 1997). In contrast, other studies have shown that forage-fed cattle shed *E. coli* O157:H7 in their feces for longer periods than do grain-fed cattle (Hovde et al., 1999, Van Baale et al., 2004).Yang et al. (2010) showed that the inclusion of high levels of corn or wheat DDGS in feedlot diets of cattle may encourage the survival of *E. coli* O157:H7 in feces.

Changes in diet may influence fecal shedding of pathogenic bacteria from ruminants (Callaway et al., 2003, Looper et al., 2006). Cattle grazing toxic endophyte-infected (EI) tall fescue, a cool-season forage found throughout the Southeastern United
States are exposed to ergot alkaloids that cause increased body temperature during summer months, reduced reproductive performance and growth rate and decreased milk production (Hoveland et al., 1983, Paterson et al., 1995). Further, dry matter intake is usually reduced in ruminants consuming toxic EI tall fescue (Paterson et al., 1995, Looper et al., 2006). Ruminants fed at below-maintenance requirements generally have reduced ruminal volatile fatty acid concentrations and increased pH in the rumen, which can result in increased prevalence of *E. coli* O157:H7 (Brownlie and Grau, 1967, Rasmussen et al., 1993) and *Salmonella* in feces (Brownlie and Grau, 1967).

Limited studies with grazing systems suggest pasture based cattle are infected with *E. coli* O157:H7 (Laegreid et al., 1999, Riley et al., 2003, Dunn et al., 2004) and *Salmonella* (Looper et al., 2003, Fossler et al., 2005). It is probable that some of these animals could shed bacteria during the feedyard phase and at harvest. Stress may predispose cattle to be more susceptible to opportunistic bacteria such as *E. coli* O157:H7 and *Salmonella* (Fitzgerald et al., 2003, Looper et al., 2005).

Barkocy-Gallagher et al. (2003) monitored the seasonal prevalence of *E. coli* O157:H7, *Salmonella*, non-O157 *E. coli* (*STEC*), and shiga toxin-harboring cells at three Midwestern fed-beef processing plants. The prevalence of *E. coli* O157:H7, *Salmonella*, and non-O157 STEC varied by season and was higher on hides than in feces, and decreased dramatically, along with pathogen levels, during processing and during the application of antimicrobial interventions. This study highlights the significance of hides as a major source of pathogens on beef carcasses.

Donkersgoed et al. (1999) found that the prevalence of *E. coli* O157:H7 in fecal samples was higher in yearling cattle than in cull cows. It was also found that the
prevalence of *E. coli* O157:H7 in fecal samples did not depend on rumen fill, body condition score, sex, type of cattle (dairy, beef) but it was generally observed to be based on the environmental conditions. *E. coli* O157:H7 was observed to be highest in the summer months indicating the ideal temperature for its growth. Generally, given the lack of data comparing food safety issues in conventional and alternative beef cattle production, results from more studies are needed before definitive conclusions can be made regarding food safety in organic livestock.

**Food Safety Status of Meat**

Little has been published on the safety of organic meat and any associated microbiological risks. Although the consumer perceives these products as safer, production methods such as access to the outdoors, restrictions on therapeutic use of antimicrobials, and the often smaller processing facilities, may contribute to a greater microbiological safety risk (Miranda et al., 2008a). Foods that are microbiologically contaminated may harbor harmful microorganisms, making them potentially risky for human food consumption. Organic meat production has the potential to have higher microbiological safety risks because of the strict restrictions in the use of pharmaceutical agents for therapeutic use (such as antimicrobials or parasiticides), raising the animals outdoors, use of slow-growing breeds and the smaller slaughtering facilities (Engvall, 2001, Institute of Food Technologists, 2006, Thamsborg, 2002). However, with proper management, such risks can be reduced (Bourn and Prescott, 2002, Winter and Davis, 2006). Verocytotoxigenic *E. coli* (VTEC) does not grow below 7 °C and so should not proliferate in beef products stored below this temperature (Rhoades et al., 2009). In minced beef stored for 72 h, Mann and Brashear (2006) observed no significant growth at
7.2 °C and a 1.0 log cfu g⁻¹ increase at 10 °C, while good survival on beef has been reported at 7 °C for 11 days and −18 °C for 84 days. (Uyttendaele et al., 2001, Dykes et al., 2001). *E. coli* O157 has no notable heat resistance: reported D-values in lean minced beef include 0.16 and 20 min at 63 and 55 °C, respectively (Smith et al., 2001), and 0.39 and 21.1 min at 65 °C and 55 °C, respectively (Juneja et al., 1997).

*Salmonella* spp. are capable of growth between 7 and 49.5 °C, although unconfirmed reports exist of growth down to 5.5 °C. The growth rate below 15 °C is greatly reduced. The organism can survive for long periods in foods under chilled or frozen storage (Rhoades et al., 2009). Barrell (1988) reported a decrease in viable count of *S. Typhimurium* of around 50% after 10 weeks storage at −8 °C to −20 °C in cooked minced beef, while Escartin et al. (2000) observed a 2–3 log cfu g⁻¹ decrease in pork after 78 weeks at −15 ± 2 °C. On chilled, vacuum or CO₂ packaged raw beef, *S. Brandenburg* and *S. Typhimurium* showed no decline in viability after storage for 6 weeks at −1.5 °C followed by 2 weeks at 4 °C (Dykes et al. 2001). *Salmonella* is not generally heat-resistant, although a few serovars such as *S. Senftenberg* have notably higher heat tolerance than others. Smith et al. (2001) reported D₆₄°C values of 0.92 and 0.16 min in high-fat minced beef for *S. Senftenberg* and *S. Typhimurium* DT104, respectively, and D₅₈°C values of 21.8 and 2.6 min, respectively.

*L. monocytogenes* is notable for its ability to grow at refrigeration temperatures, unlike most other enteric pathogens (Pal et al., 2008). This has considerable significance for food safety, as it means that chilling to 4 °C cannot be relied upon to prevent the growth of the organism to dangerous levels. It is destroyed by pasteurization or adequate cooking. Schultze et al. (2007) reported D₆₀°C values in frankfurter meat slurry of
between 0.9 min (23% fat slurry) and 2.2 min (8.5% fat slurry). On vacuum-packaged bologna, higher D60°C values of 7.4 and 14.5 min were observed (Selby et al., 2006), while on vacuum-packaged roast beef D values ranged from 0.07 min at 71.1 °C to 1.6 min at 62.8 °C (Muriana et al., 2002).

In a study conducted by Zhao et al. (2001), a total of 825 samples of retail raw meats (chicken, turkey, pork, and beef) were examined for the presence of *Escherichia coli* and *Salmonella* serovars, and 719 of these samples were also tested for *Campylobacter* spp. The samples were randomly obtained from 59 stores of four supermarket chains. Approximately 14% of the 172 turkey samples yielded *Campylobacter*, whereas fewer pork (1.7%) and beef (0.5%) samples were positive for this pathogen. A total of 722 *Campylobacter* isolates were obtained from 159 meat samples; 53.6% of these isolates were *Campylobacter jejuni*, 41.3% were *Campylobacter coli*, and 5.1% were other species. Of the 212 chicken samples, 82 (38.7%) yielded *E. coli*, while 19.0% of the beef samples, 16.3% of the pork samples, and 11.9% of the turkey samples were positive for *E. coli*. However, only 25 (3.0%) of the retail meat samples tested were positive for *Salmonella*. This study revealed that retail raw meats are often contaminated with foodborne pathogens and are potential vehicles for transmitting foodborne diseases. Based on these studies, we can say that organic meats, produced naturally without antibiotics, are at an increased risk of food safety.

**Influence of Feed on the Microbial Status of Cattle and Meat**

Epidemiologists have sought to correlate *E. coli* O157:H7 with feeding-management practices, but the statistical correlations were weak or inconsistent. In 1994, Hancock et al. surveyed cattle on farms and feedlots for *E. coli* O157:H7. Of the 32
management factors examined, only three items were statistically significant \((p < 0.10)\) namely: 1) computerized feeding, 2) manure on pasture, and 3) lack of whole cottonseed in the ration. Garber et al. (1995) noted that feeding whole cottonseed was negatively associated with \textit{E. coli} O157:H7 shedding from dairy calves, but other workers were unable to establish a link between \textit{E. coli} O157:H7 and manure on pasture (Hancock et al., 1997). The 1994 study of Hancock et al. indicated that the feeding of corn silage did not cause a statistically significant increase in \textit{E. coli} O157:H7 positive cattle, but later work by Herriott et al. (1998) indicated that a significantly higher prevalence of \textit{E. coli} O157 was noted in herds that fed corn silage to heifers compared to herds that did not feed corn silage. Dargatz et al. (1997) reported that barley feeding was associated with increased ‘likelihood’ of cattle being positive for \textit{E. coli} O157:H7, but other studies failed to link grain feeding with an incidence of \textit{E. coli} O157:H7 in feedlot cattle (Hancock et al., 1997).

Cattle must sometimes be transported long distances to be slaughtered, and during this time feed is often not provided. In the 1960s, Brownlie and Grau studied the effect of feed deprivation on the shedding of \textit{Salmonella} and \textit{E. coli} from cattle given ruminal doses of these bacteria. They noted that when animals were receiving a regular daily ration of 6.8 kg alfalfa, the organisms were rapidly eliminated from the rumen, and viable organisms in the feces were rarely detected. However, if the cattle were starved for one or more days, the \textit{Salmonellae} and \textit{E. coli} were detected in the feces. The work by Cray et al. in 1998 showed that inoculated calves that were starved were more susceptible to infection and shed more \textit{E. coli} O157:H7 organisms than calves maintained on normal diet.
Brownlie and Grau (1967) indicated that a large decrease in hay intake could also promote pathogenic *E. coli* shedding, and Kudva and colleagues examined the effect of drastic diet shifts on the shedding of *E. coli* O157:H7 from experimentally inoculated sheep (Kudva et al., 1995, 1997). In the first Kudva study, sheep were switched from alfalfa hay pellets to a sagebrush/bunch grass mixture or kochia weeds, and sheep in some cases were starved. Results indicated that feed withdrawal may induce apparently *E. coli*-negative animals to become positive, but the numbers of animals were small and statistical significance was not reported. In the second Kudva study, sheep were switched from a 50:50 alfalfa/corn ration to very poor quality grass hay and starved. Sheep fed poor quality grass shed *E. coli* O157:H7 longer and in greater numbers than sheep fed the alfalfa/corn ration, but most statistical tests were not significant.

The relevance of experimental inoculation to natural shedding has never been confirmed, and many inoculation studies have employed calves rather than mature cattle because post weaning calves seem to be more prone to shed *E. coli* O157:H7 (Armstrong et al., 1996). Zhao et al. (1998) indicated that calves previously inoculated with nonpathogenic, colicin producing *E. coli* were less likely to shed *E. coli* O157:H7 that was given orally, and this result indicates that there can be a competition between *E. coli* O157:H7 and other *E. coli* strains within the gastrointestinal tract.

Hovde et al. (1999) inoculated eight mature cattle with *E. coli* O157:H7 and showed that hay-fed animals shed *E. coli* O157 longer than grain-fed animals, and they cautioned against preharvest management that includes an abrupt dietary change from grain to hay. Traditional enumeration schemes indicated that the percentage of cattle shedding *E. coli* O157:H7 was relatively low (0 to 3%) (Armstrong et al., 1996, Hancock
et al., 1994), but these techniques are inherently insensitive. Enumerations based on immunomagnetic beads are more sensitive, and these techniques indicate that the prevalence is five to tenfold greater than previously thought (Bolton et al., 1999, Mechie et al., 1997, Chapman et al., 1997). Given this comparison, it is conceivable that potential correlation between diet and *E. coli* O157:H7 shedding was simply missed. Animals shedding large numbers of *E. coli* O157:H7 are clearly more dangerous than animals shedding only a few, but field surveys have in most cases only given animals a plus or minus score (Hancock et al., 1994, Hancock et al., 1997, Herriott et al. 1998). Further work is obviously needed to clarify the epidemiology of *E. coli* O157:H7 in mature cattle under natural conditions.

**Consumers’ Opinion on Organic versus Conventional Food**

Consumers often have the perception that organic foods are safer and healthier than conventionally grown foods, and this is the primary reason for organic food and organic meat purchases (Van Loo et al., 2010). Consumers are willing to pay premium prices for these products (Van Loo et al., 2011). Over the last few years, numerous food supply crises such as mad cow disease, foot-and-mouth epidemic, and the Belgian dioxin scandal have caused widespread anxiety among consumers about the quality of food they eat (Miles and Frewer, 2001). Moreover, growing environmental awareness in combination with concerns about safer foods has led people to question modern agricultural practices. The perceived potential hazards of modern agricultural practices, such as the use of pesticides and their residues in food, are perceived to be associated with long-term and unknown effects on health (Miles and Frewer, 2001, Wilkins and Hillers, 1994, Williams and Hammit, 2001). This has been reflected in an increasing
demand for organic produce, which is perceived as less damaging to the environment and healthier than conventionally grown foods (Schifferstein and Oude Ophuis, 1998, Williams and Hammit, 2001).

Despite there being no unambiguous evidence that organic foods are healthier than conventional foods, organic foods contain less harmful additives but more primary (e.g., vitamin C, dry matter, minerals) and secondary nutrients (i.e., phyto-nutrients) than conventional foods(Chen, 2007). In other words, organic foods at least carry no additional risk of food poisoning (Heaton, 2001). On the basis of the precautionary principle alone, choosing organic foods appears to be an entirely rational decision. This has led consumers to perceive foods labeled as organic to be healthier than conventional foods (Grankvist and Biel, 2001, Magnusson et al., 2001).

**Scientific Overview on Organic versus Conventional Food**

Much of the research conducted on organic-based foods has concluded that there is no evidence that organic food is safer, healthier, or more nutritious (Williams, 2002, Magkos et al., 2003) although others have found evidence of greater levels of antioxidants and vitamins (Callaway et al., 2009, Średnicka-Tober et al., 2016, Barański et al., 2014). Therefore, a food product produced organically is not necessarily indicative of it being safer. Consumers are often not aware that the organic standards are only based on production and processing practices and not on the final quality or safety of the product (Brennan et al., 2003). There are no stricter food safety standards for organic foods; organic foods are required to meet the same food safety standards as nonorganic foods. Food safety hazards associated with organic meats remain unclear because the results of studies comparing conventionally and organically produced meat are often
contradictory. With increasing popularity in consumption of organic, free-range, and natural meat, it is becoming more urgent to address the associated impacts on food safety and to further evaluate if the consumer perception of organic meat being safer than conventionally produced meat is warranted. In addition, if there are particular food safety hazards more closely associated with organic food production and/or processing practices, these need to be identified (Van Loo et al., 2011).

Part 2: Food Safety of Organic Cattle and Feed from an Integrated Food Production System

Though several researchers indicate the many benefits associated with the production and consumption of organic food like restricted use of antibiotics and synthetic chemicals and decreased contact to antibiotic resistant pathogens, consumers must be mindful of food safety whether it is organic or conventional food. Most often, food safety would be a concern in organic production systems if preventative practices are not employed. From previous research, we can conclude that organic food production systems are not very different from conventional food production systems in relation to microbial safety risks associated to them. With integrated crop-livestock production systems being fairly new to organic agriculture, we developed and used our model to check the food safety status of crops and livestock produced in an integrated organic food production system.

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CHAPTER 4: INFLUENCE OF UV TREATMENT ON THE FOOD SAFETY STATUS OF A MODEL AQUAPONIC SYSTEM

Abstract

Aquaponics is a growing trend in food production as it is seen as a sustainable, space- and energy-efficient approach for production of fruits, vegetables and seafood. Within aquaponics, few microbial studies have been conducted to determine the food safety status of its units. The aim of this study was to determine the food safety status and the effectiveness of ultraviolet treatment (15 watt UV light, luminous flux of 900 lumens) as a food safety intervention in reducing the microbial loads of the water system, in a model aquaponic unit that is growing lettuce, basil and barramundi (Australian Sea Bass). Large Leaf basil, Buttercrunch Bibb lettuce, water and fish swab samples were collected throughout the 118-day production period and microbial analysis was conducted for the presence of E. coli O157:H7, Aeromonas and Salmonella spp. and the prevalence of aerobic plate counts (APC), coliforms, and fecal coliforms in the systems in triplicates. Absence of foodborne pathogens was confirmed using ELISA technology (3M™ Tecra, Australia) and enumeration through petrifilms (coliform/E. coli Petrifilm™, 3M, St. Paul, MN) and agar (Aeromonas agar, OXOID, Hants, United Kingdom). A significant increase was observed in aerobic plate counts over the trial period (1 to 3 log CFU/mL), in the presence and absence of UV (p>0.05). Ultraviolet treatment did not significantly reduce the APC, Aeromonas or coliform counts when compared to the control systems samples. Though the UV intervention method was not effective in reducing microbial loads, future work should focus on improving the unit design, evaluation of bio-solid
filtration and other food safety interventions that can be effective in the presence of living
system while maintaining fish homeostatic environment.

Introduction

In 2015, 163,675 growers and farmers were reported to be marketing foods
locally (Economic Research Service, 2015). The local foods movement has encouraged
growers and farmers to diversify their farming practices and find additional market
opportunities to expand their business. An increasing popular method for diversifying a
farm is aquaponics, which is a modified form of hydroponics utilizing aquaculture.
Aquaponics is an environmentally friendly agricultural practice that involves the
cultivation of crops in a non-soil media (known as hydroponics) by feeding the plants
with nutrient-rich water from intensively cultured aquatic organisms such as fish. There
are many benefits to aquaponic crop production when compared to conventional soil
culture such as accelerated plant growth (Khater and Ali, 2015), decreased production
area requirements (Palm et al., 2015), reduced water usage (Khater and Ali, 2015),
reduced environmental effluents (Khater and Ali, 2015), reduced system production costs
(Khater and Ali, 2015), extended production season (Timmons and Ebeling, 2007),
reduced soil-borne plant pathogens (Timmons and Ebeling, 2007), and diversification of
farm products (Palm et al., 2014b).

Between 1998 and 2008, 46% of all foodborne illnesses reported were associated
with fruits, vegetables, and nuts (Painter et al., 2013). Food safety is an increasingly
important concern in the food supply globally, and very few food safety interventions
within an aquaponics system are known. A main food safety concern with aquaponics is
the cultivation of fruit and vegetable crops in water containing fish excreta and other
organic matter including fish and plant particulate residuals. *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* are the main foodborne pathogens that can be within the recirculating water system and have been shown to survive in these harsh conditions (Nesse et al., 2005, Pal and Dasgupta, 1992). Additionally fish from non-reliable sources can introduce foodborne viruses and disease (e.g. *Vibrio* spp.) that commonly are not associated with fruits and vegetables (Fox et al., 2012).

Food safety concerns related to aquaponics have emphasized the need for more research in food safety interventions such as UV-treatment (Pantanella et al., 2010), ozonation (Kim et al., 2003) and organic acids (Sirsat et al., 2013). The usage of ultraviolet light (UV-C) treatment in recirculating aquaculture has been suggested to reduce pathogen loads (Guerrero-Beltr and Barbosa, 2004) in the water column, without adding any chemicals into the water, thus maintaining fish health and decreasing the need for water exchange (Timmons and Ebeling, 2007). Research with lettuce and UV treatment at 300-500 W s m$^{-2}$ showed total coliforms counts well below 1 CFU ml$^{-1}$ and a reduction in microbial loads higher than 99% with no significant difference in the productive traits of lettuce (Pantanella et al., 2010). In 1985, UV irradiation has shown to inactivate bacteria *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Bacillus subtilis* spores, the enteric viruses poliovirus type 1 and simian rotavirus SA11, the cysts of the protozoan *Acanthamoeba castellanii*, as well as for total coliforms and standard plate count microorganisms in effluent waste water at different intensities (Chang et al., 1985). This suggests that use of UV treatment in aquaponics is a valid method to produce vegetables in water with high hygienic standards. The purpose of this study was to determine the current food safety
status in an aquaponic system and how effective UV treatment would be as a food safety intervention.

**Materials and Methods**

**Aquaponics Unit Design**

The aquaponics units (6 total) were built to be in the same ratio of crop, water, and fish as a commercial unit. The experiment was conducted in triplicates (3 UV units and 3 control systems). Each system consisted of a fish culture tank, solids filtration, biological filtration, deep water hydroponic culture unit, ultraviolet sterilizer, submersible magnetic drive centrifugal pump, and a diaphragm style aerator with 8, 6-inch air stones per system (Figures A1, A2, and A3). The fish tank and solids/biofiltration tanks are 114 L (30 gal) high density polyethylene (HDPE) cone bottom tanks measuring 27 inches deep to the cone and 38 inches deep overall and 18 inches diameter with a 45 degree conical bottom. The 760 L (200 gal) deep water hydroponic units consist of a metal constructed frame measuring 1.2 m (4 ft) wide by 2.4 m (8 ft) long by 0.3 m (1 ft) deep that is insulated with 3.8 cm (1.5 in) thick polystyrene insulation covered reflective bubble wrap called tekfoil, lined with a 12-mil rubber liner. The pumps used are 2,082 LPH (550 GPH) ActiveAqua Pumps operated without pre-filters. Water is pumped through 2.5 cm (1 in) diameter ValuTek, black braded utility hose into the UV sterilizers. The UV sterilizers are TMC Vecton brand with a 15 watt light (luminous flux of 900 lumens/ 432.6 W s m⁻²) output and are rated for a 20.8 LPM (5.5 GPM) flow rate. It was recommended by the manufacturer based on flow rate, turn over and size of the water system. Our flow rate of 20.8 LPM falls under the 33 LPM maximum recommend for the Vecton 15 watt with a turnover that is within 1.5 times per hour. The UV system was
located after the hydroponic unit so that UV would act on water after sufficient nutrients were taken up by the plants. Water flow rate into the fish tank was adjusted with a PVC ball valve. Directional water inflow into the fish tank was created using a 50 cm (20 in) section of 2.5 cm (1 in) diameter PVC pipe that is capped on the end. The water flows out of 15, 6.4 mm (0.25 in) diameter holes drilled along a single plane to create a counter clockwise flow. A dual standpipe was created with 81 cm (32 in) section of 3.8 cm (1.5 in) diameter PVC pipe as a stand pipe and an 84 cm (33 in) external standpipe with holes cut in the bottom of it surrounding the standpipe. Two air stones provide aeration and gas exchange in the fish culture tank. The water flows by gravity into the mechanical filter screen, which consists of 80% cover shade cloth that is 4 layers thick, then through an additional solids filter pad. The biofilter is located directly below the mechanical filter screens and was filled with bio-balls, bio-barrels, and blocks of filter pad to provide adequate surface area to harbor nitrogen-processing bacteria. The water depth in the biofilter is 51 cm (20 in) and is constructed in the same manner as the fish tank. Two air stones are located in the biofilter tank to provide mixing and aeration. The water then flows by gravity to the far end of the deep water hydroponic unit through a 3.8 cm (1.5 in) PVC pipe with a tee at the end to then be exposed to the plants. Four air stones are located in each hydroponic unit. The water slowly flows slowly back to the opposite end of the hydroponic unit into the pump that then completes the circuit. We performed a dye test before the study began to ensure uniform water circulation in the system. To establish the biological filtration system, four bio-barrels were added to each of the six replicated systems from a pre-established system to enhance their biological filtration performance.
After a period of 4 weeks, water chemistry testing indicated that nitrifying bacteria populations had established in each system and it was safe to add the fish.

**Fish and experimental design**

Juvenile barramundi (*Lates calcarifer*) were obtained from a local aquaculture nursery for this study. Upon arrival, the fish were acclimated to laboratory conditions for 18 days prior to the first sampling date and were fed with a Ziegler brand Finfish G 42-16 floating diet measuring 2.5 mm diameter, containing a 42 % protein, 16% lipid diet. The experiment was conducted during the winter, from November 2014 to January 2015. At the beginning of the experiment, 10 fish with an average weight of about 120-165 g were stocked in each of 6, HDPE, 114 L (30 gal) tanks. Each experimental treatment (UV treatment and control) was conducted in triplicate (2 experimental treatments × 3 tanks). The fish were fed twice daily at 8:00 and 18:00 over the 118 day experiment. Daily feeding rate was about 3% of total body weight or until the fish showed signs of satiation. Excess feed was removed to prevent water quality degradation. A daily record was kept of feed offered. A photoperiod of 16 h light (06:00–22:00 h) and 8 h dark was provided using the 400 W, high pressure sodium grow lights used for plant growth. Water temperature, dissolved oxygen and pH were monitored daily using an HQ0d water quality probe (HACH). Water chemistry parameters were measured either once (alkalinity, hardness, carbon dioxide, chloride, iron) or twice (ammonia, nitrite, nitrate) weekly. The standards for each of these parameters were as follows: pH 6.5-7, dissolved oxygen above 10 mg/L, ammonia below 1.0 ppm, nitrite below 1.0 ppm, chloride below 500 ppm, carbon dioxide below 5 ppm, water hardness between 100-300 ppm, and alkalinity between 40-300 ppm. If the water chemistry parameters fell outside of these
recommendations then a mitigation step was followed according to the parameter out of the safe zone. The mean water quality parameters were recorded as follows: temperature 23.2 ± 5.2°C, dissolved oxygen 8.1 ± 1.0 mg/L, pH 7.7 ± 1.0, ammonia 0.5 ± 0.2 ppm, nitrite 0.33 ± 0.33 ppm, chloride 250 ± 100 ppm, carbon dioxide 0.25 ± 0.2 ppm, water hardness 200 ± 100 ppm, and alkalinity 104 ± 4 ppm.

**Crops and experimental design**

Italian Large Leaf basil and Buttercrunch Bibb lettuce were used for this study because of their local marketability and value. Pelleted seeds were obtained from Johnny’s Selected Seeds (Winslow, Maine, USA). One pelleted seed of each species was germinated in 3.8 cm x 3.8 cm (1.5 in x 1.5 in) rockwool starter plugs (Grodan A-OK, Farmtek, Dyersville, IA, USA) in numbers sufficient to supply the floating rafts (8 rafts per system) on a weekly basis for the duration of the study. The 8 floating rafts were 60 cm x 60 cm x 3.8 cm (2 ft x 2 ft x 1.5 in) and had either 9 (4 rafts) or 16 (4 rafts) holes with 20 cm (8 in) or 15 cm (6 in) spacing for lettuce or basil, respectively. The plants were germinated for 14 days, then transplanted into their appropriate rafts and inserted into the system at the distal end of the influent water from the biological filter (Figure A1). Each week a new cohort of plants were germinated and the next set of seedlings were transplanted into the system and the older plants were moved one space closer to the influent end of the floating raft hydroponic unit. Finally, after four weeks (28 days) of growing, the plants and roots were harvested from the system. This weekly cycle continued for the duration of this 118 day study based on a normal growth cycle for the barramundi fish.
Microbiological analysis

Two heads of lettuce, or two bunches of basil, and 1 liter of water was collected randomly from each of the six systems per sampling period. For lettuce, a random sample of 10 grams was taken and added to 90ml of 1% peptone (HiMedia, Mumbai, India) into a sterile stomacher bag. For basil, a random sample of 5 grams was taken and added to 45ml of 1% peptone and added to a sterile stomacher bag. For water, a random 10ml sub sample was added to 90ml of 1% peptone and added to a sterile stomacher bag. For the fish samples, swabs (Biomerieux, Marcy-l’Etoile, France) were taken on both sides of the body surface including gills and alimentary canals using a 10*5 cm sterile template and added to 10 ml of 1% peptone test tube. Individual samples were homogenized either in a stomacher or vortex and enumerated using coliform/E. coli Petrifilm™ (3M, St. Paul, MN). Duplicate samples were used in this experiment. Coliform and E. coli levels were enumerated using 3M Petrifilm E. coli/ Coliform Count Plate TM (3M Microbiology Products, Minneapolis, Minnesota), following label directions (detection limit of <10 CFU/g or <1 CFU/ml or <0.1 CFU/cm²). Plates were incubated at 35°C and observed for changes at 24 and 48 h. Interpretation of the Petrifilm followed E. coli/Coliform Petrifilm label directions and AOAC Official Method 991.14. Blue to red-blue colonies associated with gas were counted as E. coli coliform colonies. Red colonies associated with gas were counted as coliform colonies. Further analysis was conducted on the samples for presence of E. coli O157:H7 and Salmonella spp. using ELISA (color change assay) system (3M™ Tecra, Australia) and 0157 latex agglutination for confirmation (Oxoid/Remel, Hants, United Kingdom), as per manufacturer’s instructions. Samples were processed through a series of enrichment and selection
methods prior to the ELISA (detection limit: 1-5 cells/25g of sample) test to reduce the presence of false positive samples. 25 g of lettuce and basil samples and 25 ml of water and fish swab samples were added to 225 ml of EC Broth (3M™ Tecra, Minneapolis, Minnesota) with 5% novobiocin supplement (MP, Salon, Ohio) and incubated at 42 ± 1°C for 15-24 h. This enrichment was used for ELISA analysis (E. coli 0157 detection). Same quantities of samples were incubated in 225 ml of Universal Pre-enrichment Broth (DIFCO, Sparks, Maryland) at 36°C for 24 h. Following incubation, 0.5 ml of sample was transferred into 10 ml TT broth (Hajna) broth (DIFCO, Sparks, Maryland) and 0.1 ml into 10 ml RV broth (DIFCO, Detroit, Michigan) and incubated at 36 ± 0.5°C for 22-24 h. Following incubation, 1ml of each were transferred to 10 ml of M Broth (HiMedia, Mumbai, India) and incubated at 36 ± 0.5°C for 22-24 h. This enrichment was used for ELISA analysis (Salmonella detection).

These rapid detection kits are approved by the U.S. Food and Drug Administration (FDA) for use on food samples. Aerobic plate counts were obtained in duplicates for each of the six systems, at suitable dilutions of BPW enrichment, incubated at 36°C for 48 h, using media made from Total Plate Count Agar (HiMedia, Mumbai, India). Aeromonas was also enumerated at suitable dilutions of BPW enrichment on Aeromonas agar (OXOID, Hants, United Kingdom) plates.

**Statistical analysis**

This study was conducted between November 2014 and February 2015 and experiments were conducted in triplicates (3 UV and 3 No UV (control)). Statistical analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, NC). Microbial counts were obtained for basil, lettuce and water samples on day 0, 28, 42, 54, 63, 76, 88, 102
and 118 in duplicate for each of the six UV/control systems, and data were analyzed using the least square means method. Direct swabs of fish and microbial counts from 5 different fish were performed in duplicates. The effects of day and treatment were studied for aerobic plate counts and coliform counts. Combinatorial effects of day and treatment was also studied. All statistical analyses were conducted at 95% level of confidence (p<0.05).

Results

Pathogenic Microbial Status of the basil, lettuce, and water. There were no detectable levels of *E. coli* coliforms, *E. coli* O157:H7 or *Salmonella* spp. found in any of the lettuce, basil, or water samples over the 118 study period.

Aerobic Plate Counts of the basil, lettuce, and water. Table A1 displays the aerobic plate counts in the basil, lettuce, and water samples over the 118 day study. There is a general trend of increasing aerobic plate counts (1 to 3 log CFU/mL) from day 0 to day 63 and a decrease in aerobic plate counts (1 to 3 log CFU/mL) from day 63 to day 118 of the trial for the basil, lettuce, and water samples. There were no environmental changes between day 0 and day 63 samples, as determined by water temperatures and chemistry (ammonia, nitrite, nitrate, alkalinity, pH or dissolved oxygen levels, data not shown), therefore these difference can be attributed to normal environmental flora variations.

There was no significant difference between the UV and No UV units for aerobic counts with the basil and water samples throughout the study (p>0.05). When observing the aerobic plate count in lettuce samples, there was a significant difference observed between the UV and No UV treatments during the 118 day study (p<0.05; Table A1).
Specificially, UV treatment had a significantly higher aerobic count (0.24 log CFU/g) for day 63 than No UV treatment for day 63. When the No UV treatment systems were evaluated alone, day 63 showed a significantly higher in aerobic count (0.65-3.30 log CFU/g) than other days (0, 28, 42, 54, 76, 88, 102, or 118) and day 76 has a significantly higher aerobic count (1.74-2.65 log CFU/g) than days 42 and 118. When the UV treatment systems were evaluated alone, day 63 has a significantly higher aerobic count (1.09-2.83 log CFU/g) than other days (0, 28, 42, 54, 76, 88, 102, or 118).

When the UV and No UV treatments are combined (Table A2), the lettuce samples had a significant increase in aerobic plate counts (0.55-3.01 log CFU/g and 1.25-2.05 log CFU/g, respectively) between days 54 and 76. There are no significant changes in the water quality for the basil or water samples when the UV and No UV treatments are combined throughout the 118 days (data not shown).

Coliform Counts of the basil, lettuce, and water. There was no significant difference between the UV and No UV units in general for coliform counts in general (lettuce, basil and water samples) (p>0.05). Table A3 displays the coliform counts for the basil, lettuce, and water samples over the 118 day study. There was a significant increase in coliform counts (0.61-2.12 log CFU/g) observed in all the samples (basil, lettuce, and water) on day 28 of the trial when compared to all the other days. There was a significant decrease (0.24-1.87 log CFU/g) in the coliform counts in all the samples on day 76 when compared with the day 28, 42, and 54 (p<0.05), which had a significant increase in coliform counts (0.50-1.78 log CFU/g) on day 88 for all the samples. With the water sample, there was a significant decrease in coliform counts (1.13-1.67 log CFU/g) between day 88 and 118 of the trial in the presence and absence of UV treatment. It
must be noted that there were no significant temperature or environmental changes on day 76 during the study, therefore these difference can be attributed to normal environmental flora variations.

**Aeromonas Counts of water.** There was a significant difference (p<0.05) in *Aeromonas* count in water samples, over the culture period, in the presence and absence of UV. However, there was no significant change based on the type of treatment used (Table A4). Day 63 showed a decrease in the *Aeromonas* count in both treatments. However, this was followed by an increase in the counts.

**Microbial Status of fish.** There were no *E.coli* coliforms, *E.coli* O157H7 or *Salmonella* spp. found in any of fish samples over the 118 study period. Table A1 and A3 displays the aerobic plate counts and coliform counts for the barramundi fish on days 0 and 118 of the trial. There was a significant increase of aerobic counts (0.65 log CFU/g) in the fish sample with the presence and absence of UV on day 0 and day 118 (p>0.05). There was no significant increase or decrease in the coliform counts on day 0 and day 118 in the presence or absence of UV treatments.

**Discussion**

Effective usage of a UV sterilizer has been suggested to reduce the abundance of many bacterial pathogens suspended in water in aquaponic operations, and thus reduce the probability of cross contamination between water and plant tissue (Bintis et al., 2000, Friedberg et al., 2005, Guerrero-Beltr and Barbosa, 2004, Moeller et al., 2010). Our results show high variation in the aerobic plate counts and coliform counts and that UV was not effective at reducing both aerobic and coliform counts on the lettuce, basil, water and fish samples when compared to the control system. Further, there was a general
trend of microbial increase within the study period followed by a decrease after 76 days (Table A2 and A3). We attribute the fluctuation pattern within both the hydroponic and aquaponic units to normal microbial community changes. The inconsistent patterns and variability between and amongst treatments observed in the microbial counts is likely due to the dynamic ecosystem interactions that occur in a living system like aquaponics. The aquaponics unit is a living system in which the biosolids and rich microbial community is critical to producing the ideal growing conditions for both crops and fish. If these biosolids and microbial community are disrupted, it can result in poor growth rates and lack of nutrients for crops and fish. Additionally, a recirculating aquaculture systems water management is critical to ensure the health of fish and/or crops is continuous. To maintain that homogeneity there requires a greater understanding of all life support processes that make up the biological filtration systems. Schreier, Mirzoyan and Saito (2010) explain that the biological filtration systems rely on the interaction of microbial communities with each other and their environment as a consequence of nutrient input (fish waste output) and, as such, are not easily controlled.

Since this microbial community is rich with different microorganisms, if zoonotic pathogens were introduced into the system, the risk for foodborne illness from the fish and/or food crop is higher (Hollyer et al., 2009). There have been multiple foodborne outbreaks with *E. coli* and *Salmonella* associated with fruits and vegetables that have been attributed to water sources (CDC, 2015). If the water source is contaminated with one of these zoonotic pathogens, then the entire system and the biofiltration system can continuously contaminate the food crops and fish (Nesse et al., 2005, Pal and Dasgupta, 1992). Within our study, we did not observe any foodborne bacteria (*E.coli* O157:H7
and *Salmonella* spp.), or fecal coliforms over the trial period. Water testing is critical to ensure the system is pathogen free.

Aerobic plate counts are typically utilized within the food industry as an indicator for shelf life and for sanitation practices. Aerobic plate counts above $10^7$ CFU/gram are seen as unacceptable for fish and produce (ICMSF, 1986). Fecal coliforms (*E.coli*) above 500 CFU/gram in fish and produce are also seen as unacceptable (ICMSF, 1986). Fecal coliforms are an indicator of poor water supply and poor sanitation practices (Varga and Anderson, 1968). Within this study, our counts remained below the APC, coliform and fecal coliform limit, indicating our system had good sanitation conditions and the food is safe for consumption, but the high variability in the microbial counts requires additional research to solidify the theory of normalization in the system. At present there are no standards for *Aeromonas* counts in food, as its occurrence is not common. However, with the introduction of aquaponics where food systems come in contact with fish pathogens, it is important to establish limits for their presence following adequate research. Morgan et al. (1985) showed that *A. hydrophila* induced moderate diarrhea in only 2 out of 57 human volunteers at high levels of $10^7$ - $10^9$ CFU when administered orally in a double blind study. Throughout our study, *Aeromonas* counts remained well below this infection causing level.

So why was our treatment not effective in our system? Even with prior knowledge of the need for a rich microbial community for aquaponics to be successful from a plant and fish perceptive, the presence of particles within the system can inhibit the penetration of UV into the system water, thus reducing its effectiveness (Petrea et al., 2013, Stermer et al., 1987). Timmons and Ebeling (2007) suggest that the water should
be filtered through a 50 micrometer (um) screen prior to exposure to UV irradiation to improve UV efficacy in a recirculating aquaculture system (Timmons and Ebeling, 2007). Within this model system there was no filtration system at 50 μm, but one to collect the larger biosolids from the unit through our mesh system. Enhanced filtration could help with the effectiveness of the UV technology. Another method for improving effectiveness of the UV irradiation treatment is through an increase in the intensity of the rays even in the presence of high biosolids. UV systems as high as 36 watt light output have been used in a recirculating aquaculture system (Petrea et al., 2013). Our unit was only at 15 watt light output based on manufacturer’s recommendation for a flow rate of 20.8 LPM and stability of nutrients in the system. Coagulating agents can also be used to collect the suspended particles which can be periodically removed. Chemical precipitation using lime, alum, or ferric chloride is the method most commonly used by municipalities which can be extended to aquaponics if modified for living systems (Adler et al., 2000). This method could clarify the circulating water allowing for deeper penetration of UV rays, but these substances would need to be monitored to ensure the change in pH would not affect the plants or fish units through additional research.

The results of this study found that the UV treatment used in our model aquaponic unit was not effective in reducing coliform and aerobic plate counts. However, clarifying the water or reducing the flow rate might improve the penetration of UV or increasing the intensity of the radiation may control microbial populations to a greater extent. Future studies can be conducted using this method alone or in combination with other food safety interventions such as ozone and organic acids. Overall, given the many benefits associated with aquaponic food production systems, determining a stable system that
produces safe food would be a great asset in increasing economic, social, and environmental sustainability. Further studies in similar area of research are encouraged.

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References


CHAPTER 5: EVALUATING THE FOOD SAFETY STATUS OF ORGANIC FEED AND LIVESTOCK IN AN INTEGRATED AGRICULTURAL SYSTEM

Abstract

Though several researchers have indicated the many benefits associated with the production and consumption of organic food such as restricted use of antibiotics and synthetic chemicals; it must be kept in mind that these benefits do not address the issue of microbial safety. With integrated crop-livestock production systems being relatively new practice in organic agriculture, the aim of this study was to develop and use a model agricultural system to check the food safety status of crops and beef and dairy calves (6-10 months old) produced in an integrated environment in Minnesota (MN), Iowa (IA), and Pennsylvania (PA). Pasture and fecal samples were collected 3 months apart and evaluated for presence of *E. coli* 0157:H7 and *Salmonella* using miniVIDAS and confirmation tests were performed according to FDA BAM and USDA standards. Results indicated very low probability for (0.0173-IA, 0.0032-MN, 0.0039-PA) *E. coli* 0157:H7 and (0.0077-IA, 0.0027-MN, 0.0022-PA) *Salmonella* occurrence (overall Pr<0.1). The three states were studied individually for occurrence of *E. coli* 0157:H7 or *Salmonella*. The probabilities of occurrence were again very low (0.0048-IA, 0.0003-MN, 0.0009-PA). Also, there was no significant difference between the three research sites (p>0.05) in terms of *E.coli* O157:H7 or *Salmonella* occurrence. At present, this model has low chance of *E.coli* O157:H7 and *Salmonella* being present in the feed and fecal matter, but long term studies including evaluation of meat products and rotational crops might help us better understand the stability of this system.
Introduction

Driven by consumer demand, the U.S. organic food industry has grown substantially in popularity. According to USDA-ERS (2014), organic food purchases were estimated to be more than $35 billion in 2014 alone. Several factors have led to this increased consumption of organic foods in the U.S., including consumer preference for lower pesticide residues (Baker et al., 2002), nutrition and health concerns (Williams, 2002, Magkos et al., 2003), negative environmental impacts associated with intensive conventional production (Venterea and Rolston, 2000), and the assurance of organic integrity through consistent federal organic standards (USDA-AMS, 2014). Farmers also are interested in producing organic crops that meet the “triple bottom line” of environmental sustainability, economic viability, and social equity.

In recent years, organic farmers have become increasingly concerned about farm product/food safety, particularly important for farmers practicing integrated crop/livestock production (Pereira et al., 2013). Studies comparing organic and conventionally raised livestock and pasture crops have found, in general, no significant food safety differences between conventional and organic systems (Bourn and Prescott, 2002, Maffei et al., 2013, Oliveira et al., 2010, Blanco-Penedo et al., 2012). In a livestock comparison in Spain, there were no food safety differences in organic or conventional beef cattle, but organic beef was reported to have higher quality (Blanco-Penedo et al., 2009).

Another concern is the presence of pathogenic bacteria, such as Salmonella and toxin-producing Escherichia coli in forage crops and silages (Pauly, 1999). Feed has been reported as a vehicle for transmission of Salmonella enterica in cattle (Glickman et al.,
1981, Jones et al., 1982, Anderson et al., 1997, Krytenburg et al., 1998, Lindqvist et al.,
1999, Hinton, 2000, Kidd et al., 2002), and several lines of evidence suggest that feed can
be a vehicle for transmitting *E. coli* O157:H7 (O157) as well (Hancock, Rice et al., 1997,
Hancock et al., 2001, Lynn et al., 1998). Because food-producing animals are the primary
source of O157 and pathogenic *Salmonella* infections in humans, it follows that bacterial
contamination of animal feed may contribute to the burden of foodborne illness (Crump
et al., 2002). Pathogenic strains of *E. coli* can cause severe illness in humans and
animals, and the toxin-producing organism *E. coli* O157:H7 is of special concern; if
conditions in silage are favorable for growth of this bacterium, it may cause intestinal
disorders and mastitis in animals that consume the silage (Lindgren, 1991). Cattle are a
primary source of pathogenic *E. coli* O157:H7 (Bach et al., 2002), but this organism may
be transmitted to crops and their products via shedding or through fertilization of fields
with manure (Russell et al., 2000).

The present study has been designed to facilitate the development of organic
agriculture production methods that emphasize a whole-systems approach by integrating
crops and livestock, and evaluate the food safety status to analyze microbial
contaminants. This is a 3-year study including a rotation from pasture to small grains (rye
and wheat) and to row crop production (corn and soybeans). This experiment represents
the first phase of the rotation when calves were on pasture. Fecal samples were studied
from dairy and beef calves (6-10 months old) from Iowa, Minnesota and Pennsylvania, at
the beginning and end of a three month interval (summer-fall 2015).
Materials and Methods

Experimental Design and Field Operations

Research began in fall of 2014 on University Experiment Station sites in Minnesota (MN) and Iowa (IA). Because of the complexity of experimental design and the large land commitment that would be required for an organic farmer, we utilized the Rodale Institute’s living farm (PA), which mimics a typical organic farm of the region, for our on-farm site. An integrated pasture-livestock design was established at each site, based on local farmer input and compliance with certified organic rules. The 3-year experiment included a rotation from pasture to small grains (rye and wheat) grazing to row crop production (corn and soybeans). This experiment represents the first phase of the rotation when calves were on pasture.

For the animal component, dairy beef steers were accessed from the Morris, MN, station and the Rodale Farm, while the Iowa Experiment Station utilized beef cattle steers from the Erlandam Farm, Greenfield, Iowa. All the calves, at all three sites were 6-7 months old in August, 2015 when the study began and 9-10 months old by November, 2015. Iowa, Minnesota and Pennsylvania had 8, 12 and 11 calves each which were reflective of their treatment herd sizes.

Following certified organic rules, cattle were provided access to 100% organic pasture. Minerals were fed free choice. Organic feed were provided if insufficient pasture conditions necessitated additional feeding. Cattle remained in their treatment group during the study with no comingling with other cows.
Forage and Fecal Samples

Feed samples from the three experimental sites were tested for microbial quality and all three sites had insufficient forage development, therefore additional organic feed was provided to all the calves in the study. Calves at Iowa Experiment Station were on an organic pasture, hay and corn grain diet throughout the study period. Calves at Minnesota station were on an organic pasture diet alone. Calves at Pennsylvania station were fed organic forage, hay, kelp, nutri-balancer and salt. Two of the calves at Pennsylvania station fell ill during the study, therefore additional nutrient supplementation (nutri-balancer) was provided to aid in their recovery. First batch of Iowa and Minnesota samples were collected on 19 August, 2015 and Pennsylvania samples were collected on 24 August, 2015. Second batch of Iowa samples were collected on 2 November, 2015, Minnesota samples were collected on 10 November, 2015 and Pennsylvania samples were collected on 17 November, 2015.

Each feed was sampled randomly to ensure uniformity. Specifically, a 100 g sample was taken randomly from daily feed distribution. Fecal grab samples were collected following methods in Narvaez-Bravo et al. (2013). Rectal palpation was used to obtain approximately 100 g of fecal samples for microbial analysis. All samples were stored on ice in an insulated cooler until completion of sampling and transported to the Iowa State University Food Microbiology Lab (Ames, Iowa) where the samples were temporarily stored at 4° C until analysis. Samples were processed within 48 hours of collection.
Microbial Analysis

Sub-samples (25 g) were randomly taken from the shipped feed and livestock feces for microbial analysis. A selective enrichment protocol for *Salmonella* and *E. coli* O157 detection in feed and fecal samples was followed which were then subjected to miniVIDAS *Salmonella/E. coli* O157 (Biomerieux, Marcy-l’Etoile, France), a rapid PCR detection method used to detect the presence or absence of these harmful bacteria. If a sample was positive for either of the tests, further confirmation was performed according to FDA BAM and USDA standards (Barkocy-Gallagher et al., 2002, USDA, 2014).

For identifying the presence of *Salmonella*, 25 g of feed or fecal sample was aseptically transferred into a stomacher bag containing 225 mL of 2% buffered peptone water or BPW (HiMedia, Mumbai, India) with 5% *Salmonella* supplement (Biomerieux, Marcy-l’Etoile, France) and incubated at 41.5°C for 18-24 h. Post incubation, 1 mL of sample was transferred into 10 mL of premade SX2 broth (Biomerieux, Marcy-l’Etoile, France) and incubated at 41.5°C for 6-24 h.

Post incubation, 0.5 mL of enrichment was transferred into the VIDAS test strip-SPT (Biomerieux, Marcy-l’Etoile, France) and was run on the VIDAS machine along with controls (positive and negative) as instructed by the manufacturer (miniVIDAS, Bimerieux, Marcy-l’Etoile, France). Results were qualified as positive or negative which were further isolated for confirmatory tests. The 50% detection limit for *Salmonella* using this method is between 0.3 to 1.3 cells/ 25 of sample.

For performing the confirmatory tests (USDA, 2014), 25 g of the positive samples were aseptically transferred into a stomacher bag containing 75 ml of mTSB broth (OXOID, Hants, United Kingdom) with 5% novobiocin supplement (MP, Salon, Ohio).
and incubated at 42 ± 1°C for 15-24 h. Following incubation, 0.5 ml of sample was transferred into 10 ml TT broth (Hajna) broth (DIFCO, Sparks, Maryland) and 0.1 ml into 10 ml RV broth (DIFCO, Detroit, Michigan) and incubated at 42 ± 0.5°C for 22-24 h.

Contents of tubes were mixed after incubation and streaked onto brilliant green sulfa agar or BGS (DIFCO, Sparks, Maryland) and Xylose lysine tergitol™ 4 agar or XLT4 (DIFCO, Sparks, Maryland) agar plates using a 10 μl loopful of inoculum for each plate and incubated at 35 ± 2°C for 18-24 h. Well-isolated Salmonella colonies were picked from BGS and XLT4 plates based on manufacturer instructions (DIFCO, Sparks, Maryland). Triple sugar iron agar or TSI (DIFCO, Sparks, Maryland) and lysine iron agar or LIA (OXOID, Hampshire, England) slants were inoculated in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation. Screw cap tubes were loosened and incubated at 35 ± 2°C for 24 ± 2 h. TSI and LIA slants were examined as a set and analyzed for positives based on manufacturer instructions (DIFCO, OXOID).

For identifying the presence of E.coli 0157:H7, 25 g of feed or fecal sample was aseptically transferred into a stomacher bag containing 225 mL of 2% buffered peptone water or BPW (HiMedia, Mumbai, India) with vancomycin (Biomerieux, Marcy-l’Etoile, France), cefixime (AK Scientific, Union City, California) and cefsulodin (MP, Salon, Ohio) supplements and incubated at 41.5°C for 18-24 h.

Post incubation, 0.5 mL of enrichment was transferred into the VIDAS test strip-ECPT (Biomerieux, Marcy-l’Etoile, France) and was run on the VIDAS machine along with controls (positive and negative) as instructed by the manufacturer (miniVIDAS,
Bimerieux, Marcy-l’Etoile, France). Results were qualified as positive or negative which were further isolated for confirmatory tests (Barkocy-Gallagher et al., 2002). The 50% detection limit for *E. coli* 0157:H7 using this method is between 0.2 to 1.6 cells/25 of sample.

Sorbitol-MacConkey (SMAC) agar is the medium of choice for isolation of *E. coli* O157:H7 for performing confirmatory tests (Barkocy-Gallagher et al., 2002, Reynnells et al., 2014). To isolate *E. coli* O157:H7 on SMAC, 25 g of sample was taken in a sterile stomacher bag containing 225 ml of EC broth (3M) containing 5% novobiocin supplement (MP, Salon, Ohio) and incubated at 42 ± 1°C for 15-24 h. Following incubation, the sample was inoculated onto CT (Cefixime-tellurite)-SMAC agar (DIFCO, Sparks, Maryland) containing 5% CT mixture (OXOID, Hampshire, England), and incubated for 18-24 hours at 35-37°C. Sorbitol-negative colonies were selected from SMAC with latex reagents (O157 antibody-coated latex and control latex) according to the procedures recommended by the manufacturer (Dry Spots, OXOID, Hants, United Kingdom). Specimens from which sorbitol-negative colonies were isolated, that agglutinates in O157 latex reagent, and was biochemically *E. coli*, was reported as presumptively positive for *E. coli* O157:H7.

**Statistical Analysis**

Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC). The probability of occurrence of *E. coli* 0157:H7 and *Salmonella* spp. in the feces of calves from Iowa, Minnesota and Pennsylvania were studied individually. The probability of occurrence of either one of the pathogens in the feces of calves was studied in each state individually. These probabilities were obtained using least square mean
analysis. Tukey-Kramer analysis (unequal sample size) was performed on fecal samples from the three states, at 95% level of confidence (p<0.05) to identify if there were any significant differences between the states in terms of *E. coli* 0157:H7 or *Salmonella* occurrence.

**Results and Discussion**

In this model, we developed and studied an integrated-organic food production system to check the microbial safety status of pasture and livestock feces produced from beef and dairy calves which were 6-7 (August, 2015) and 9-10 months old (November, 2015). The probability of occurrence of *E. coli* 0157:H7 or *Salmonella* in the fecal samples collected from the calves in all three states were very low, (0.0173-IA, 0.0032-MN, 0.0039-PA) *E. coli* 0157:H7 and (0.0077-IA, 0.0027-MN, 0.0022-PA) *Salmonella* during trial period (overall Pr<0.1) (Table B1). Among the three states, there was a lower probability of *Salmonella* occurrence than *E. coli* 0157:H7 occurrence (Table B1). Also, the overall probability of *E. coli* 0157:H7 or *Salmonella* occurrence is very low for each state individually (0.0048-IA, 0.0003-MN, 0.0009-PA, Table B2).

Dunn et al. (2004) and Cobbold et al. (2001) reported the increased prevalence of STEC O157 during spring and summer seasons in the northern hemisphere in their study. More frequent recovery of STEC O157 were from young cattle (<3.5 months) and the fecal shedding decreased as they matured (Cobbold and Desmarchelier, 2000, Wells et al., 1991, Garber et al., 1995, Rugbjerg et al., 2003, Hancock et al., 1997). The pasture phase of our research was conducted during summer and early winter, similar to the stated researches. Our study was conducted in similar weather and geographical locations, and hence the results can be compared to previous studies (Cobbold and
Desmarchelier, 2000, Wells et al., 1991, Garber et al., 1995, Rugbjerg et al., 2003, Hancock, Besser et al., 1997). We observed lower counts of *E. coli* 0157:H7 in our study when compared to other studies (3.8% in Garber et al., 1995, 1.8 % in Hancock, Besser et al., 1997). Our calves were older (7 to 10 months) than calves from other studies that indicated higher fecal shedding of STEC 0157 (<3.5 months) (Cobbold and Desmarchelier, 2000, Garber et al., 1995). Also, our calves were predominantly pasture fed in comparison to other studies where they were mostly grain fed (Rugbjerg et al., 2003, Hancock, Besser et al., 1997). Differences in age and feed, as indicated by the above mentioned researches lead to differences in microbial load in feces.

Also, our study is similar to a national study of the U.S. dairy cow population where fecal samples were collected from representative cows on 91 dairies and 97 cull dairy cow markets in 19 states. *Salmonella* spp. were recovered from 5.4% of milk cows, 18.1% of milk cows expected to be culled within 7 days, and 14.9% of culled dairy cows at markets. It was found that *Salmonella* fecal shedding was higher during the sampling period from May through July, in herds with higher number of milk cows, and in the Southern region (Wells et al., 2001). Our study samples were collected during the warmer period of August and early winter period of November and our study predominantly has dairy calves (Morris, MN station and Rodale Farm, PA) and hence results can be comparable to the previously mentioned study (Wells et al., 2001). We observed a low probability (Pr< 0.01) of *Salmonella* occurrence in the fecal samples from all three states during August and November, 2015 (Table B1). Fessler et al. (2005) conducted a multi-state study to evaluate associations between herd characteristics and the isolation of *Salmonella* from dairy cows in Minnesota, Wisconsin, Michigan, and New York.
Seasonal associations were present as cows were more likely to be *Salmonella*-positive in summer, spring, and fall compared to winter. Our study was only conducted during summer and winter and hence we may not be able to observe the influence of seasonal changes on the *Salmonella* status of our fecal samples. Additionally, Fossler group found that herd size was not associated with *Salmonella* shedding (Fossler et al. 2005).

Between the three states (Iowa, Minnesota and Pennsylvania), there was no significant difference (p>0.05) in *E. coli* 0157:H7 or *Salmonella* occurrence (Table B3). We choose three distinct geographic locations to see if our results supported research that showed regional difference in prevalence levels of *E. coli* O157:H7 and *Salmonella* in similar seasons. Edrington et al. (2006) conducted a research representing three regions in North America (southern Canada, midwestern United States, and the southern United States/Mexico) and found that a positive correlation existed between day lengths and, to a lesser extent, ambient temperature and *E. coli* O157:H7 prevalence in fecal shedding. Islam et al. (2014) performed a meta-analysis on global data and found different pooled prevalence estimates of *E. coli* O157:H7 in Africa 31.20% (95% CI, 12.35–50.04), Northern America 7.35% (95% CI, 6.44–8.26), Oceania 6.85% (95% CI, 2.41–11.29), Europe 5.15% (95% CI, 4.21–6.09), Asia 4.69% (95% CI, 3.05–6.33) and Latin America-Caribbean 1.65% (95% CI, 0.77–2.53) respectively. Our three sites showed no significant difference (p>0.05) in pathogen occurrence in contrast to the mentioned studies indicating no regional differences.

As organic farmers cannot employ conventional safety interventions like antibiotics and pesticides, relying on crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, mechanical cultivation and mineral-
bearing rocks (maintain soil fertility) can be useful to improve crop productivity and reduce pathogen occurrence (Hill and MacRae, 1991). Grazing systems that reduce the larval or shed load of internal parasites will enhance cattle productivity on organic pastures (Larsen, 2006). Continuously grazed pastures fail to break the life cycle of these parasites, whereas rotational grazed pastures frequently reduce the parasite larval load (Stromberg and Averbeck, 1999). Subsequent use of long term rotation can further reduce the probability of *E. coli* 0157:H7 and *Salmonella* occurrence in the agricultural system (Stromberg and Averbeck, 1999).

Most often, cattle excretions are used as manure for crop growth. This is particularly popular in organic agriculture as manure can be a natural nutrient source (Ongeng et al., 2014). However, the use of contaminated livestock wastes such as manure and manure slurry in crop production is believed to be one of the principal routes of fresh vegetable contamination with *E. coli O157:H7* and *S. enterica* at preharvest stage because both ruminant and non-ruminant livestock are known carriers of *E. coli O157:H7* and *S. enterica* in the environment (Ongeng et al., 2014). If contaminated livestock waste is used as manure in our study, it can increase the probability of *E. coli O157:H7* and *Salmonella* contamination in feed (Ongeng et al., 2014) which is eventually consumed by the grazing livestock in the system. USDA-AMS-NOP has mandated that unless composted, raw manure should be incorporated into the soil not less than 120 days prior to the harvest of a product whose edible portion has direct contact with the soil surface or soil particles; or not less than 90 days prior to the harvest of a product whose edible portion does not have direct contact with the soil surface or soil particles (USDA-AMS-
NOP, 2016). Following this manure rule should help us further reduce pathogens in our feed and livestock system.

In the future, an important component of this study will be to test the meat for microbial quality post animal slaughter. Organic meat production has the potential to have higher microbiological safety risks because of the strict restrictions in the use of pharmaceutical agents for therapeutic use (such as antimicrobials or parasiticides), raising the animals outdoors, use of slow-growing breeds and the smaller slaughtering facilities (Engvall, 2002, Doyle et al., 2006, Thamsborg, 2002). Ensuring proper management in the future including storage and refrigeration, can help reduce these risks (Bourn and Prescott, 2002).

**Conclusion**

Consumers often have the perception that organic foods are safer and healthier than conventionally grown foods, and this is the primary reason for organic food purchases (Van Loo et al., 2010). Over the last few years, numerous food supply crises such as mad cow disease, foot-and-mouth epidemic, and the Belgian dioxin scandal have caused widespread anxiety among consumers about the quality of food they eat (Miles and Frewer, 2001). Use of integrated organic crop-livestock agricultural systems might possess the key to solving this problem of producing safe vegetables and meat. At present, our system has shown very low probabilities (0.0173-IA, 0.0032-MN, 0.0039-PA) for *E. coli* 0157:H7 and (0.0077-IA, 0.0027-MN, 0.0022-PA) for *Salmonella* occurrence. Long term studies analyzing rotational feed crops and meat microbial quality will help us determine the stability and success of the proposed model in producing crops and marketable meat free of *E. coli* 0157:H7 and *Salmonella*. 
Acknowledgements

Support from OREI, USDA is highly appreciated.

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CHAPTER 6: SUMMARY AND CONCLUSION

The increasing popularity of organic food has led us to develop and analyze several modern agricultural methods which are known to be more energy efficient than their conventional counterparts. Analyzing their safety is of prime importance as there have been several occurrences of pathogens associated with organic food like *E. coli* 0157:H7, *Salmonella*, *Campylobacter*, and *Listeria monocytogenes*. In this research work, we have focused on two important foodborne pathogens, *E. coli* 0157:H7 and *Salmonella*, and have analyzed the overall microbial profile of the system in aquaponics using APC, coliform and *E. coli* (fecal coliform) counts.

In aquaponics research, we found that UV treatment used in our model aquaponic unit was not effective in reducing coliform and aerobic plate counts. However, clarifying the water using flocculating agents or reducing the flow rate might improve penetration of UV or increasing the intensity of the radiation might have an enhanced microbiocidal effect. Ozonation and addition of organic acids or natural antimicrobials may be a potential alternative for sterilizing the aquaponic system. Future studies can be conducted using this method or in combination with UV treatment; along with the testing of *Listeria monocytogenes* and aquaculture pathogens. However, we found that there was no *E. coli* 0157:H7 or *Salmonella*, and APC, coliform and generic *E. coli* levels were well below the standards (ICMSF, 1986). Overall, given the many benefits associated with aquaponic food production system, determining a stable system producing safe food would be a huge asset in increasing economic, social, and environmental sustainability. Further studies in similar areas of research are encouraged.
In the first year of our integrated crop-livestock research, our system has shown very low probabilities of *E. coli* 0157:H7 and *Salmonella* occurrence during the months of August and November, 2015. There was no statistical difference between the study sites of Iowa, Minnesota and Pennsylvania in terms of pathogen occurrence (p>0.05). However, long term studies evaluating the safety of the resulting meat products and grains from the different rotational crops will help us better determine the stability and success of our proposed model. Testing the meat quality post-slaughter will help us in understanding the microbial profile of the marketable product. Given the many benefits associated with organic/sustainable agriculture, it should be encouraged more among farmers, with adequate importance given to food safety.
Table A1: Log$_{10}$ of aerobic plate counts collected from basil, water, lettuce, and fish over 118 days in an aquaponic model system.

<table>
<thead>
<tr>
<th>Days of Sampling</th>
<th>Treatment</th>
<th>0</th>
<th>28</th>
<th>42</th>
<th>54</th>
<th>63</th>
<th>76</th>
<th>88</th>
<th>102</th>
<th>118</th>
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<tr>
<td></td>
<td><strong>Lettuce</strong></td>
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<tr>
<td></td>
<td>NO UV</td>
<td>4.97$^{Aa}$</td>
<td>4.80$^{Aa}$</td>
<td>3.78$^{Aa}$</td>
<td>5.37$^{Aa}$</td>
<td>6.17$^{Ba}$</td>
<td>5.52$^{Ca}$</td>
<td>4.21$^{Aa}$</td>
<td>4.32$^{Aa}$</td>
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<tr>
<td></td>
<td>UV</td>
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<td>4.51$^{Aa}$</td>
<td>3.81$^{Aa}$</td>
<td>4.11$^{Aa}$</td>
<td>6.41$^{Bb}$</td>
<td>5.32$^{Aa}$</td>
<td>4.12$^{Aa}$</td>
<td>4.80$^{Aa}$</td>
<td>3.58$^{Aa}$</td>
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A,B Different letters indicate significant differences (p < 0.05) between within the same row.

a,b Different letters indicate significant differences (p < 0.05) between within the same column.
Table A2: Log$_{10}$ of aerobic plate counts of the ultraviolet (UV) sterilizer and non-ultraviolet (No UV) sterilized lettuce samples over 118 days in an aquaponic model system.

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<th>54</th>
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<th>76</th>
<th>88</th>
<th>102</th>
<th>118</th>
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<tr>
<td>NO UV</td>
<td>4.97$^{Aa}$</td>
<td>4.80$^{Aa}$</td>
<td>3.78$^{Aa}$</td>
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<td>6.17$^{Ba}$</td>
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$^{A,B}$ Different letters indicate significant differences (p < 0.05) between within the same row.

$^{a,b}$ Different letters indicate significant differences (p < 0.05) between within the same column.
Table A3: Log₁₀ of coliform counts collected from basil, water, lettuce, and fish over 118 days in an aquaponic model system.

<table>
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<td>1.59&lt;sup&gt;Aa&lt;/sup&gt;</td>
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</tr>
<tr>
<td>NO UV</td>
<td>0.07&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.83&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.19&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>UV</td>
<td>0.18&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.43&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.77&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.79&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO UV</td>
<td>1.00&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>2.26&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.11&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.11&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.30&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.12&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>2.12&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.30&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.21&lt;sup&gt;Ba&lt;/sup&gt;</td>
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<tr>
<td>UV</td>
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<td>2.09&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.90&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.62&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;Ba&lt;/sup&gt;</td>
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<td>Fish</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NO UV</td>
<td>1.17&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.50&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>UV</td>
<td>1.17&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.85&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A,B</sup> Different letters indicate significant differences (p < 0.05) between within the same row.

<sup>a,b</sup> Different letters indicate significant differences (p < 0.05) between within the same column.
Table A4: $\log_{10}$ of *Aeromonas* counts collected from basil, water, lettuce, and fish over 118 days in an aquaponic model system.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>NO UV</td>
<td>3.19$^{Aa}$</td>
</tr>
<tr>
<td>UV</td>
<td>3.08$^{Aa}$</td>
</tr>
</tbody>
</table>

$^{A,B}$ Different letters indicate significant differences ($p < 0.05$) between within the same row.

$^{a,b}$ Different letters indicate significant differences ($p < 0.05$) between within the same column.

Figure A1: Overview of the Iowa State University model aquaponic system utilized to grow lettuce, basil, and Barramundi over 118 days.
Figure A2: Overview of the aquaculture unit utilized to house the Barramundi over 118 days.
Figure A3: Overview of the hydroponic culture unit utilized to grow lettuce and basil over 118 days.
APPENDIX B: CHAPTER 5

Table B1: The probability of *E. coli* 0157:H7 and *Salmonella* occurrence in the feces of calves on a forage diet during the months of August and November.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>State</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> 0157:H7</td>
<td>Iowa</td>
<td>0.0173</td>
</tr>
<tr>
<td><em>E. coli</em> 0157:H7</td>
<td>Minnesota</td>
<td>0.0032</td>
</tr>
<tr>
<td><em>E. coli</em> 0157:H7</td>
<td>Pennsylvania</td>
<td>0.0039</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Iowa</td>
<td>0.0077</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Minnesota</td>
<td>0.0027</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Pennsylvania</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

Table B2: The probability of *E. coli* 0157:H7 or *Salmonella* occurrence in the feces of calves in Iowa, Minnesota and Pennsylvania, on a forage diet, during the months of August and November.

<table>
<thead>
<tr>
<th>State</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>0.0048</td>
</tr>
<tr>
<td>Minnesota</td>
<td>0.0003</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Table B3: Comparison between Iowa, Minnesota and Pennsylvania for significant difference in the presence of *E. coli* 0157:H7 or *Salmonella* using Tukey-Kramer analysis at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>State 1</th>
<th>State 2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>Minnesota</td>
<td>0.9479</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pennsylvania</td>
<td>0.9315</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Pennsylvania</td>
<td>0.7289</td>
</tr>
</tbody>
</table>
Table B4: *E. coli* 0157:H7 and *Salmonella* status of each feed analyzed during the months of August and November, 2015 from Iowa, Minnesota and Pennsylvania.

<table>
<thead>
<tr>
<th>State</th>
<th>Ration</th>
<th>Month</th>
<th><em>E. coli</em> 0157:H7</th>
<th><em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa</td>
<td>Hay</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Corn Grain</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Pasture</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Pasture</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Hay</td>
<td>August</td>
<td>Presence</td>
<td>Presence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Hay</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Fertrell North Atlantic Kelp</td>
<td>August</td>
<td>Presence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Fertrell North Atlantic Kelp</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Redmond Salt Fertrell</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Redmond Salt Fertrell</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Nutri-balancer</td>
<td>August</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Iowa</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Minnesota</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
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</table>
Table B4 continued.

<table>
<thead>
<tr>
<th>Location</th>
<th>Product</th>
<th>Month</th>
<th>Absence 1</th>
<th>Absence 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minnesota</td>
<td>Pasture</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Hay</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Forage</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Redmond SaltFertrell</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>Fertrell NorthAtlantic Kelp</td>
<td>November</td>
<td>Absence</td>
<td>Absence</td>
</tr>
</tbody>
</table>