MEDWIN PUBLISHERS
Committed to create value for Researchers

Food Science and Nutrition Technology
ISSN: 2574-2701

Microbial Risk Analysis of Produce Grown on a Sustainable Chicken Production Farming System

Tong Ding[1], Xinyu Diao[1] and David J Baumler[1,2,3,*]

[1]Department of Food Science and Nutrition, University of Minnesota-Twin Cities, United States

[2]Microbial and Plant Genome Institute

[3]Biotechnology Institute

Short Communication

Volume 2 Issue 3

Received Date: July 25, 2017

Published Date: August 09, 2017

***Corresponding author:** David J Baumler, Department of Food Science and Nutrition, University of Minnesota-Twin Cities, 1354 Eckles Ave, St. Paul, MN 55108, Tel: +1 612 624 3086; Email: dbaumler@umn.edu

Abstract

Sustainable agriculture encourages the use of organic fertilizers instead of synthesized chemicals, and poultry manure can be recycled as an economical organic fertilizer due to its high nutrient content. However, fruits and vegetables in direct contact with manure-fertilized soil can easily be contaminated by coliforms and food-borne pathogens such as *Salmonella spp., Escherichia. coli., and Listeria spp*. These human pathogens may lead to serious food-borne disease outbreaks in susceptible individuals (e.g. children, the elderly, pregnant women, or immunocompromised), making them a concern for sustainable farming using poultry manure. In this study, we tested for the presence of coliforms and the aforementioned pathogens in soil samples collected from a sustainable farming system in Minnesota over five months in 2015, and analyzed the risk of microbial contamination on spinach and cantaloupes grown in this soil. Overall, this study identified those additional control parameters, such as heat/chemical treatment of poultry manure, should be implemented into the practice of sustainable farming using chicken manure to improve the food safety of produce items.

Keywords: Sustainable farming system; Organic fertilizer; Microbial contamination

Introduction

Sustainable agriculture is defined as "an integrated system of plant and animal production practices having a site-specific application" by USDA National Institute of Food and Agriculture [1]. To perform sustainable farming practices, organic fertilizer, which is a soil amendment derived from natural sources, such as animal by-products, has been widely used. A well-designed sustainable faming system should lead to lower energy costs, environmental preservation, and many other benefits. However, the system designed for one specific farm may not work for another, and; therefore, the unique features of the farm must be considered when designing a system to fit that farm [2]. In this study, the farming system was designed for property located in Northfield, MN, possessing free range chickens, perennial plantings of hazelnuts and elderberries, and annual vegetable crops.

The organic fertilizer used for sustainable farming was poultry manure, a good source of nutrients that foster the growth of crops, but also a natural reservoir of human pathogens such as *Salmonella* [3-5]. For the purpose of energy-saving and financial benefits in a sustainable system, the manure had been collected from a local meat broiler and stockpiled outside throughout the winter period. With temperatures that can fall as low as −60°F (−51°C) and heavy snowfalls (2.3 to 170 inches on average) [6], this stockpiling process was integrated within the farming system to inhibit the growth and multiplication of potential pathogens in the manure fertilizer under the extremely cold winter climate in Minnesota.

To evaluate the biological safety of this sustainable farming system, microbiological hazards must be identified, because pathogens present in the air, water, soil, animals, and animal feces can cause microbial contamination that may result in food borne illness outbreaks. Coliforms, including *Escherichia coli (E. coli)* that was found in the gut flora of warm-blooded animals, are commonly used as indicator microorganisms to predict the level of fecal contamination in food and water [7]. In this study, the fecal coliform *E. coli* was tested considering its pathogenicity related to human illness. The presence of *Salmonella* was also tested, because it is widely found in poultry products and can inhabit a wide range of niches from warm-blooded animals to plants [8]. *Listeria monocytogenes* was also tested, due to its ability to survive and multiply under refrigeration temperature [9]. Chickens can be asymptomatic disease-carriers for these bacteria, and the pathogens can contaminate fresh produce through fecal contamination. Reused chicken manure can possess up to 9.7×10^4 CFU/g *E. coli* [3]. The resulting food borne illnesses pose a threat to high-risk population including young, old, pregnant, and immuno-compromised individuals [10].

Materials and Methods

In this study, microbial risk was analyzed by detecting the presence or absence of *Salmonella*, *Listeria*, and coliforms including *E. coli* in samples received from a sustainable farming test field that uses chicken manure as soil fertilizer. According to the farm worker, the poultry litter used in this test field was harvested from a meat broiler unit at Mirasol Farm in October 2014, applied in a 3 inch thick layer, and left between elderberry rows throughout winter. In spring 2015, the manure was turned lightly and applied to crops as fertilizer. Starting in May 2015, one bag of fertilized soil and one bag of unfertilized soil were collected by the farm worker and delivered to our Biosafety Level 2 Laboratory for microbial detection until September. In addition to the comparison between fertilized and unfertilized soils, tests were also done for the chicken manure sample collected in May, the spinach sample collected in June, and the cantaloupe samples collected in August to better interpret the analysis result.

Salmonella detection was done qualitatively over the 5 months based on the current FDA Bacteriological Analytical Manual (BAM) [11]. 3M™ Coliform Count Plates were used for quantitative coliform detection in May, and then 3M™ E. Coli/coliform Count Plates were used instead until September, so the presence or absence of *E. coli* can be detected as well. One detail not included is this type of petrifilm does not specifically indicate whether any O157 strain is present. 3M™ Petrifilm™ Environmental *Listeria* Plates were used for qualitative *Listeria* spp. detection from June to September, after a decision was made to test *E. coli* and *Listeria* other than *Salmonella* and coliforms. Therefore, the manure sample collected in May was not detected for *Listeria* spp. and *E. coli*. 0.1% peptone water was used in serial dilution that was done in duplicate for petrifilm inoculation without enrichment, and the average amount of detected bacteria was calculated for each sample.

Results and Discussion

Coliforms commonly inhabit the intestinal tract of chickens, and; therefore, they can be found in chicken litter and soil that was contaminated by the manure. By comparing the amounts of coliforms in normal soil, manure-fertilized soil, chicken manure used as soil amendment, and fresh produce cultivated in the amended soil, the potential of fecal contamination caused by applying poultry manure as organic fertilizer can be estimated. Table 1 shows detailed counts of coliforms and *E. coli* detected in normal soil, amended soil, and fresh produce samples over five months. On average, the coliform population detected in the samples of manure-fertilized soil ($1.53\times10^4 \pm 4.26\times10^3$ CFU/g) is slightly higher than the amount of coliforms found in the unfertilized soil samples ($1.37\times10^4 \pm 4.77\times10^3$ CFU/g), while the pure poultry manure that was used as soil amendment contains 5.50×10^5 CFU/g coliforms.

	May	June	July	Aug.	Sept.
Fertilized soil[1]	9.75×10^{3}/ N.A.[2]	1.93×10^{4}/ BDL[3]	3.75×10^{3}/ BDL	5.10×10^{4}/ BDL	8.38×10^{3}/7.50×10^{1}
Unfertilized soil[4]	4.00×10^{2} / N.A.	8.50×10^{3}/ BDL	4.98×10^{4}/ BDL	1.05×10^{4}/ 5.00×10^{1}	8.13×10^{2} / BDL
Spinach	– [5]	2.35×10^{4}/ BDL	–	–	–
Cantaloupe 1	–	–	–	5.00/ BDL	–
Cantaloupe 2	–	–	–	3.10×10^{2}/ BDL	–
Fertilizer[6]	5.50×10^{5} / N.A.				

Table 1: The amount of coliforms/*E. coli* (CFU/g) quantitatively detected in fertilizer, manure amended soil, unfertilized soil, and fresh produce samples over five month

1. Soil amended with poultry litter, used for planting fresh produce
2. Not Applicable – no test was performed
3. Below Detection Limit – the presence of colonies was not observable on test
4. Soil without chicken manure amendment, collected from the same farm
5. No sample of such type was collected for the test
6. Organic fertilizer (poultry manure) used for crop cultivation, tested only once

The number of coliform counts detected in fertilized soil fluctuated around the average value over the five months as shown in Figure 1, while for unfertilized soil the number increased from May to July and then dropped till September. This trend may relate to the climate changes over the five months, in which the temperature and moisture level picked around July [12]. The fertilized soil probably kept a consistent moisture level over that period due to irrigation compared to the unfertilized soil that was not used for farming, so its microorganism content was relatively stable. On a log scale, the (Figure 1) better indicated that the coliform populations detected in the samples of manure-fertilized soil and unfertilized soil samples were not statistically different compared to the amount of coliforms found in the poultry manure sample.

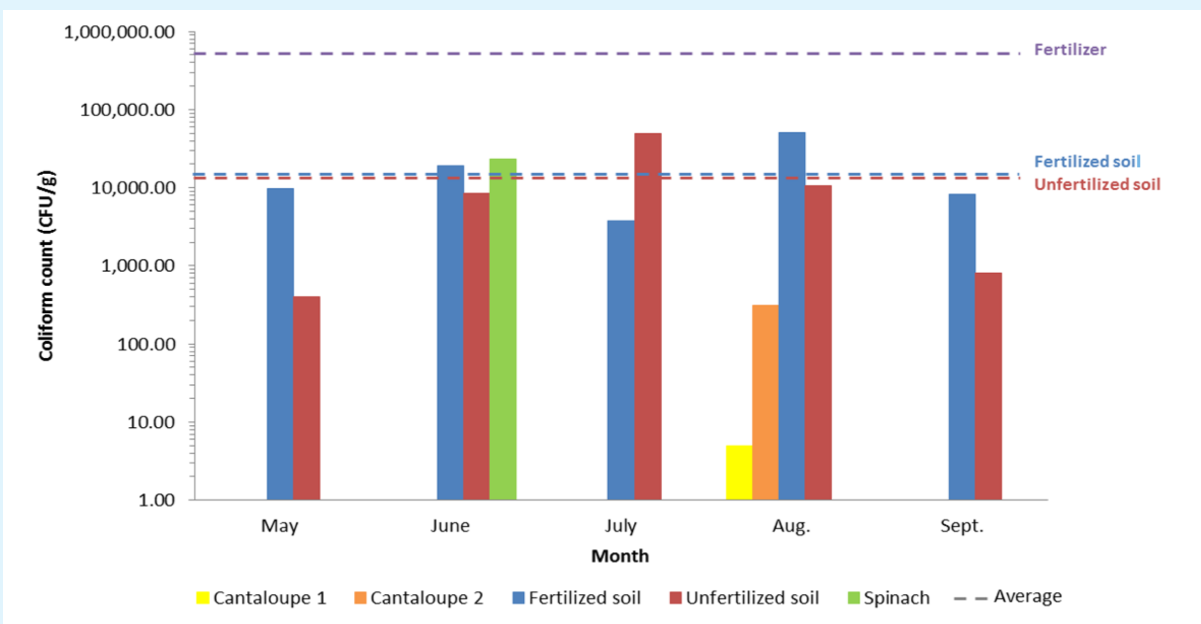

Figure 1: The amount of coliforms (CFU/g) quantitatively detected in fertilizer, manure amended soil, unfertilized soil, and fresh produce samples over five months.

Both cantaloupe and spinach grow outside on the ground, exposing them to soil, rain, and wild animals like birds; therefore, they are at great risk for biohazards related to contaminated environment. In 2006, a multistate outbreak of *E. coli* O157:H7 infections occurred and the source was identified to be fresh spinach [13]. The number of coliforms detected on the spinach sample collected in June was higher than the amount of coliforms found in the amended soil in the same month, but no *E. coli* was found in both samples. The two cantaloupe samples tested in August also do not contain detectable *E. coli*, and the amounts of coliforms they possess were much lower than the value estimated for the fertilized soil sample.

Similarly, the experimental results indicated that *Salmonella* should not be a concern for planting crops using chicken manure as fertilizer in this farming system, as there was no *Salmonella* qualitatively detected on either manure-treated soil or fresh produce samples. In contrast to the *Salmonella* results, the presence of *Listeria* was verified in the unfertilized soil sampled in the last three months of the study, and it was also found in one of the cantaloupe samples in August (Table 2).

	May	June	July	Aug.	Sept.
Fertilized soil[1]	N[2]/N.A.[3]	N/N	N/N	N/N	N/N
Unfertilized soil[4]	N/N.A.	N/N	N/Y[5]	N/Y	N/Y
Spinach	– [6]	N/N	–	–	–
Cantaloupe 1	–	–	–	N/N	–
Cantaloupe 2	–	–	–	N/Y	–
Fertilizer[7]	Y/Y				

Table 2: The presence of *Salmonella*/*Listeria* qualitatively detected in manure amended soil, unfertilized soil, fertilizer and food samples over five months.

1. Soil amended with poultry litter, used for planting fresh produce
2. Not qualitatively detected
3. Not Applicable – no test was performed
4. Soil without chicken manure amendment, collected from the same farm
5. Qualitatively detected
6. No sample of such type was collected for the test
7. Organic fertilizer (poultry manure) used for crop cultivation, tested only once

The Petrifilm used for *Listeria* detection in this study detects environmental *Listeria* spp. including *L. monocytogenes*, *L. innocua*, and *L. welshimeri*. Although the *Listeria* spp. found in the samples of this study cannot be further identified, the presence of *Listeria* spp. provides evidence that the environment is suitable for the occurrence of *L. monocytogenes.* This pathogen is tolerant to refrigeration temperature [14], and it is possible for them to survive under the winter climate in Minnesota. Cantaloupes contaminated with *Listeria monocytogenes* have caused a multistate outbreak of listeriosis in 2011 [15].

Although previous studies have stated that *L. monocytogenes* should not be a concern for chicken manure-based organic fertilizers, considering it is usually absent from chicken waste [3], due to the limitations in sampling we cannot identify the source of *Listeria* contamination. As the unfertilized soil samples showed positive in *Listeria* presence, it is possible that the cantaloupe was contaminated by factors other than the use of manure-amended farming soil. Practices such as soil amendment application, irrigation before harvest, gathering, handling, and processing after harvest are factors that may influence the microbial safety of the fresh produce. Climate change, wildlife interference, and geographical location can also affect the safety of food.

The stockpiling process utilized in this sustainable chicken production farming system is intended to suppress the growth of pathogens with cold temperature throughout the winter in Minnesota. Nevertheless, small amounts of bacteria may regenerate from a small population after surviving temperature-dependent processes when they encounter suitable conditions [16]. Therefore, the time interval between raw manure application and harvest has an impact on the risk of crop contamination. For crops in contact with the soil, the FDA Food Safety Modernization Act proposed an application interval of nine months but later compromised with a 120-day interval for the farmers complying with the USDA's National Organic Program standards [17]. An interval of at least one year for the use of raw manure is suggested by The Leafy Greens Marketing Agreement, considering the long survival period of bacteria in raw manure that poses a risk for lettuce and leafy greens [18].

As Chen *et al*. suggested, subsequent treatments should be added to more efficiently inactivate pathogenic *microorganisms* in chicken litter [3]. Additional control parameters, such as heat or chemical treatment, are recommended to minimize the risk of contamination and improve safety [5, 16]. Field experiments by Nicholson *et al*. showed that if the temperature in solid manure heaps surpasses 55 °C, the level of *E. coli, Salmonella*, and *Listeria* will be undetectable within one week [19]. Destruction of the three microorganisms can also be greatly increased by decreasing the moisture content and exposing the manure to ammonia gas [20].

Conclusion

Overall, this work found the proposed sustainable farming system is unlikely related to *E. coli* and *Salmonella* contamination. Although there currently is no standard for the tolerant amount of *Listeria* on the surface of cantaloupes, it is suggested that additional biohazard control approaches should be implemented into the practice of sustainable farming to improve the safety of produce items.

References

1. (2011) United States Code Title 7.
2. Rigby D, Cáceres D (2001) Organic farming and the sustainability of agricultural systems. Agricultural systems 68: 21-40.
3. Chen Z, Jiang X (2014) Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review. Agriculture 4: 1-29.
4. Jung KS, Heu SG, Roh EJ, Kim MH, Gil HJ, et al. (2013) Survival of Salmonella enterica and Listeria monocytogenes in Chicken and Pig Manure Compost. Korean Journal of Soil Science and Fertilizer 46: 469-473.
5. Wilkinson K, Tee E, Tomkins R, Hepworth G, Premier R (2011) Effect of heating and aging of poultry litter on the persistence of enteric bacteria. Poultry science 90: 10-18.
6. (2017) Climate of Minnesota. 5/22/2017 ed. Wikipedia: The Wikimedia Foundation, Inc.
7. Larney FJ, Yanke LJ, Miller JJ, McAllister TA (2003) Fate of coliform bacteria in composted beef cattle feedlot manure. Journal of environmental quality 32: 1508-1515.
8. Guard-Petter J (2001) the chicken, the egg and Salmonella enteritidis. Environmental microbiology 3(7): 421-430.
9. Bortolussi R (2008) Listeriosis: a primer. Canadian Medical Association Journal 179(8): 795-797.
10. Lund BM, O Brien SJ (2011) the occurrence and prevention of food borne disease in vulnerable people. Food borne Pathogens and Disease 8(9): 961-973.
11. FDA (2016) BAM: Salmonella. Laboratory Methods: U.S. Food and Drug Administration.
12. Annual Weather Averages near Minneapolis. www.timeanddate.com.
13. Control CfD, Prevention (2006) ongoing multistate outbreak of Escherichia coli serotype O157: H7 infections associated with consumption of fresh spinach--United States, September 2006. MMWR Morbidity and mortality weekly report 55(38): 1045.
14. Hudson J, Mott S (1993) Growth of *Listeria monocytogenes, Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiology 10: 61-68.
15. Control CfD, Prevention (2011) multistate outbreak of listeriosis associated with Jensen Farms cantaloupe--United States, August-September 2011. MMWR Morbidity and mortality weekly report 60(39): 1357.
16. Kim J, Shepherd MW, Jiang X (2009) Evaluating the effect of environmental factors on pathogen regrowth in compost extract. Microbial ecology 58(3): 498-508.

17. FSMA Final Rule on Produce Safety. https://www.fda.gov: U.S. Food and Drug Administration.

18. (2010) Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens. Leafy Greens Marketing Agreement.

19. Nicholson FA, Groves SJ, Chambers BJ (2005) Pathogen survival during livestock manure storage and following land application. Bioresource technology 96: 135-143.

20. Himathongkham S, Riemann H (1999) Destruction of Salmonella Typhimurium, Escherichia coli O157: H7 and Listeria monocytogenes in chicken manure by drying and/or gassing with ammonia. FEMS microbiology letters 171(2): 179-182.