Bacteria in Runoff following Simulated Rain Events using Greenhouse and Small Field-Plot Poultry Litter Application

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Abstract

Poultry rearing in the United States is approximately a 30 billion dollar per year industry. Land application of poultry litter is an economically viable use of this manure byproduct. However the recent concern associated with organic food and pathogenic bacterial contamination has led to increased scrutiny regarding land applied manures. Runoff following a rain event is one possible source of environmental contamination resulting from manure application. In this study a series of treatments involving litter (two rates), inorganic fertilizer, and no fertilizer controls were added to bermudagrass troughs and plots held in a greenhouse and field scale environments, respectively. A rainfall simulator was used to simulate precipitation events and following each rain event, runoff samples were collected for microbial analyses. Total Heterotrophic Plate Count (HPC) bacteria, antibiotic resistant bacteria (ARB), thermal-tolerant coliforms, enterococci, staphylococci, and *Clostridium perfringens* were investigated. Over a period of 30 days, 5 rain events were simulated and results indicated that staphylococci, enterococci, and clostridia correlated directly with manure application. Traditional indicators such as thermal-tolerant and total coliforms performed poorly as fecal indicators relative to the other bacteria assayed in this study. No "frank" pathogens such as *Salmonella* or *Campylobacter* were detected in the applied litter or runoff. This study indicated that poultry litter land application can contribute to microbial runoff, however proper land and agronomic management practices can mitigate this.

Introduction

Land application of waste byproducts as fertilizer has been a common agricultural practice since land applying "night-soil" began centuries ago. Manure from bovine, ovine, and poultry sources are some of the most common sources of organic fertilizers. These wastes are ripe with N, P, and other plant nutrients which make the application of these manures as fertilizer the most efficient method of manure reuse. Precautions must be taken to avoid runoff of these manure-borne nutrients as well as manure-borne microorganisms, which can survive land application and potentially be moved horizontally via runoff following rain events.

Though many manure-borne microorganisms are environmentally labile organisms, some such as spore forming bacteria can survive for extremely long periods of time. In addition, the presence of antibiotic resistance bacteria (ARB) can compound the issue as potentially all manure-borne bacteria (pathogenic and non-pathogenic) can harbor antibiotic resistance (both intrinsic and acquired).

The purpose of this research was to identify the potential for manure borne bacteria to be horizontally transported via simulated rain water runoff. Runoff samples were collected from simulated runoff troughs and plots and analyzed for the presence of a wide range of bacteria and selected isolates were analyzed for antibiogram characteristics.

Objectives

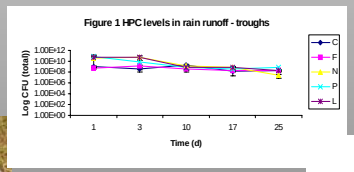
1. To determine the microbial runoff associated with simulated rain events on poultry-litter applied runoff troughs under artificial conditions
2. To determine the microbial runoff associated with simulated rain events on poultry-litter applied land under field conditions
3. To determine the antibiotic resistance biograms associated with commonly isolated poultry litter bacterial isolates.
4. To determine the suitability of runoff trough simulations vs. field experiments.

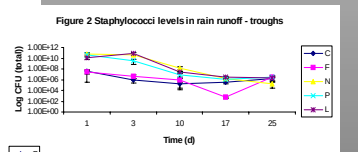
Materials and Methods

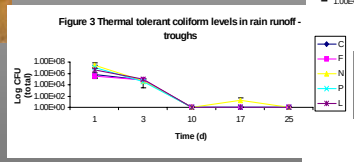
1. Simulated runoff troughs, greenhouse:
   1. Runoff troughs = 3.1 ft²
   2. 3 troughs per treatment, randomized trough design
      A. Treatments = Litter-Nitrogen 250 lb/acre (N), Litter-Phosphorus 50 lb/acre (P), Chemical fertilizer (F), Lime (10% weight) (L), Control (C)
      B. Troughs = Bermudagrass – grown 1.5 months
2. Field runoff plots:
   1. Plot size = 84 ft²
   2. 3 plots per treatment, randomized plot design
      A. Treatments = Litter-Nitrogen 250 lb/acre (SF-N), Litter-Phosphorus 50 lb/acre (SF-P), Chemical fertilizer (SF-F), Control (SF-C)
3. Poultry Litter:
   1. Poultry litter stored for five days prior to application
4. Rain Simulation:
   1. Rain events = 1/week, 27-30 mm/hr
      Time = 25 min/rain event
5. Sample collection
   1. Samples collected – sterile 250 ml bottles
      A. Trough runoff collected via collectors at edge of the trough.
      B. Field runoff collected via runoff trays buried at edge of the plot.

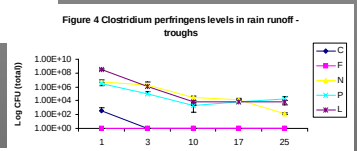
6. Assays
   Culture
   1. Heterotrophic Plate Count Bacteria (HPC)
   2. *Staphylococcus*
   3. *Enterococcus*
   4. Total and Thermotolerant coliforms
   5. *Escherichia coli (E. coli)*
   6. *Clostridium perfringens*
   7. *Salmonella*
   8. *Campylobacter*
   9. Antibiotic Resistant Bacteria (ARB)
      A. Antibiogram
         - Kirby-Bauer Method
           E, P, PB, CIP, TE, AM, AN, GM, N, CF, K, and VA

Results – Simulated Rain Runoff Troughs









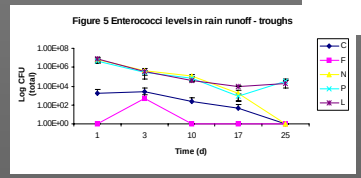
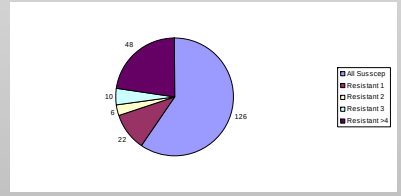


Figure 7: Antibacterial resistance (antibiogram) associated with bacterial isolates from litter and runoff.



Conclusions

1. Microbial runoff occurred for 4 weeks
   1. No *Salmonella* or *Campylobacter* detected in applied litter or runoff
      A. Fresh poultry litter – *Salmonella* detected
      B. Storage reduced to non-detect levels
   2. Though runoff occurred, samples were collected at the edge of the troughs/plots without grass borders – not indicative of surface water contamination
   3. Border (grass) strips can limit potential runoff to surface water

2. Traditional ("fecal") coliform indicators were not suitable
   1. Alternates needed
      A. *Clostridium perfringens* – Best alternative
         1. Direct correlation with litter application
         2. Has source tracking capabilities – positive aspect
      B. Possible other indicators: Staphylococci, Enterococci
         1. More ubiquitous sources – negative aspect
         2. Have source tracking capabilities – positive aspect

3. ARB could be a concern – depends on litter or manure source and previous antibiotic use
   1. Most bacterial isolates were susceptible to antibiotics tested.
   2. Most resistant isolates were resistant to 4 antibiotics.
   3. Most had intrinsic resistance, possible sign of minimal antibiotic use

4. Runoff simulation troughs can be used in lieu of field simulations.
   1. Runoff data was similar for both runoff greenhouse simulation and field plot trials

Future Work

1. 16S rRNA analysis of microbial runoff samples
2. Investigate fingerprinting profiles
3. Source track methodologies
4. Repeat simulation(s) using alternative agronomic rates and practices.

Acknowledgements

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