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Destruction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in chicken manure by drying and/or gassing with ammonia

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Abstract

Escherichia coli O157:H7 and *Listeria monocytogenes* were able to grow for a period of 2 days in fresh chicken manure at 20°C with a resulting 1–2 log units increase in CFU; *Salmonella typhimurium* remained stable. Prolongation of the storage time to 6 days resulted in a 1–2 log decrease of *S. typhimurium* compared to the initial count and a 3–4 log decrease of *E. coli* O157:H7; the number of *L. monocytogenes* did not decrease below the initial. These changes were accompanied by an increase in pH and accumulation of ammonia in the manure. The destruction of the three microorganisms was greatly increased by drying the manure to a moisture content of 10% followed by exposure to ammonia gas in an amount of 1% of the manure wet weight; *S. typhimurium* and *E. coli* O157:H7 were reduced by 8 log units, *L. monocytogenes* by 4.

Keywords: *Salmonella*; *Escherichia coli*; *Listeria*; Feces; Ammonia; Chicken; Water; Growth; Development

1. Introduction

Poultry as well as other animals shed *Salmonella* and other enteric microorganisms in the feces if they become colonized. This may facilitate horizontal transmission and has also caused concern in connection with the practice of feeding chicken manure to cattle or applying it as a fertilizer for growing produce for human consumption; this may result in disease unless the manure is sterilized or pasteurized. Composting or other processing procedures can reduce the number of viable pathogens in manure but only a limited amount of manure is composted and the process may not always be under strict control. Drying in itself reduces the number of *Salmonella* in manure and litter [1–3] but it has been found that drying is only effective at certain intermediate levels of water activity; when most of the water has been removed *Salmonella* will survive for long periods of time [4]. Ammonia above a certain concentration also has a killing effect [2]. However, there does not seem to be any published report related to the application of ammonia and drying to reduce human pathogens in manure. The aim of this report is to provide an evaluation of the effect of drying and/or exposure to ammonia on *Salmonella typhimurium*,

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Escherichia coli O157:H7 and *Listeria monocytogenes* in chicken manure.

2. Materials and methods

2.1. Manure and bacterial strains

Fresh chicken manure was obtained from the Department of Avian Science facility, University of California, Davis. It was found to be free of natural contamination with the three test pathogens. *E. coli* O157:H7 was obtained from Dr. B. Walsh, Department of Population Health and Reproduction, University of California, Davis; *S. typhimurium* was isolated in a chicken house in California; *L. monocytogenes* was obtained from Dr. D. Hirsh, Department of Pathology, Microbiology and Immunology, University of California, Davis.

2.2. Bacterial inocula and counting procedures

Inocula to be added to manure were grown on blood agar at 37°C for 24 h and the cells were suspended in sterile saline. The numbers of microorganisms in the manure before and after treatments were determined by suspending a 10-g manure sample in 100 ml sterile saline and surface plating on brilliant green novobiocin agar (Difco plus 20 mg novobiocin per liter) for *Salmonella*, violet red bile agar (Difco) for *E. coli* and lithium chloride phenyl ethanol moxalactam agar [5] for *L. monocytogenes*. Quantification of low numbers was done by a most probable number procedure [6] where four 10-fold dilutions, two units per dilution level, were cultured in pre-enrichment broth followed by confirmation using the plating media mentioned above. Pre-enrichment of *Salmonella* and *E. coli* took place in lactose broth (16 h at 37°C); for *Salmonella* this was followed by selective enrichment in tetrathionate broth (Difco) for 24 h at 37°C. Pre-enrichment of *Listeria* took place in *Brucella* broth (Difco) for three weeks at 4°C.

2.3. Inoculation and treatment of manure

Ten grams of manure was distributed evenly on the bottom of a standard petri dish (100 mm × 15 mm) and five 20-μl volumes of bacterial suspensions, containing approximately 10^{10} CFU per ml, were distributed on the surface of the manure. For counting bacteria, pH measurements and determination of ammonia the entire manure sample was suspended in 60 ml sterile saline.

Manure was dried by placing the open petri dish on the laboratory bench at 20°C and under normal ventilation (relative air humidity was about 50%); This resulted in a drop of the moisture content in the manure from 60–70% to about 10% in 24 h, this corresponds to water activity levels of 0.91–0.99 and 0.37 respectively (unpublished data).

Exposure to ammonia gas was done by placing a small petri dish with calculated amounts of ammonium sulfate and potassium hydroxide and water in the dish containing the manure sample; a matching half of a standard petri dish was then used to close the dish with the manure sample and sealed in place with electric tape. After the unit, which had an air space of 122 cm^3, had been closed it was tilted to allow the potassium hydroxide to react with water and ammonium sulfate to release the desired amount of ammonia. When combinations of drying and exposure to ammonia was used the drying was done first.

2.4. Determination of moisture, pH and ammonia in manure

Total moisture was determined by drying a manure sample at 105°C for 2 h; pH was determined with a glass electrode. Ammonia was determined by using an improvised diffusion chamber; 2 ml of the manure suspension was placed in a small, open petri dish (3.5 cm diameter), 1 ml diluted sulfuric was placed in another open, small petri dish. Both were placed in a standard petri dish, potassium hydroxide pellets were put into the manure suspension and the standard petri dish was immediately closed with a matching half of a petri dish that was sealed in place with electric tape. After 24 h at room temperature the amount of volatile bases (ammonia) was determined by titrating the sulfuric acid with 1 N sodium hydroxide using bromocresol purple as an indicator.

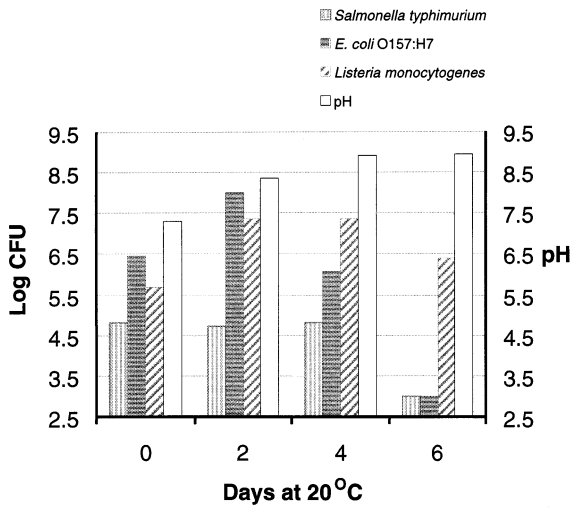

Fig. 1. pH changes and growth/survival of pathogens at 20°C in fresh chicken manure with 71% moisture content.

3. Results

3.1. Growth and survival of microorganisms in fresh manure

L. monocytogenes and *E. coli* O157:H7 grew for a limited time in the manure and reached levels 10–100 times higher than the initial level in 2 days (Fig. 1). Following that a decline set in and *S. typhimurium* and *E. coli* O157:H7 declined to below the initial level in 6 days, *L. monocytogenes* was more persistent. The decline was accompanied by an increase in pH to close to 9.5, probably due to an increase in ammonia (Fig. 2) that reached levels of 3.4 mg g^{-1} in 48 h.

3.2. Destruction of microorganisms in manure by drying and/or gassing with ammonia

The separate and combined effects of drying and gassing with ammonia were tested in a factorial experiment with two replications. Drying reduced the water content of the manure to 10%, gassing was done by generating ammonia in amounts corresponding to 1% of the wet manure weight. The results, expressed as changes in log CFU, are shown in Fig. 3. The untreated controls exhibited growth of the microorganism which is in agreement with the

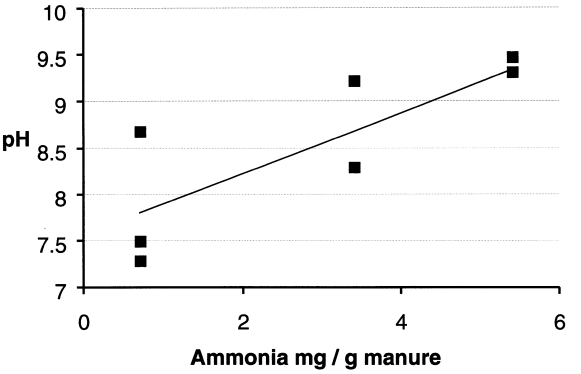

Fig. 2. pH and ammonia levels (mg g^{-1}) in fresh chicken manure.

results for fresh manure (Fig. 1). Drying alone and gassing with ammonia alone resulted in less than two log reductions. Drying followed by gassing with ammonia resulted in a 4-log reduction of *E. coli* O157:H7, 3 logs for *S. typhimurium* and 2.5 logs for *L. monocytogenes*. These results were obtained with a 24-h treatment and a partial replication of the experiment was done where the treatment with ammonia gas was extended to 72 h (Fig. 4). This resulted in an almost 8-log reduction of *S. typhimurium* and *E. coli* O157:H7 and more than 4-log reduction of *L. monocytogenes* from an initial number of 10^9 CFU g^{-1}.

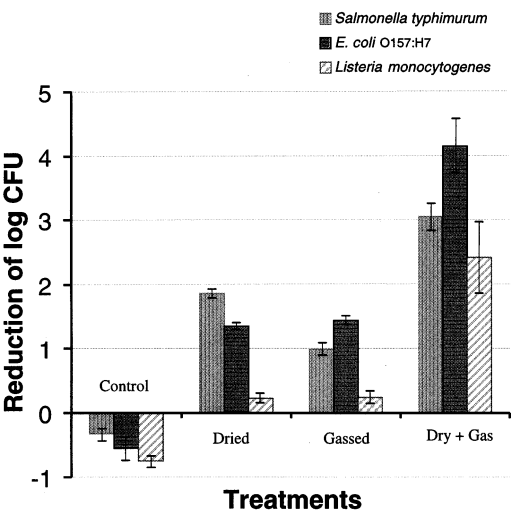

Fig. 3. Reduction of log CFU g^{-1} of pathogens in manure exposed to drying to 10% moisture in 24 h at 20°C and/or exposed to 1% ammonia gas for 24 h at 20°C.

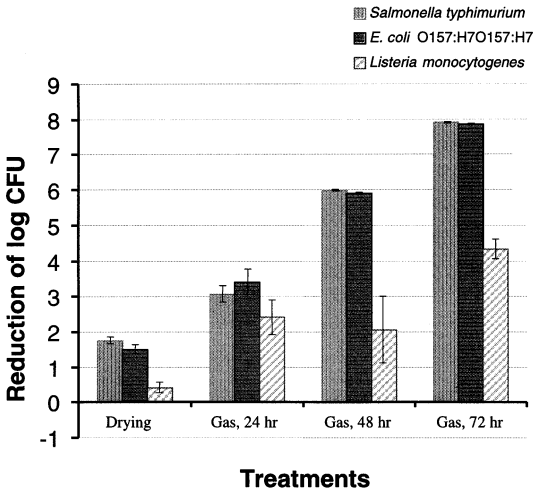

Fig. 4. Survival of pathogens in chicken manure after drying at 20°C to a moisture level of 10% followed by exposure to 1% ammonia gas for 24 h, 48 h and 72 h, at 20°C.

4. Discussion

Ammonia is naturally generated by indigenous microorganisms in moist chicken manure at appropriate temperature [7] and can cause a significant reduction of non-spore forming pathogens in stacked manure [2,7]. However, it is practice in commercial poultry production to let the manure dry in order to reduce the detrimental effect ammonia on the birds [8]; under these circumstances the destruction of pathogens becomes less predictable. The present study indicates that treatment of the dry manure with ammonia results in a significant reduction of common pathogens. The amount of added ammonia in these experiments corresponds to 10 kg, or 13 l liquid ammonia, per ton manure as compared to a natural content of total nitrogen in chicken manure of 2–60 kg per ton [9]. By extending the exposure time it might be possible to decontaminate manure with concentrations of ammonia smaller than 10 kg per ton manure.

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